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Unravelling and exploiting astrocyte dysfunction in Huntington's disease

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Abstract

Astrocytes are abundant within mature neural circuits and are involved in brain disorders. Here, we summarise our current understanding of astrocytes and Huntington's disease (HD) with a focus on correlative and causative dysfunctions of ion homeostasis, calcium signaling, and neurotransmitter clearance, as well as on the use of transplanted astrocytes to produce therapeutic benefit in mouse models of HD. Overall, the data suggest astrocyte dysfunction may be an important contributor to the onset and progression of some HD symptoms in mice. Additional exploration of astrocytes in HD mouse models and humans is needed and may provide new therapeutic opportunities to explore in conjunction with neuronal rescue and repair strategies.

Keywords

astrocyte; HD; GAT3; Kir4.1; calcium; GLT1; EAAT2; KCNJ10; GCaMP

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Astrocytes are critical components of neural circuits and are known to be involved in brain disorders [1, 2]. Astrocytes may represent about 40% of all brain cells and tile the nervous system [3]. In mice, cortical astrocytes are born soon after neurogenesis at around embryonic day 16, and following migration along radial glia they occupy well defined territories with properties and locations that may be tailored to the needs of nearby neurons [4]. Each individual mature astrocyte comprises a cell body, six or so major branches, a thick blood vessel-associated endfoot, and myriad finer branchlets and leaflets [3]. Although astrocytes are highly complex structures, they are not polarised like neurons and their complexity is largely equivalent throughout individual territories [5] that overlap very little in healthy rodent tissue [6] or in models of trauma [7]. It is these stereotypical features that give rise to “bushy” astrocyte appearances in healthy tissue (Figure 1). The loss of some of these anatomical features has been documented in disease [8], including in HD [9].

There has been progress in deciphering how astrocytes contribute to the nervous system since the first electrophysiological studies ~50 years ago [10, 11]. Established, proposed and debated roles for astrocytes include neurovascular coupling, stabilizing the blood brain barrier, regulation of synaptic function, ion homeostasis, neurotransmitter clearance, metabolic support of neurons, regulation of action potential waveforms, synaptic plasticity and regulation of behaviors such as sleep [3, 12–18]. In addition, it is now well established that astrocytes respond to all forms of injury, trauma and infection via a process termed astrogliosis [19].

Herein, we summarise recent work that is beginning to unravel how astrocyte dysfunction accompanies and contributes to neuronal deficits in Huntington’s disease model mice (Box 1). We do not consider other glia (e.g. microglia, oligodendrocytes, NG2 cells, Schwann cells, Bergmann glia, Müller cells), because these are beyond the scope of this review that concerns astrocytes. However, broad changes in glia are to be expected from human studies showing profound gene expression changes in HD: perhaps around 20% of all genes are altered [20]. In addition, there is currently a paucity of available data on these specific forms of glia in HD models. Furthermore, by necessity, we focus on recent aspects and seek to emphasize specific astrocyte dysfunctions rather than astrogliosis, which itself is a highly complex, often all-embracing general response [8] that could be secondary to neuronal changes in the context of HD mouse models [21]. We believe this distinction has value in order to form, test and ultimately prove or refute specific hypotheses. However, there has been recent progress in identifying reactive astrocytes classified as “A1” being potentially damaging in HD: the neurotoxic substance they release and A1 astrocytes themselves now also merit evaluation in HD [22].

Huntington’s disease (HD)

Huntington’s disease is a neurodegenerative disease characterized by progressive motor, psychiatric and cognitive dysfunction [23, 24], caused by an expansion of a polyglutamine-encoding CAG repeat (>36) in exon 1 of the huntingtin gene (HTT) [The Huntington’s Disease Collaborative Research Group, 25]. HD is primarily a basal ganglia disorder: cortico-striatal-thalamo-cortical neural circuits appear particularly affected [26] (Figure 2). Cortical thinning and basal ganglia degeneration are obvious (Figure 2). We have restricted

our discussions to the striatum, because of the dearth of data exploring cortical astrocytes in HD mouse models.

The molecular and physiological role of normal HTT, or the known pathogenic mechanisms induced by mutant HTT (mHTT), have thus far failed to identify precise events that contribute to the subsequent stereotyped progression of the disease. Nonetheless, several general points merit discussion. There is profound loss of GABAergic medium spiny neurons (MSNs) in the caudate-putamen (the *Corpus striatum*) as well as of neurons in the *Globus pallidus* [27], in addition to heterogeneous grey and white matter loss, especially in cortical regions [reviewed in 28]. In accord, most effort has focused on the study of mHTT expression in, and on, neuronal function in the corticostriatal inputs and output MSNs. However, it is now clear from human imaging data [23] that HD related structural brain changes (Figure 2) are more widespread and heterogeneous than first appreciated [reviewed in 28]. Expression of HTT and mHTT is ubiquitous throughout the body: additional pathology (including peripheral pathology) is thus expected and known. However, the exquisite vulnerability of particular neural circuits and relative sparing of others remains largely unexplained. It remains to be clarified whether or not astrocyte dysfunction and loss of support of these circuits plays a significant role in the known patterns of pathology.

Astrocyte changes in humans with HD

A hallmark of HD neuronal pathology is the presence of intracellular mHTT aggregates. However, inclusions are also present in cortical and striatal astrocytes [29] and detected to the same extent as in neurons in adult-onset HD patient tissue [30], although neuronal inclusions are generally larger. Fibrillary astrocytosis, as assessed by microscopic analysis of cresyl violet stained sections within the *Corpus striatum*, is a classifying feature of HD: absent in Grade 0, but progressing from mild, to moderate and severe from Grade 1 – 4 [27, 31, 32]. At Grade 0, there is loss of neurons with no detectable astrocytosis [32]. Individual astrocytes imaged using GFAP immunostaining in HD brains display thicker processes and larger somata [9] than in control striatal tissue, where astrocytes were lightly labelled with GFAP and displayed a fine lace-like appearance, similar to healthy rodent astrocytes [33]. In Faideau et al, GFAP density markedly increased two-fold by Grades 2 and above [9]. In another study, increased GFAP immunoreactivity was noted in Grade 0–1 tissue and was confined to striatal striosomal rather than matrix regions [34]. In this section, we have used the terms astrocytosis and astrogliosis in accord with the original papers, but each term is poorly defined.

In the adult rodent forebrain, ~90% of glutamate uptake is via the high-affinity electrogenic transporter known as EAAT2 or GLT1 [35] (gene name *Slc1A2*), which is highly expressed in astrocytes. A consistent finding between multiple studies in human HD postmortem tissue is the loss of GLT1 [9, 29, 36]. The loss of GLT1 increased with HD severity, but was already substantially reduced by ~40% in Grade 0 specimens compared to healthy controls [9], making this the earliest astrocyte dysfunction detected in HD patients.

The aforementioned findings are from postmortem tissue in patients with a confirmed clinical diagnosis of HD, and it is important to reiterate that neuropathological HD Grade is

not comparable to the clinical UHDRS rating of HD patients, which confusingly also uses a 0 – 4 scale [37]. Indeed, based on the firm clinical diagnosis of HD in postmortem Grade 0 patients, clinical symptoms are likely to precede astrocyte changes. Hence, to clarify if astrocyte dysfunction precedes, accompanies or follows that of neuronal pathology in human HD, and for the establishment of a relationship to clinical symptoms, improved *in vivo* imaging tools to assess astrocyte (dys)function need to be developed. Such work is highly relevant, because human and mouse astrocytes markedly differ.

Pathophysiology induced by expression of mHTT in astrocytes

Several pioneering rodent studies expressing mHTT specifically in astrocytes suggest that astrocyte dysfunction directly contributes to HD pathology. Heterologous expression of mHTT using lentiviruses *in vivo* reproduced many of the morphological features observed in human HD specimens, suggesting that the changes seen in HD patient astrocytes are cell-autonomous and perhaps not the result of neuronal damage [9]. Expression of mHTT in astrocytes resulted in death of co-cultured striatal neurons [29], and expression of a mHTT N-terminal fragment with either 98 or 160Qs in astrocytes *in vivo* using the human GFAP promoter lead to reduced GLT1 expression, astrogliosis, and HD-like pathology [9, 38, 39]. Loss of BDNF release from astrocytes has also been reported [40]. Behavioural alterations and premature death were observed in the hGFAP-Q160 expressing model [38, 41]. Further, in cynomologus monkeys, lentiviral overexpression of a N171-Q82 mHTT fragment triggered neuronal death and astrogliosis via activation of the JAK/STAT3 pathway [42]. However, these studies do not directly test if astrocyte physiology is altered in conventional mouse models of HD. Notably, transgenic mice using the GFAP promoter also target neuronal progenitors, some mature neurons and peripheral organs [43, 44]. Hence, an important task was to test the hypothesis that astrocyte physiology is altered in standard models of HD that, like the human disease, reflect mHTT expression in multiple cell types.

Neuronal changes with potential involvement of astrocytes in HD model mice

Rodent HD models have been invaluable to identify the earliest physiological changes that occur due to mHTT, and to clarify the sequence of primary and secondary pathological alterations that may occur. The plethora of available models include: transgenic mice overexpressing either full length, or fragment, mouse, human or hybrid mHTT under various promoters, and ‘knock-in’ mice expressing full length mHTT under control of the endogenous promoter. Box 1 summarizes astrocyte-related HD phenotypes found in popular and widely used mouse models.

The similarity of murine HD models to human HD is particularly obvious with regard to the progressive loss of GLT1 [33, 45–51] (Box 1). Also reminiscent of findings from HD post-mortem tissues, nuclear inclusions are present in astrocytes in HD mouse models [29, 30, 33]. Striatal astrocytes from R6/2 mice contained mHTT nuclear inclusions as early as 4–5 weeks of age [29], i.e. when the mice are just becoming symptomatic, but before detectable evidence of astrogliosis and overt tissue loss. These astrocytic inclusions are less abundant than in neurons at the same age, although the proportion of astrocytes showing inclusions

increased progressively to 30–50% of S100β-positive astrocytes, while this proportion was much less in GFAP-positive astrocytes [29, 33]. Morphological analysis in humans and HD mice shows that there is neither glial cell death, nor strong evidence of reactive astrogliosis at early symptomatic ages when GLT1 loss occurs [30, 33].

Regarding a potential contribution of astrocytic dysfunction to neural dysfunction in HD, the first suggestion came from studies in awake behaving HD model mice during recording of cortical and striatal activity [47, 52]. Recording extracellular spike activity and local field potentials (LFPs) in R6/1, R6/2 and full-length (knock-in) mouse models, Rebec and colleagues discovered that signal processing in the HD striatum was altered. The HD-related changes included an increase in the mean firing frequency of units within the striatum, while coincident M1 and PFC cortical and striatal bursting, which measures the temporal overlap between bursting pairs, was reduced. The increased firing frequency deduced from LFP recording in the striatum of awake HD mice may have several causes: increased intrinsic excitability of MSNs, tonically elevated extracellular [K^+] levels and a weakness of tonic inhibition via extrasynaptic GABA(A) receptors, among others. The impairment of cortical and striatal firing synchronicity also indicated reduced corticostriatal connectivity. Of particular interest, ceftriaxone administration to R6/2 mice (a beta-lactam antibiotic known to elevate GLT1) attenuated several HD behavioral phenotypes; paw clasping and twitching were reduced, while motor flexibility and open-field climbing were increased [53]. This was correlated with an improvement in striatal and cortical GLT1 levels [48], and increased striatal glutamate uptake, as measured by no-net-flux microdialysis [48, 53]. Although correction of the neurophysiological phenotypes were not explicitly evaluated, improvements in the behavioral phenotype implied a correction of the basal ganglia motor circuitry via GLT1 upregulation.

Many neurophysiological abnormalities in the HD corticostriatal connections and MSN excitability can be observed in acute slice preparations from HD models. HD MSNs show a progressive phenotype of emerging intrinsic hyperexcitability, whereby cells become modestly depolarized, exhibit reduced rheobase in response to current injection, and exhibit significantly higher membrane resistance around resting states, implying the loss of hyperpolarizing conductances that maintain quiescence. These findings are progressive and have been demonstrated in both R6/2 mice as early as 5–7 weeks [54–58] and in Q175 KI mice around 4 months of age [59–61]. It is likely that a loss of intrinsic MSN potassium conductances at least in part underlies this phenomenon; loss of inwardly rectifying potassium channels (Kirs) are likely contributors [56, 62] as they are largely responsible for maintaining Vm, and respective gene expression is known to be decreased [56, 62]. However, extracellular basal K^+ levels measured *in vivo* in the striatum of symptomatic R6/2 mice are elevated by ~0.7–1.5 mM, which would also be expected to depolarize MSNs and contribute to this phenotype [33, 63].

Regarding corticostriatal connectivity, MSNs in symptomatic R6/2, YAC128 and Q175 mice also display a progressive impairment in responsiveness to cortical stimulation ex vivo, whereby equivalently sized cortically evoked EPSCs (relative to WT age-matched controls) can only be achieved with higher stimulus intensities [55, 59, 64, and reviewed in 65]. Multiple HD mouse models in addition show reduced frequency of mEPSCs and sEPSCs

onto MSNs, indicating a paucity of striatal glutamatergic input, in R6/2 by 6–8 weeks [55], in Q175 het by 6 months [59, 60], and in YAC128 at 7 months [64]. *In vivo*, R6/2 mice show reduced glutamate release as measured by microdialysis in response to K^+ stimulation by 6 weeks [52, 66]. These data point to an impairment in corticostriatal and/or thalamostriatal glutamatergic inputs.

Complex alterations in striatal GABAergic transmission are also apparent in HD mouse model striatum [58]. Unitary eIPSC amplitudes in MSNs in response to intrastriatal stimulation are reduced in R6/2 and Q175 mice, with a clear deficit in the probability of release, as indicated by a higher paired-pulse ratio, failure rate and coefficient of variation [67]. Pharmacological interrogation identified this suppression of synaptic GABA release as a glutamate- and endocannabinoid-dependent process [49, 67]. However, spontaneous action potential-dependent GABAergic synaptic activity is enhanced in these models [58, 67], likely due to the aberrant activity of MSNs and/or GABAergic interneuron populations [58]. There is also a noted decrease in tonic inhibition of HD MSNs, most likely due to reduced GAT3-mediated astrocytic release of GABA [49, 67].

In summary, the early neurophysiological changes within the striatum of HD models coincident with symptom onset are (*i*) increased intrinsic MSN excitability, (*ii*) increased $[K^+]$ and decreased tonic inhibition in the striatum, (*iii*) impairment of glutamatergic corticostriatal input, and (*iv*) altered GABAergic synaptic transmission. Given the proposed and established roles of astrocytes in the control of synapse stability, ion homeostasis, neurotransmitter clearance, synaptic modulation and plasticity, a role for mHTT-induced astrocyte dysfunction in contributing to the striatal circuit phenotypes seemed likely, and recent studies have confirmed this in some aspects.

Astrocyte K^+ homeostasis and Ca^{2+} signaling in HD mouse models

K^+ homeostasis

Astrocyte membrane conductance was decreased by loss of weakly inwardly rectifying K^+ channels in astrocytes that express mHTT in HD mouse models [33]. Kir4.1 channels are astrocyte enriched (gene name *KCNJ10*), developmentally regulated, regionally variable in expression and involved in important functions, including bulk and local K^+ homeostasis [17]. The importance of Kir4.1 channels is emphasized by studies of knock-out mice, which die three weeks after birth and display severe motor deficits, including jerky movements, loss of balance/posture, body tremors and frequent falls and rollovers [68, 69]. Some of these phenotypes are mediated by Kir4.1-expressing astrocytes [70, 71] although some are due to loss of Kir4.1 in oligodendrocytes [69]. Conditional Kir4.1 knock-out mice displayed deficits in K^+ homeostasis in the brain [72]. Notably, humans with mutant Kir4.1 channels display Epilepsy, Ataxia, Sensorineural deafness and Tubolopathy – *EAST* syndrome [73], and Jack Russell terriers carrying Kir4.1 mutants suffer from juvenile-onset spinocerebellar ataxias [74]. Thus, it is clear that relatively simple changes in Kir4.1 function can produce profound neurological symptoms in mice, dogs and humans [17].

Reminiscent of the aforementioned Kir4.1 knock-out studies, evaluations of astrocytes in HD model mice (Box 1) revealed contributions of Kir4.1 channels to astrocyte membrane

properties and basal K^+ homeostasis [33]. However, it should be noted that further evaluations are needed to explore other mechanisms known to contribute to K^+ homeostasis such as the Na^+/K^+ ATPase, gap junctions or other K^+ channels [17]. There are several additional observations that are worth noting. At early stages, striatal astrocytes from symptomatic R6/2 mice displayed resting membrane potentials depolarized by a few millivolts and lower membrane conductances [33]. These electrophysiological differences between wild-type and R6/2 astrocytes could be accounted for by lower functional expression of Kir4.1 channels, and the alterations were recovered by AAV2/5 mediated delivery of Kir4.1 specifically to astrocytes [5, 33]. Extracellular basal K^+ levels in the striatum at P60–80 were elevated by ~1.5 mM. This suggests that increased K^+ may contribute to neuronal hyperexcitability phenotypes in HD model mice, a possibility that gains support from the empirical observations that increasing K^+ concentrations in brain slices from healthy wild-type mice by ~1.5 mM reproduced some features of altered striatal medium spiny neuron (MSN) hyperexcitability observed in R6/2 mice [33]. The key experiments were also repeated in Q175 mice (Box 1) [33]. Recently, observations on changes in expression of Kir4.1 and GLT1 have been extended with next generation sequencing in HD model mice [62].

Perhaps the most interesting aspect of Tong *et al.* was the discovery that expression of Kir4.1-GFP in astrocytes also reversed some excitability MSN deficits observed in R6/2 mice, and that expression of Kir4.1-GFP within striatal astrocytes *in vivo* attenuated some HD–like motor deficits and prolonged survival in R6/2 mice [33]. Rescue of Kir4.1 function also appears effective after systemic AAV9 delivery to improving HD astrocyte phenotypes and a simple motor function *in vivo* [75]. These data provide proof-of-concept that correcting a key astrocyte homeostatic function may lead to general benefits that can be observed at the level of neural circuits and animal behavior. At a conceptual level, this adds credence to the hypothesis that astrocytes can be targeted for therapeutic benefit in complex multicellular diseases at their early stages [3]. Early stage dysfunctions may be critical to target, because later stage astrogliosis may also reflect external cues resulting from local damage [21]. More broadly, the data provide evidence for the hypothesis that some key aspects of altered MSN excitability in HD are secondary to astrocyte homeostasis dysfunction.

Ca^{2+} signaling

Intracellular Ca^{2+} signaling is an important aspect of astrocyte biology in brain slices, *in vivo* [12, 14] and during disease [76]. Astrocyte Ca^{2+} signaling was increased in mouse models of Alzheimer's disease (AD) and stroke that are accompanied with strong astrogliosis [77–80]. In these settings, it was proposed that elevated astrocyte Ca^{2+} signaling may contribute to pathology. In contrast, little was known about striatal astrocyte Ca^{2+} signaling in HD model mice, prompting the exploration of the relationship between astrocyte Ca^{2+}, Kir4.1 and GLT1 [81]. Astrocyte spontaneous Ca^{2+} signals were reduced in R6/2 mice, but evoked Ca^{2+} signals were increased in a manner that was dependent on GLT1, at ages when Kir4.1 and GLT1 deficits were noted [81]. The action potential-dependent evoked Ca^{2+} signals were mediated by astrocyte mGluR2/3 receptors and likely due to glutamate spill-over to distal sites where greater numbers of receptors were likely

recruited (Figure 3). In accord, the lifetime of electrically-evoked glutamate release near the surface of astrocytes was prolonged in R6/2 relative to wild-type mice, likely reflecting loss of GLT1 function within astrocytes and reduced glutamate clearance [81]. Of note, the prolonged astrocyte iGluSnFR signals and elevated mGluR2/3-mediated evoked Ca^{2+} signals observed in R6/2 mice could be mimicked in wild-type mice by blocking GLT1, implying that reduced glutamate uptake may be the likely cause of these astrocyte dysfunctions in R6/2 mice. Some of the iGluSnFR and Ca^{2+} signal alterations observed in R6/2 mice could be rescued by astrocyte delivery of Kir4.1 [81]. Thus, it appears that Kir4.1 can set into effect a sequence of events that results in restoration of normal astrocyte ion and membrane potential gradients, recovery of GLT1 function, reduced perisynaptic glutamate levels as well as altered synaptic transmission and plasticity [82] in HD model mice. However, there was no evidence for elevated astrocyte glutamate release, which had been suggested based on cell-culture work [83].

Impaired glutamate uptake and possible consequences in HD mice

As mentioned, studies in humans and in several mouse models of HD consistently show a disease-related down-regulation of the gene encoding GLT1 (*Slc1A2*) [9, 29, 46, 51, 62, 84–87]. As HD is also characterized by a marked decrease in the density of glutamatergic synapses in the dorsal striatum [60, 88–90] the question arises as to what extent the observed reduction in striatal glutamate uptake may be an adaptation to the loss of synaptic glutamate release, or a risk factor to be eliminated by novel therapies of HD.

GLT1-related alterations in synaptic transmission would argue for the latter possibility, especially if the changes lead to significant loss in the corticostriatal coherence or the appearance of spontaneous firing at rest [47, 52]. While in normal mice glutamate transport is hardly ever overwhelmed [91], slowed glutamate uptake and spillover to perisynaptic sites are now being considered in some mechanisms of synaptic plasticity [82] and in neurological conditions, including HD. Recent experiments with the fluorescent glutamate sensor iGluSnFR illuminate the reduced glutamate clearance capacity of striatal astrocytes in R6/2 mice [81]. However, Raymond and colleagues [39] found no evidence for impaired clearance of synaptically released glutamate when analysing the glutamate transients elicited by local EFS in iGluSnFR-expressing MSNs [92].

Further evidence in support of HD-related changes in striatal glutamatergic synaptic transmission was provided by recording corticostriatal EPSCs in the absence of NMDA receptor blockers. In Q175 mice, EPSC decay was slowed [93], notably when the EPSC included NMDAR-mediated currents at membrane potentials of +50 mV and higher. This observation is in line with the results from normal mice and rats where pharmacological block of GLT1 with TBOA induced EPSC prolongation [94] and changes in the preferentially expressed type of plasticity [82]. It was suggested that GLT1 blockade results in the recruitment of a critical number of perisynaptic NMDA receptors to elicit non-Hebbian forms of plasticity at the expense of spike-time dependent plasticity (STDP). Finally, it was proposed that in HD, glutamate spillover to perisynaptic mGluR5 could account for the heterosynaptic CB1-dependent depression of synaptic GABA release, as observed in R6/2 and Q175 HD mice [67].

A very sensitive indicator of failing glutamate uptake is the level of nonsynaptic release of GABA from astrocytes [49]. It was found that astrocytic GAT3-mediated release of GABA contributes to the maintenance of a tonic $GABA_A$ receptor-mediated conductance ($I_{Tonic(GABA}$) in MSNs (Figure 4A). The driving force for the nonsynaptic GABA release through GAT3 is established by the $[Na^+]$ elevation at co-localized GLT1 clusters (see [95]), since pharmacological block of GLT1 reduces ($I_{Tonic(GABA}$) to the HD level (Figure 4B). We tentatively conclude that, in HD mice, glutamate uptake is insufficient as it leads to unwarranted alterations in glutamatergic and GABAergic synaptic transmission. Notably the loss of spike-time-dependent plasticity could contribute to the impaired corticostriatal control.

Additional studies are needed to evaluate if the observed changes in glutamate spillover are due to insufficient or inappropriate function of the transporter itself, or reflect changes in the basic properties of astrocytes, such as membrane depolarization and loss of high membrane conductance due to Kir4.1 deficiency. This question was addressed by recording glutamate transporter currents (GTC; Figure 4C–F) in single sulphorhodamine-labelled astrocytes [93]. Addition of Ba^{2+} to the recording solution decreased the impact of Kir4.1, thereby unmasking a functional deficit of GLT1 itself. In Q175 homozygous mice, the decrease in the GTC maxima amounted to about one third of the WT control. The deficit should be larger under conditions allowing for a combined effect of reduced GLT1 and Kir4.1 deficiency as is the case in the recordings of glutamate-induced $[Na^+]_i$ transients in unperturbed WT and HD astrocytes (Figure 4G,H).

In addition to the impact of electrochemical driving force on GLT1 [96–98], the level of GLT1 transport would be expected to depend on the number of transporter molecules at synaptic sites, their distance from the sites of synaptic release, their transport rate, diffusion and a variable dependence on cAMP-mediated vesicular replenishment of transporter aggregates [99]. Astrocytes exhibiting the usual negative membrane potential at E_K are assumed to restrict glutamate signalling to postsynaptic receptors [91], even though the synaptic glutamate concentration can increase by 5 orders of magnitude [89, 100, 101]. Nevertheless, excitotoxicity due to pathological activation of extrasynaptic NMDA receptors remains a frequently discussed, but mechanistically unclear consequence of GLT1 deficiency [102]. Considering the powerful glutamate clearance capacity still remaining in symptomatic HD (Figure 4F), one may wonder why astrocytic glutamate uptake does not cope with the levels of glutamate needed to activate the NR2B–containing extrasynaptic NMDA receptors (EC_{50} for L-glutamate ~3 μM [103]). However, if extracellular $[K^+]$ and intracellular $[Na^+]$ both increase to ~30 mM, an astrocyte depolarization of ~30 mV would suffice to silence glutamate uptake and to initiate a reverse mode of operation [104, 105]. Such extreme elevation of extracellular $[K^+]$ is indeed possible during states of spreading depression in cortical structures, but modelling of neurotransmission-induced $[K^+]$ transients and respective astrocyte membrane potentials predict much smaller changes [105].

Another basis for the excessive activation of NMDARs in HD could be a particularly low density of astrocytic glutamate transporters at extrasynaptic sites. It is already well known that GLT1 immunoreactivity is not evenly distributed [53, 106], but forms clusters adjacent to the areas of presynaptic vesicle accumulation (Figure 4I). Whether synapse loss in HD is

associated with a change in the distribution of astrocytic glutamate transporters remains to be determined. Overall, there appears to be good evidence for GLT1-specific roles in HD pathology that add to the deficits imposed by altered K^+ homeostasis [81, 93].

Cholesterol, astrocytes and HD

In the adult brain, astrocytes play a critical role in *de novo* synthesis of cholesterol, which regulates neuronal function. Dysregulation of cholesterol metabolism is observed in HD astrocytes, both in terms of gene expression and levels of various cholesterol intermediates, possibly via an undefined mechanism involving mHTT and the SREBPs transcription factors [107, 108]. A change in cholesterol levels have been inconsistently reported in HD models, although lower 24-hydroxy-cholesterol levels in plasma have been described in patients and in rodent models, supporting deficits in cholesterol metabolism [107, 109]. The pathogenic impact of cholesterol pathways in HD models is supported by a recent study showing that enhanced degradation of cholesterol in the striatum of R6/2 mice via AAVrh10 expression of CYP46A1 (the enzyme that converts cholesterol into 24-hydroxy-cholesterol) significantly modifies core features of the disease [110]. It remains to be determined how other metabolic astrocytic processes may be adversely influenced by the presence of mHTT.

Stem cell approaches in HD model mice

Notwithstanding the wealth of data pertaining to the nature of glial dysfunction in rodent models of HD, the relative role and importance of human glial pathology in HD has remained unclear. Furthermore, *in vivo* models for assessing the degree to which glial pathology might be necessary or sufficient for disease progression have been lacking, as has the ability to investigate HD glia with regards to their cell-autonomous pathology in live adults. This lack of understanding of the role of glial pathology in HD has reflected the lack of *in vivo* models that permit the separate interrogation of glial and neuronal functions in HD, particularly so in humans.

To address this fundamental gap in our knowledge, Benraiss and colleagues used human glial chimeric mice, in which neonatal implantation of human glial progenitor cells leads to substantial replacement of resident murine glia by their human counterparts [111, 112], to assess the role of human striatal glia in the pathogenesis of HD [63]. To that end, both the striatal neuronal physiology and behavior of immunodeficient mice xenografted at birth with mutant HD-expressing human hGPCs were assessed, and compared to that of mice engrafted with normal HTT hGPCs [63]. In this study, the motor behavior of mice engrafted with human embryonic stem cells (hESCs) HD-derived (48 CAG) GPCs was first contrasted to that of controls engrafted with hGPCs derived from an unaffected sibling line (18 CAG). The HD GPC-engrafted mice manifested impaired motor learning relative to control hGPC-engrafted mice, that was sustained and profound. Furthermore, mice then engrafted with human striatal astrocytes transduced to overexpress mutant HTT (73Q) exhibited sharply increased neuronal input resistance and excitability, relative to their controls engrafted with normal HTT (23 CAG)-transduced striatal glia.

Since striatal chimerization with HD-derived glia proved sufficient to recapitulate aspects of disease phenotype in normal mice, one might anticipate neonatal chimerization of HD mice with normal hESC-derived glia might delay or rescue aspects of disease phenotype. Using R6/2 transgenic HD mice as hosts [113], Benraiss and colleagues then found that the substantial replacement of diseased striatal glia with wild-type human glia indeed resulted in a slowing of disease progression and corresponding increment in survival in transplanted R6/2 mice. Of note, the glial donor cells were isolated on the basis of CD44, the hyaluronan receptor, so as to capture a more astrocyte-biased donor cell population [114]. This neonatal engraftment by CD44-defined glia yielded a transplant-associated fall in neuronal input resistance, and a corresponding drop in interstitial K^+ in the R6/2 striatum, with an attendant rescue of the otherwise hyperexcitable phenotype of R6/2 striatal neurons [63]. This was accompanied not only by delayed disease progression and enhanced survival, but by improved cognitive and motor function on a variety of metrics, relative to untreated R6/2 mice. Normal glial progenitor cells and their derived astrocytes thus proved able to substantially delay and lessen the pathology of resident HD striatal neurons, and by so doing defined a substantially non-cell autonomous component to neuronal pathology in HD. Together, these studies indicate a critical role for glial pathology in the progression of HD. Going forward, they suggest the potential for glial cell replacement as a therapeutic strategy in Huntington's disease, and more broadly, to other neurodegenerative diseases in which glial pathology might be causally contributory.

Concluding remarks

The last few years have witnessed the start of the beginning in our understanding of how astrocytes contribute to HD in mouse models and these studies signal the possibility that astrocytes may be exploited to derive therapeutic effect in HD. However, it is still necessary to determine the functions of normal HTT within astrocytes and it will be informative to overexpress and delete the disease causing mHTT only within astrocytes in the fully developed mature brain. These experiments are now feasible based on the availability of pan-astrocytic *Aldh1l1*-Cre/ERT2 mice [115], which better overcome the limitations of using *Gfap*-Cre lines that target only some astrocytes, but also neural progenitors, some neurons and some peripheral organs [43, 44]. In a complex disease such as HD, even modest expression in neurons would be a serious confounding factor and should be avoided at the outset when designing astrocyte studies. It is worth noting that healthy striatal astrocytes display low levels of GFAP [33, 113], which suggests that *Gfap*-Cre lines may be suboptimal in HD studies. Hence, the pursuit and availability of genetic tools to specifically and reliably study astrocytes will be important in explorations in the context of HD. We suggest that all molecular and cellular evaluations need to be performed in mature mice at ages relevant to the onset and progression of HD symptoms in disease models. Of course, analyses of human HD specimens is critical to place into context the data from mouse models. Furthermore, it remains to be determined if the changes in K^+, glutamate and GABA homeostasis mediated by astrocytes in HD model mice are causative or correlative to disease. We suggest both scenarios are likely: it seems difficult to imagine how marked changes in extracellular K^+, glutamate and GABA would not contribute causatively to neuronal dysfunction, as has been discussed in the preceding sections in detail. Additional

mechanisms that warrant further study are astrocyte mitochondrial and nuclear alterations [116, 117].

The astrocyte studies in the last few years have important implications for therapeutic strategies in HD and suggest that approaches that target only neuronal disturbances are likely to be inadequate or suboptimal for restoring function. Strategies that target key mechanisms in neurons and astrocytes are likely to be most successful, because astrocytes and neurons function synergistically in the healthy brain and appear to have done so throughout the evolution of complex nervous systems [118]. We propose that restoring astrocyte-mediated homeostasis and normal synergistic relationships of neurons and astrocytes may provide new therapeutic strategies for brain disorders. This seems appropriate given that mHTT is found within neurons and astrocytes in humans with HD.

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Glossary

Cre	short for cre recombinase, which catalyzes recombination between two LoxP sites in DNA
Cre/ERT2	a type of tamoxifen inducible Cre
GFAP	a type of astrocyte-enriched intermediate filament protein
GLAST	a particular type of plasma membrane glutamate transporter found in astrocytes
GLT1	a particular type of plasma membrane glutamate transporter strongly enriched in astrocytes
GCaMP3	a type of genetically-encoded calcium indicator
iGluSnFR	a type of genetically-encoded glutamate sensor
HTT	huntingtin protein
Kir4.1	a type of weakly inwardly rectifying potassium channel found in astrocytes
mHTT	mutant huntingtin protein carrying disease causing glutamine expansion
MSN	medium spiny neuron, frequently also called striatal projection neurons or spiny projection neurons
UHDRS	Unified Huntington’s disease rating scale

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Box 1: Commonly used HD mouse models where astrocyte assessments are available

Model (background) [key reference]	Available strains (JAX stock #)	Brief description	Known striatal astrocyte dysfunction
R6/2 (CBAxC57/BL6, C57/BL6) [113]	002810	Uses HD promoter - Human Exon 1 *mHTT* transgene.	Reduced Kir4.1 protein, function and K+ homeostasis [33]
	006494		Reduced GLT1 mRNA and protein [33, 46, 53, 84]
	027419		Impaired glutamate transport efficiency in striatum [46, 53, 81, 84, 93], contested in [39]
	027423	Multiple strains with various CAG (Q) sizes exist. The 120Q line is widely used.	GAT3-mediated/GLT1-dependent GABA release impaired, resulting in reduced tonic GABA conductances [49]
			Increased extracellular [Gln] [84]
			Decreased GS, altered GS immunoreactivity [46, 84]
			Decreased astrocyte spontaneous Ca^{2+} signals [81]
			Enhanced cortically-driven striatal astrocyte Ca^{2+} signals [81]
			No overt astrogliosis at symptomatic ages [33, 113]
			mHTT astrocyte inclusions [33]
			Glial replacement with wild-type human glia slowed disease progression and increased survival [63].
R6/1 [113]	006471	Uses HD promoter - Human Exon 1 *mHTT* transgene.	Reduced GLT1 protein [46]
		CAG of ~ 120 is more stable than R6/2	Decreased GS [46]
N171-82Q (C57/BL6) [119]	003627	Predominantly neuronal expression through PrP promoter – N terminal 171 *mHTT* transgene.	N171-82Q phenotype is exacerbated by crossing to [aphenotypic]
			N208-98Q glial mHTT line [38]
YAC128 (C57/BL6, FVB/N) [120]	027432	Full length HTT YAC transgene	Impaired GLT1 function and impaired

Model (background) [key reference]	Available strains (JAX stock #)	Brief description	Known striatal astrocyte dysfunction
		2 strains, CAG100 and 126	glutamate uptake from 3 months reported [50, 51], contested in [102]
BACHD (FVB/N) [121]	008197	Full length human HTT BAC transgene with 97 polyQ encoded by mixed CAGCAA - 2 strains	Glutamate uptake deficiency in the subthalamic nucleus [122]
zQ175 (C57/BL6J) [59, 86]	027410	Knock-in lines. Human HTT exon 1 sequence with an ~190 pure CAG tract replacing the mouse Htt exon 1.	Reduced Kir4.1 protein and function in Q175 homozygotes [33] GLT1 loss [33, 86] Impaired glutamate transport efficiency in striatum [93] GAT3-mediated/ GLT1-dependent GABA release impaired, resulting in reduced tonic GABA conductances [49] Glutamate uptake deficiency in Q175 het STN [122] Increased expression of GFAP, slight astrogliosis in striatum and cortex in Q175 homozygotes [75]

Note to table: we have only highlighted mouse models for which astrocyte data are available. Additional models that have been assessed for neuronal properties are not listed. Key to table: GS = Glutamine synthetase. PrP = prion promoter. Gln = glutamine. Glu = glutamate. mHTT = mutant huntingtin.

Trends box

- Astrocytes are involved in Huntington's disease (HD)
- Astrocyte dysfunctions contribute to HD pathophysiology in mice
- Transplanted astrocytes produce therapeutic benefit in HD model mice
- Targeting astrocyte dysfunction may provide new therapeutic targets in HD

Outstanding Questions Box

Molecules

* What is the molecular profile (RNA and protein) of striatal astrocytes in healthy tissue and at the various stages of disease progression in HD mouse models?
* What is the molecular profile of astrocytes from humans with HD at various clinical stages?
* Can selective Kir4.1 and GLT1 positive allosteric modulators be discovered and can they be used pharmacologically to mitigate the decrease of these proteins in HD?
* What is the neurotoxic substance released from A1 reactive astrocytes [20] and does this contribute to HD?

Cells and circuits

* How do astrocytes affect striatal synapses and circuits?
* Do astrocytes interact with and “listen” to specific neural inputs?
* Do striatal astrocytes release glutamate via reversal of transporters or via Ca^{2+}-dependent exocytosis in adult brain?
* Do astrocyte Ca^{2+} signals serve distinct functions in distinct circuits?

Mice

* Can astrocyte biology be exploited to produce desirable effects in neural circuits associated with specific brain disorders?
* Do altered astrocytes contribute to specific neurodegenerative diseases or do they have the same effect in all neurodegenerative disorders?
* How do astrocytes contribute to dopaminergic control in intact mice?
* Can astrocyte modifications prevent the loss of glutamatergic and GABAergic synapses?

Humans

* Can paradigms be developed to evaluate if combined astrocyte and neuronal strategies are more effective than either one alone?
* Can astrocytes be exploited for selective delivery of small molecules via intracellular gap-junctional transport specifically to synaptic sites?
* Is it possible to design an astrocyte-specific PET probe to study astrocytes in humans as disease progresses and as treatments are administered?

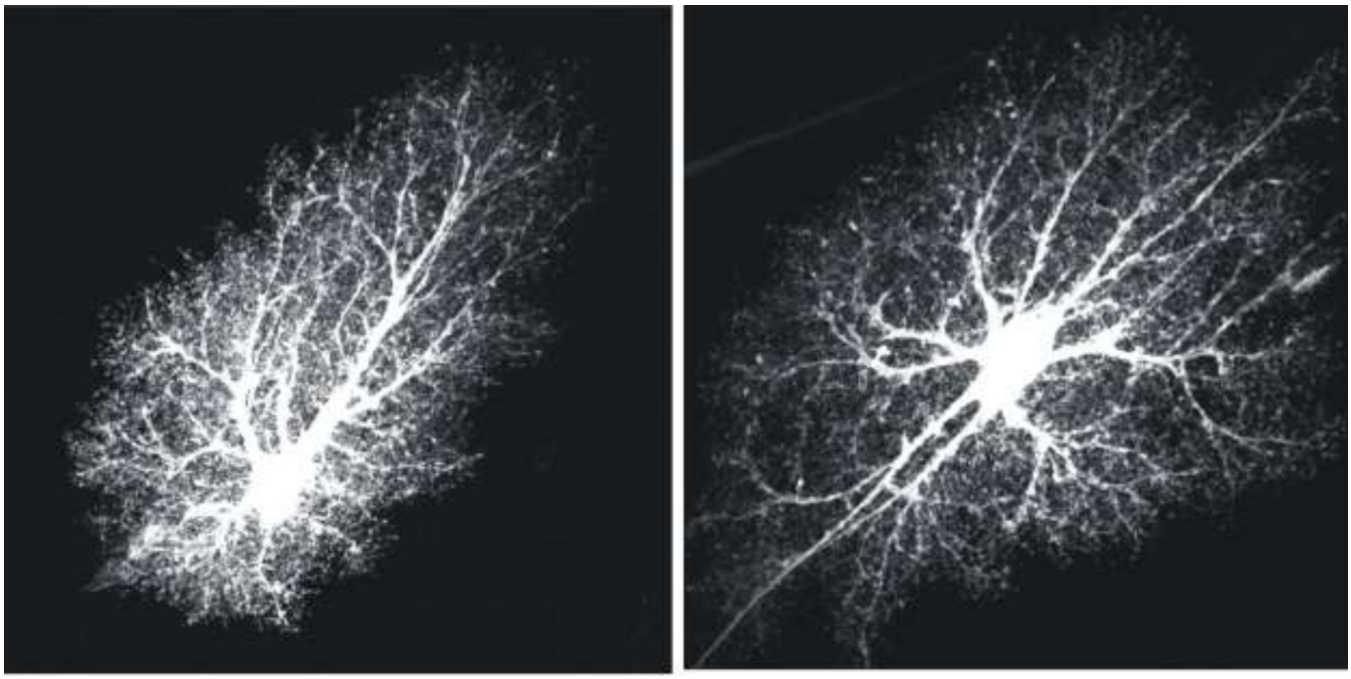

Figure 1. Bushy astrocyte morphology

The images show hippocampal CA1 molecular layer protoplasmic astrocytes loaded with a fluorescent dye (Lucifer yellow). The images are from the Cell Centered Database [6, 123, 124] at the National Center for Microscopy and Imaging Research (http://ccdb.ucsd.edu/index.shtm) and have Accession #1066, 1063. One can observe a soma, several major branches and thousands of branchlets and leaflets, which are discussed in the text. These two images are reproduced from a published paper [3].

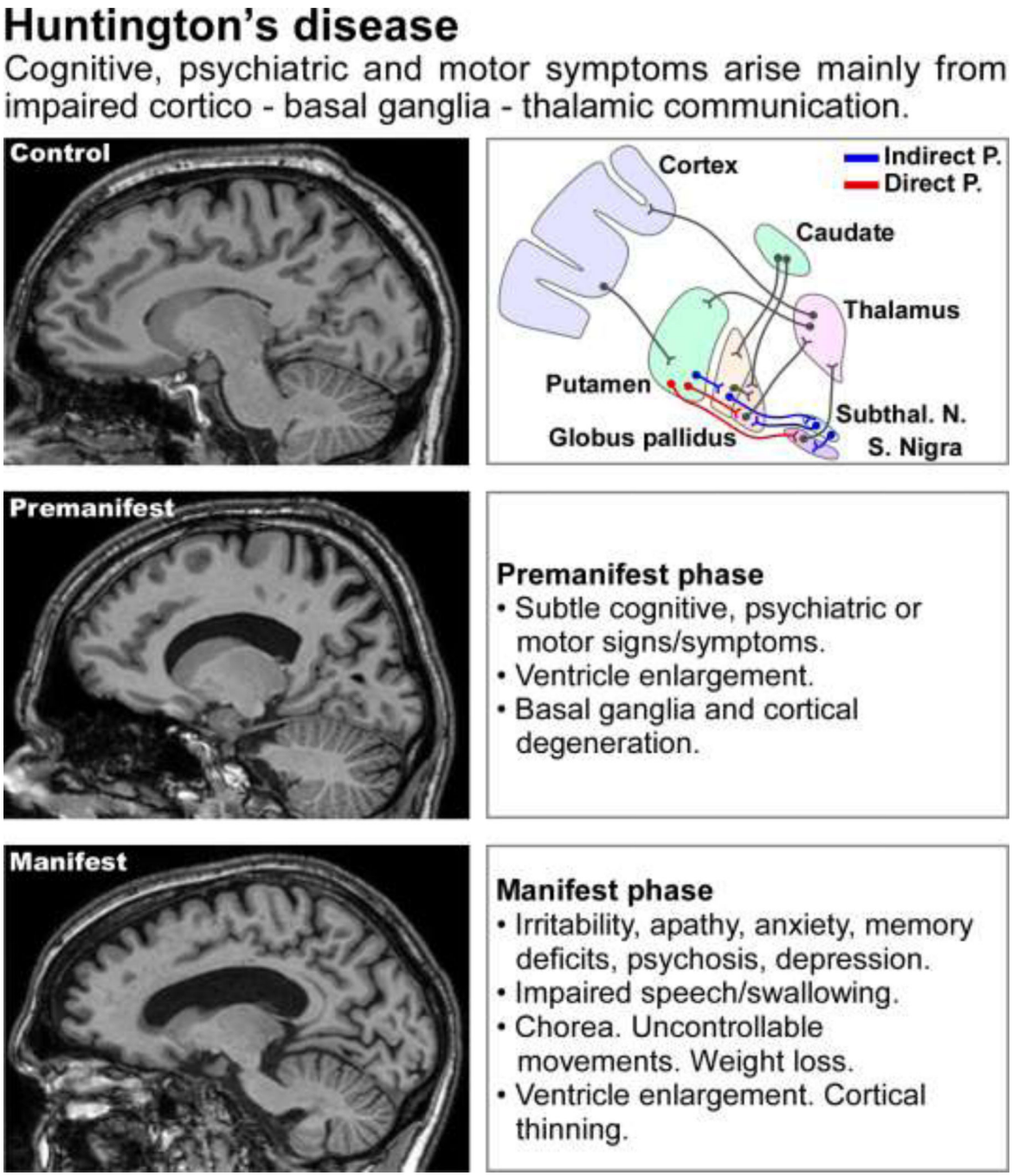

Figure 2. Imaging and clinical manifestations of Huntington's disease

Top row: MRI scan from a healthy control subject (left) and a scheme of the connectivity between cerebral cortex, basal ganglia and thalamus, indicating the indirect and direct pathways, the balance of which is thought to be altered in HD (right). Subthal. N. = subthalamic nuclei, S. Nigra = substantia nigra. Middle and bottom rows: MRI scans from a subject in the pre manifest stage of the disease (CAG length: 42; middle left) and a subject in the manifest stage of the disease (CAG length: 43; bottom left), and descriptions of the most common clinical findings at each stage (middle and bottom right). The MRI images are

from the TRACK-HD study [23], courtesy of Dr. Sarah Tabrizi (University College of London).

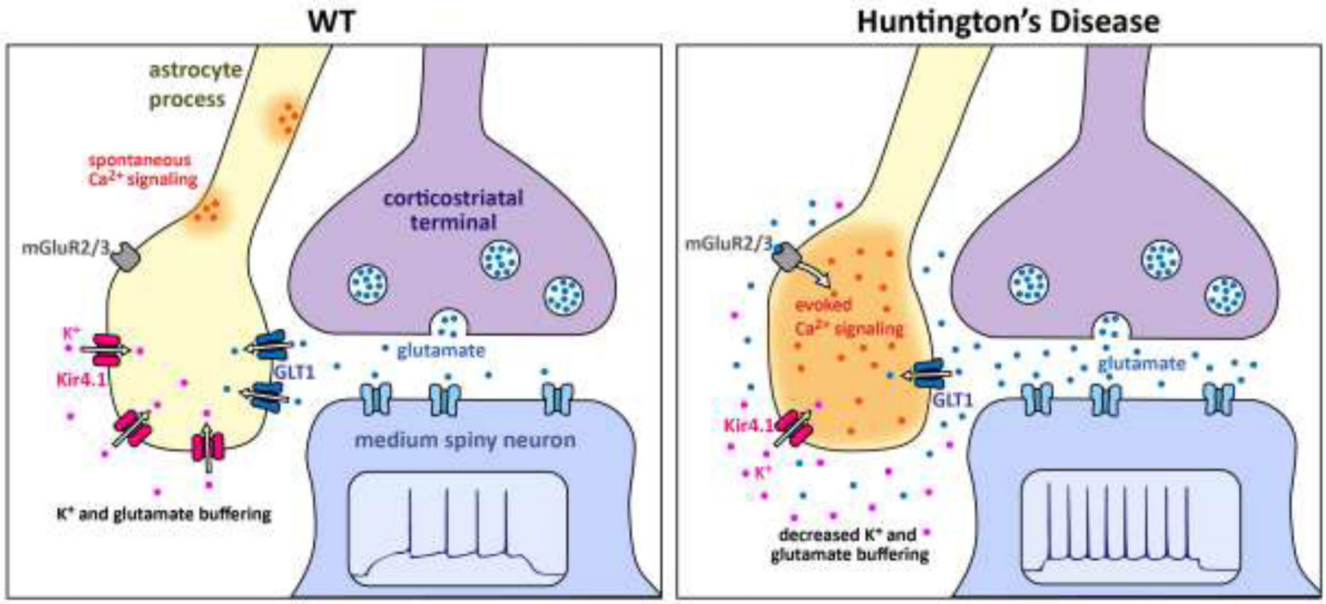

Figure 3. Schematic summary of the major Kir4.1, Ca^{2+} and GLT1 astrocyte changes observed in R6/2 mice at ~P70

Only the key features are schematized and other differences between WT and HD mice are discussed in the text. In the WT striatum, normal expression levels of Kir4.1 and GLT1 maintain low levels of extracellular K^+ and glutamate near synapses. As a result, electrical stimulation of cortico-striatal axons does not evoke robust mGluR2/3-mediated astrocyte Ca^{2+} signals. However, there are abundant spontaneous Ca^{2+} signals in striatal astrocytes. In HD mice, expression levels of Kir4.1 and GLT1 are reduced, and this has a number of observable consequences for astrocytes. Reduced GLT1 levels mean that astrocytes display robust mGluR2/3-mediated Ca^{2+} signals during stimulation of cortico-striatal axons. This gain of evoked Ca^{2+} signals is accompanied by a loss of spontaneous Ca^{2+} signals. The elevated glutamate and K^+ levels in the extracellular space are proposed to contribute to increased excitability of MSNs. The cartoon is based on published work [33, 75, 81, 93].

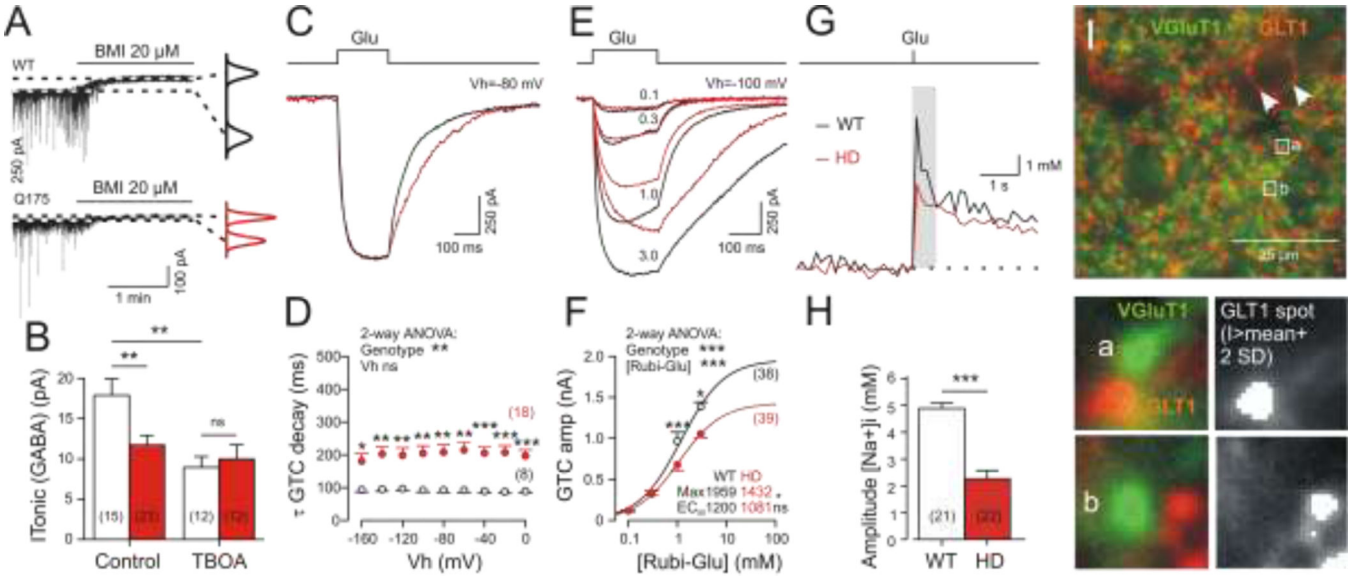

Figure 4. Altered function of GABA and glutamate transporters in HD model mice

(A, B) Quantification of $I_{Tonic(GABA)}$ from WT and HD MSNs [49]. Experiments in the presence of DNQX (10 µM), APV (50 µM), LY341495 (40 µM); no K^+ channel block. Graphs on the right: Gaussian fits to all-points histograms derived from 30 s recording periods under control conditions and in the presence of BMI. The difference between the peaks of the fitting curves represents the amplitude of $I_{Tonic(GABA)}$. Block of GLT1 with TBOA (100 µM) in WT reduces $I_{Tonic(GABA)}$ to HD level. (C–H) Records and quantification of glutamate uptake activity in single sulphorhodamine-labelled astrocytes from the striatum of 1-year-old Q175 wild types and homozygotes [93]. (C–F) Experiments with somatic patch-clamp recording of glutamate transporter currents (GTCs) in the presence of carbenoxolone (100 µM) to block gap junctions and Ba^{2+} (100 uM) to block Kir4.1 channels. The latter reduces the possible impact of reduced driving forces (low Kir4.1 expression) in HD. Glutamate was applied by flash photolysis of Rubi-glutamate (3 mM). The stimulus covered the entire astrocyte. (C, D) HD-related prolongation of GTC decay. (E, F) Dose-response relationship to estimate the transport maximum and respective HD-related differences. (G) Average traces of $[Na^+]_i$ from individual astrocytes in one wild-type and one HD section after loading of SBFI-AM into sulphorhodamine-labelled cells. The recordings were performed in the presence of the gap junction blocker carbenoxolone and the glutamate receptor blockers DNQX and MK101. The signals were acquired from the soma and proximal processes before and after optical uncaging of Rubi-glutamate (3 mM) within the same area. (H) Quantification of results. Numbers in brackets: number of cells (data from 9 sections from 3 animals per group). (I) View field from the dorsal striatum showing vGLUT1 (green) and GLT1 (red) immunofluorescence (Dvorzhak and Grantyn, unpublished). Clusters of GLT1 are found in co-localization with glutamatergic terminals (see boxed regions of interests at larger magnification below) and in contact with blood vessels (arrowheads). The GLT1 spots in co-localization with vGLUT1 terminals were delineated and evaluated by a threshold algorithm.