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Mitochondrial bioenergetics and dynamics in Huntington's disease: tripartite synapses and selective striatal degeneration

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Abstract Preferential striatal neurodegeneration is a hallmark of Huntington's disease (HD) pathogenesis, which has been associated with mitochondrial dysfunction. Evidence from genetic HD models suggest that mutant huntingtin (mHtt) compromises mitochondrial bioenergetics and dynamics, preventing efficient calcium handling and ATP generation in neuronal networks. Striatal neurons receive abundant glutamatergic input from the cortex, forming tripartite synapses with astrocytic partners. These are involved in bidirectional communication, play neuroprotective roles, and emerging evidence suggests that astrocyte dysfunction supports non-cell autonomous neurodegeneration. In addition to mHtt effects, inherent mitochondria vulnerability within striatal neurons and astrocytes may contribute for preferential neurodegeneration in HD. Dysfunctional astrocytic mitochondria in cortico-striatal tripartite synapses might be particularly relevant in the pathogenesis of juvenile/infantile HD, frequently associated with seizures and abnormally large mHtt polyglutamine expansions. This review discusses our work, primarily addressing in situ mitochondrial function in neurons and astrocytes, in the context of related work within the HD-mitochondria field.

Keywords Huntington's disease · Mitochondria · Striatum · Neurons · Astrocytes

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Introduction

Huntington's disease (HD) is an inherited neurodegenerative disorder caused by a polyglutamine (polyQ) repeat expansion mutation in huntingtin (Htt). HD pathophysiology associates loss of normal Htt function and a dominant gain of function in mutant Htt (mHtt), which is prone to aggregate and cleaved into toxic N-terminal fragments. The most prominent HD neuropathological feature is the preferential death of GABAergic medium spiny neurons, the prevailing neuronal population in the striatum, which becomes progressively atrophic in strong correlation with typical choreic motor symptoms. The selective targeting of striatal neurons by mHtt is intriguing, namely because polyQ expansion in other proteins induces different neurodegeneration patterns, e.g. hereditary spinocerebellar ataxias. Given that mutant protein distribution, expression levels and shared polyQ expansions do not explain differential vulnerability within and across these disorders, HD selective striatal targeting is unlikely related to the polyQ tract per se, but instead involves the full-length mHtt protein context in coordination with vulnerability factors intrinsic to the striatum (Gusella and MacDonald 2000). Significantly, the striatum is also preferentially damaged by acute metabolic insults, such as hypoxia, hypoglycemia and global ischemia (Calabresi et al. 2000). Multiple pathogenic mechanisms are putatively linked to mHtt and striatal vulnerability, but here I shall focus on those pertaining to the mitochondria.

Mitochondria dysfunction is associated with HD pathogenesis, being arguably a common theme to several neurodegenerative disorders (Lin and Beal 2006). HD patients exhibit profound weight loss despite sustained caloric intake, strongly suggesting metabolic abnormalities (Djousse et al. 2002). The concept of mitochondria

dysfunction in the HD brain stemmed from postmortem brain data (Gu et al. 1996), being assisted by data from animal models induced with mitochondrial toxins (Brouillet et al. 2005) or genetically engineered to express full-length/truncated mHtt constructs (Browne 2008). The nature and cause of HD striatal mitochondria deficits is multifactorial, involving direct mHtt-mitochondria interactions and indirect effects via transcriptional dysregulation and trafficking impairment, which compromise mitochondria bioenergetics and dynamics (Oliveira 2010a). Neurons are highly dependent on mitochondria ATP production (oxidative phosphorylation, OXPHOS) and Ca^{2+} buffering to maintain excitability and synaptic communication (Kann and Kovacs 2007), and rely on dynamic trafficking of mitochondria to adapt this limited resource to the variable needs of distant processes in vast neuritic networks (Chang and Reynolds 2006). Moreover, neurons require efficient biogenesis and mitophagy (mitochondria autophagy) to renew or adapt mitochondria levels throughout their lifespan, and proper fusion and fission dynamics to allow mitochondria functional and spatial segregation (Chen and Chan 2009), including mitochondria quality control mechanisms (Twig et al. 2008). Thus, the general designation 'mitochondrial dysfunction' encompasses several distinct mitochondrial roles in neuronal physiology that are potentially affected by mHtt. Also, glial mitochondria dysfunction may compromise tripartite synapses, involving pre- and post-synaptic neurons as well as astrocytic partners (Bambrick et al. 2004; Perea et al. 2009), thus contributing for neuronal demise. Our recent and ongoing work is primarily focused on functional assays with in situ mitochondria from HD models, aiming to identify specific mitochondrial deficits as well as vulnerability factors in striatal neurons and astrocytes. The present review summarizes and discusses our keys findings in the context of related research from other groups.

The bioenergetics of isolated and in situ HD mitochondria

Mitochondrial proton motive force (Δp), primarily membrane potential ($\Delta\psi_m$), is at the core of the organelle's key bioenergetic properties. Ion gradients and proton fluxes across the inner mitochondrial membrane govern respiration (oxygen consumption), ATP production and Ca^{2+} buffering. The latter two properties compete for $\Delta\psi_m$ and become compromised when this common resource decreases. Thus, findings of decreased $\Delta\psi_m$ and depolarization at low Ca^{2+} loads in mitochondria from HD patients' lymphoblasts (immortalized lymphocytes) or YAC72 HD mice brain, along with evidence for mHtt—mitochondria association, and polyQ length dependent depolarization with glutathione S-transferase fusion proteins, strongly supported the hypothesis of a direct mHtt toxicity decreasing Ca^{2+} buffering capacity (Panov et al. 2002). However, contrasting findings of increased Ca^{2+} buffering capacity were subsequently reported for brain mitochondria in several HD mice (R6/2—expressing truncated mHtt, and various knock-ins expressing full-length mHtt; Brustovetsky et al. 2005). Mitochondrial Ca^{2+} buffering capacity is limited by permeability transition (mPT) onset, which is strongly influenced by experimental conditions. A previous study with rat brain mitochondria showed that slow Ca^{2+} infusion into dilute mitochondria suspensions provides highly reproducible measurements of Ca^{2+} buffering capacity, by minimizing the bioenergetic demands comparatively to Ca^{2+} bolus addition that result in depolarization or NAD(P)H oxidation (Chalmers and Nicholls 2003). Thus, we used this slow Ca^{2+} infusion technique with forebrain mitochondria suspensions (0.1 mg/ml), from different HD mice models and respective wild type littermates, performing the assay in the presence of adenine nucleotides, oligomycin and albumin to optimize mitochondrial stability (Oliveira et al. 2007). Full-length Htt and/or mHtt were present in the mitochondrial preparations (Fig. 1*a*) and likely associated with mitochondria since the isolation procedure, with multiple dilution and centrifugation steps, should eliminate virtually all unbound Htt/mHtt. We found a significantly increased Ca^{2+} buffering capacity in mitochondria from R6/2 and YAC128 HD mice vs. wild-type, but not in those from homozygous or heterozygous knock-in mice ($Hdh^{150/150}$ or $Hdh^{150/+}$, respectively). Meaningfully, wild-type mitochondria from all three genetic backgrounds (B5CBAF1/J, FVB/N and C57BL/6J—$Hdh^{+/+}$, respectively) displayed similar Ca^{2+} loading capacities (Oliveira et al. 2007). These results suggest that the HD mitochondria population as a whole displays an increased Ca^{2+} buffering capacity, detectable in dilute suspensions (0.16 mg/ml, Brustovetsky et al. 2005; 0.1 mg/ml, Oliveira et al. 2007). However, in more concentrated suspensions (0.5 mg/ml, Panov et al. 2002; 1 mg/ml, Choo et al. 2004), Ca^{2+} abruptly released by a vulnerable mitochondria subset undergoing premature mPT may reach nearby mitochondria, propagating mPT across the whole population as in a nuclear chain reaction, and suggesting a decreased rather than increased Ca^{2+} buffering capacity in the whole population. In addition to the pathophysiological relevance of identifying a vulnerable subset of mitochondria (Panov et al. 2002), a pressing question is why the isolated HD brain mitochondria population has increased Ca^{2+} buffering capacity (Brustovetsky et al. 2005; Oliveira et al. 2007). A plausible explanation is that in a stressful environment mitochondria may change qualitatively, increasing their resistance to mPT, as documented for liver mitochondria (Klohn et al.

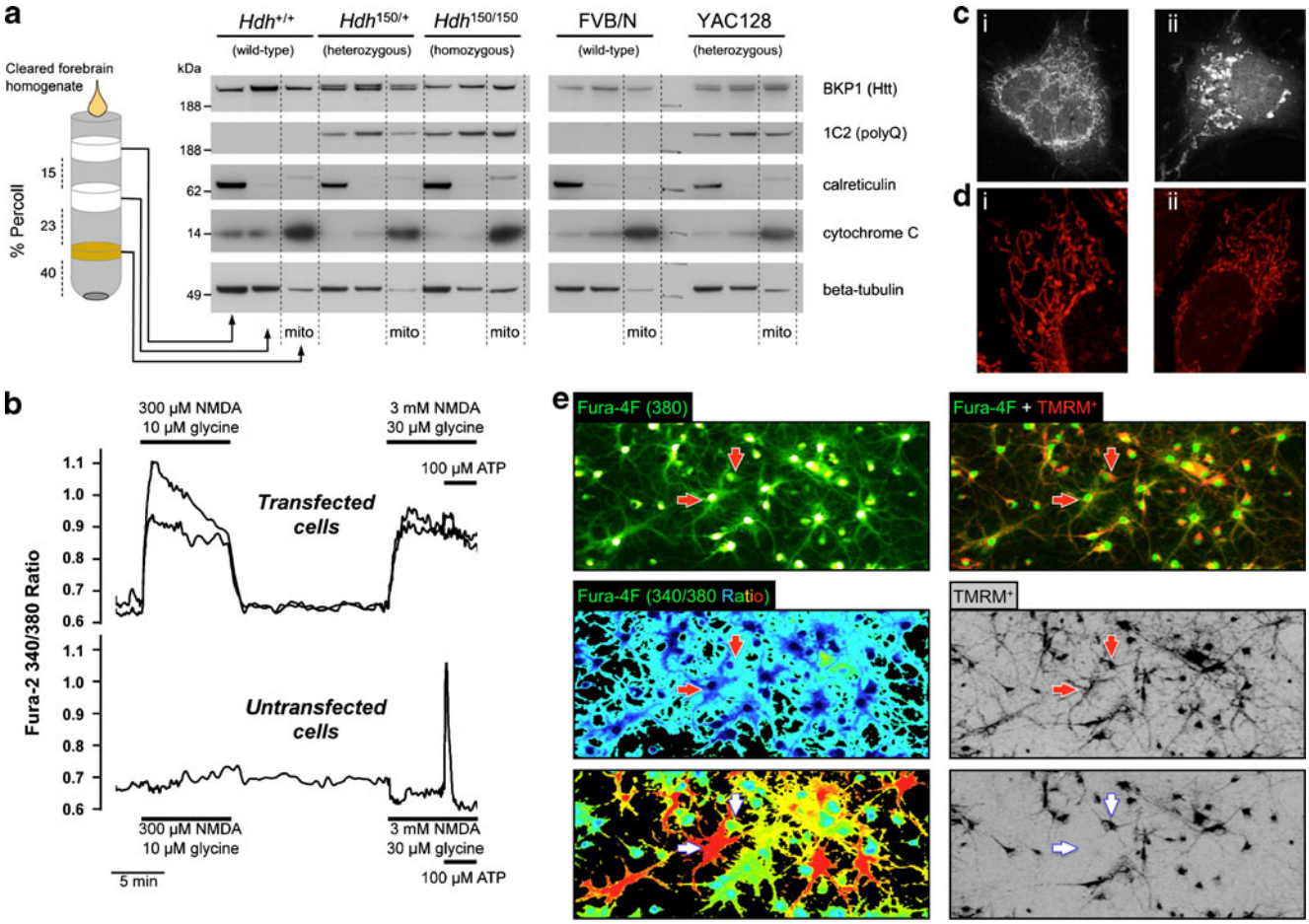

Fig. 1 Isolated brain mitochondria and in situ mitochondria in cell lines, neurons and astrocytes. ***a***, Western blot characterization of non-synaptic mitochondrial fractions (*mito*; delimited by dashed lines) isolated from wild-type and HD mice forebrain via Percoll gradient centrifugation. Note the presence of full-length Htt or mHtt in the mitochondrial fractions, which exhibit the highest cytochrome *c* content. BKP1 antibody recognizes both wild-type and mHtt (higher molecular weight), explaining double band in heterozygous ($Hdh^{150/+}$ and YAC128) vs. single band in homozygous ($Hdh^{150/150}$) HD mice and wild-type ($Hdh^{+/+}$ and FVB/N). The anti-polyQ antibody 1C2 recognizes only mHtt, which has a much longer polyQ tract (Q128 in YAC128 and Q150 in *Hdh*150) than wild-type Htt (Q7); See Oliveira et al. (2007) for further details. ***b***, Changes in intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$; Fura-2 340/380 Ratio) in striatal cell lines (ST*Hdh* Q7) transiently co-transfected with plasmids encoding NR1-GFP and NR2B receptor subunits (Domingues et al. 2007) vs. untransfected cells within the same imaging field. Efficient transfection was detected by GFP fluorescence. Note that untransfected ST*Hdh* cells do not respond with Ca^{2+} elevation to NMDA up to 3 mM, even though a positive Ca^{2+} response to 100 μM ATP confirms cell viability (Oliveira et al. 2004). For NMDA and ionophore Ca^{2+} responses in HD striatal neurons and ST*Hdh* cells, respectively, see Oliveira et al. (2006). ***c***, COS-7 cells labeled with mitotracker red under control conditions (*i*) or following transient transfection with plasmid encoding truncated mHtt Q138 via CMV promoter (*ii*). Expression of mHtt was detected by EM48—Alexafluor 488 labeling (not shown). Note the abnormal globular morphology of mitotracker-labelled structures in the cell overexpressing mHtt (*ii*) vs. threadlike mitochondria in (*i*). ***d***, ST*Hdh* striatal cells expressing Htt Q7 (*i*) or mHtt Q111 (*ii*) under endogenous promoter control. Note the similar threadlike mitochondrial morphology labeled with mitotracker red in both cell-lines. ***e***, Co-cultures of striatal neurons and astrocytes from wild-type rats loaded with Fura-4F and $TMRM^+$ for simultaneous recordings of changes in $[Ca^{2+}]_i$ (*left panels*) and $\Delta\psi_m$ (*right panels*), respectively. Note the earlier loss of Ca^{2+} homeostasis (*red cells in bottom left*) and $\Delta\psi_m$ collapse (*loss of TMRM$^+$ signal in bottom right*) in astrocytes vs. neurons (*arrows*) under conditions of critical dependence of mitochondria for Ca^{2+} buffering; See Oliveira and Gonçalves (2009a) for further details

2003). Also, considering the heterogeneous composition of isolated brain mitochondria preparations, increased population resistance might stem from quantitative changes in the proportion of synaptic vs. non-synaptic mitochondria (Brown et al. 2006), or neuronal vs. glial mitochondria (Bambrick et al. 2006; Oliveira and Gonçalves 2009a). To further explore this issue it is valuable to assess how HD mitochondria perform in situ, i.e., within intact cells.

We determined in situ mitochondrial respiratory parameters in populations of intact striatal neurons cultured from $Hdh^{150/+}$ HD mice or $Hdh^{+/+}$ littermates (Oliveira et al. 2007), using a cell respirometer (Jekabsons and Nicholls

2004), and with glial proliferation inhibited by cytosine arabinoside to allow recordings of "pure" neuronal respiration. Both neuronal populations respired in the basal state at about 20% of maximal capacity, with no significant differences in uncoupling (~10% respiration driving basal H^+ leaks in HD or wild-type neurons). Only 10% of maximal ATP generating capacity (inferred from FCCP respiration) was used in resting wild-type neurons and 14% in HD neurons, thus both exhibited a large spare respiratory capacity (Oliveira et al. 2007). Still, because FCCP respiration is not limited by ATP synthase activity, but represents maximal substrate oxidation capacity (Oliveira 2010b), it is conceivable that deficits in ATP synthesis or export in individual neurons might not be detected in population oxygen consumption recordings. Meaningfully, in single cell $[Ca^{2+}]_i$ and $\Delta\psi_m$ recordings, under conditions of critical dependence on mitochondria for Ca^{2+} handling, a significantly larger proportion of *Hdh*150 neurons failed to recover from transient NMDA receptor (NMDAR) activation when compared with wild-type (Oliveira et al. 2007). Similar findings were obtained in YAC128 HD neurons transiently challenged with NMDA (Oliveira et al. 2006), and ST*Hdh* Q111 HD striatal cell lines challenged with low Ca^{2+} ionophore concentrations (Oliveira et al. 2006). In spite of previous immunoblot evidence for NMDAR subunits expression in ST*Hdh* striatal cell lines (Gines et al. 2003), we only detected NMDAR dependent Ca^{2+} mobilization in these cells following transient transfection with NMDAR subunits (Fig. 1*b*; Oliveira et al. 2004), partly explaining our option for ionophore induced Ca^{2+} mobilization (Oliveira et al. 2006). Interestingly, in addition to the above differences between HD and wild-type striatal neurons, in situ mitochondrial Ca^{2+} buffering capacity is lower in wild-type striatal vs. cortical neurons (Oliveira and Gonçalves 2009a). Thus, it seems that the increased vulnerability of striatal neurons to HD combines mHtt effects with an inherent vulnerability of their mitochondria to Ca^{2+} overload, as previously suggested based on data from mixed neuron-glia isolated mitochondria preparations (Brustovetsky et al. 2003). In addition to effects on mitochondria bioenergetics, interfering with neuronal ATP production and Ca^{2+} handling, mHtt may also modify mitochondria dynamics, as addressed bellow.

Mitochondrial dynamics in HD

Recent evidence suggests that HD affects mitochondrial fission-fusion and trafficking dynamics, being yet uncertain the contribution of direct or indirect mHtt effects on mitochondria. HeLa cells overexpressing truncated mHtt with pathological length polyQ expansions (Q74 or Q138) displayed increased mitochondrial fragmentation and decreased ATP levels, both rescuable by promoting mitochondrial fusion via overexpression of mitofusin-2, or by inhibiting fission via overexpression of dominant-negative Drp-1^{K38A} (Wang et al. 2009a). Accordingly, we found abnormal mitochondrial morphology, namely decreased filamentous mitochondria and more globular structures in COS-7 cells overexpressing truncated mHtt Q138 (Fig 1*c*). However, in ST*Hdh* striatal cell-lines, expressing full-length mHtt Q111 under endogenous promoter control, we found no apparent changes in mitochondrial morphology (Fig 1*d*). Plausible explanations are that overexpression of mHtt constructs induces a much higher cellular stress than endogenous levels of mHtt (Tobin and Signer 2000), and that high glycolytic activity in ST*Hdh* cells may compensate for mitochondrial functional deficits, as previously shown for Ca^{2+} handling abnormalities (Oliveira et al. 2006). Still, given the morphological and gene expression differences, some data from cell-lines may not apply to neurons. While fission inhibition might rescue mHtt induced bioenergetic defects in HeLa cells (Wang et al. 2009a), it may prevent mitochondria from moving through narrow processes on their way to synapses in neurons (Fig. 2). Indeed, perinuclear accumulation of elongated mitochondria may be without significant functional consequences in cells such as fibroblasts, but in highly polarized and OXPHOS-dependent neuronal cells this abnormal distribution likely compromises mitochondrial availability, Ca^{2+} handling and energy supply in distant processes (Wang et al. 2009b).

HD mitochondrial fission is not necessarily the direct result of mHtt-mitochondria interactions. Mitochondria fission in neurons treated with the complex II inhibitor 3-nitropropionic acid stems from secondary excitotoxicity (Liot et al. 2009), since glutamate induced Ca^{2+} rise via NMDAR causes neuronal mitochondrial fission (Rintoul et al. 2003; Reynolds et al. 2004), and so might be the case in HD neurons expressing mHtt. Regardless of abnormalities in fusion-fission dynamics, there is strong evidence that mHtt in either aggregate or soluble form impairs mitochondrial trafficking in neurons, by physically obstructing mitochondrial passage and by sequestering trafficking components or disturbing their normal association with mitochondria (Trushina et al. 2004; Chang et al. 2006; Orr et al. 2008) (Fig. 2). Interestingly, mitochondrial trafficking in cortical neurons is reduced specifically at sites of mHtt aggregates (Chang et al. 2006), whereas in striatal neurons mitochondrial trafficking is reduced even in regions without mHtt aggregates (Orr et al. 2008). Also, we observed a slower mitochondrial trafficking in resting wild-type striatal vs. cortical neurons (Oliveira and Gonçalves 2009a, b), which is compatible with the hypothesis of inherent striatal vulnerability to mitochondrial trafficking impairment. Taken together, available

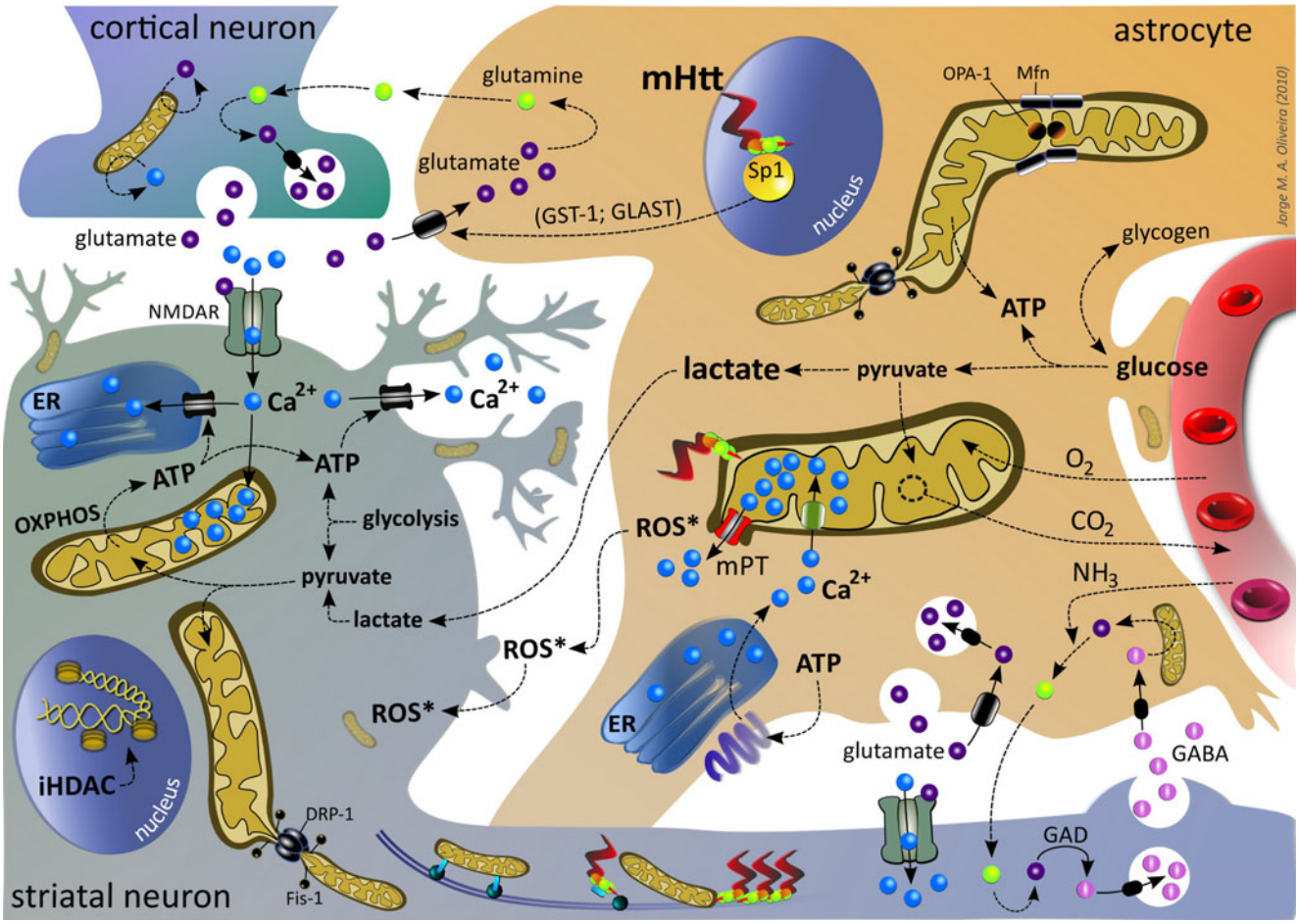

Fig. 2 Mitochondrial dysfunction in HD cortico-striatal tripartite synapses. Cortical glutamatergic neuron (*top left*) communicating with a GABAergic neuron (*bottom*) in the presence of an astrocyte (*right*). Synaptic glutamate (purple spheres) is removed via astrocytic transporters (GST-1 or GLAST) whose expression is decreased by mHtt (red protein with green polyQ) acting on nuclear Sp1 (Shin et al. 2005; Bradford et al. 2009). Circulating glucose (*middle right*) is taken by astrocytes, converted into lactate and transferred to neurons for energy production. Note mitochondrial ATP production, Ca^{2+} (blue spheres) handling, fission (via DRP-1 and Fis-1), fusion (via Mfn and OPA-1), and trafficking dynamics (impaired by mHtt; *bottom*). ER, endoplasmic reticulum; GAD, glutamic acid decarboxylase; iHDAC, HDAC inhibitor; mPT, mitochondrial permeability transition; ROS*, reactive oxygen species. See text for further details. *Author's own drawing*

evidence suggests that HD trafficking impairment is not homogenous across neuronal types, and that a mechanism underlying the increased vulnerability of striatal neurons might be the increased susceptibility of their mitochondria to trafficking impairment.

Astrocytic mitochondria and the vulnerability of striatal tripartite synapses

The HD vulnerable striatum receives massive glutamatergic input from the cortex. The resulting connections integrate not only cortical and striatal neurons, but also a third party, the astrocytes, involved in bidirectional communication with neurons, and thus forming functional tripartite synapses (Perea et al. 2009). HD astrocytes also express mHtt, which seems to compromise their normal neuroprotective role in synaptic glutamate clearance (Shin et al. 2005). In fact, astrocytic dysfunction is increasingly considered to contribute for non-cell autonomous neurodegeneration in HD (Lievens and Birman 2007; Lobsiger and Cleveland 2007; Bradford et al. 2009, 2010; Ilieva et al. 2009; Hsiao and Chern 2010). Following on previous studies evidencing an increased Ca^{2+} vulnerability of non-synaptic mitochondria isolated from striatum vs. cortex, possibly contributing for selective striatal vulnerability in HD (Brustovetsky et al. 2003), we investigated whether this vulnerability resulted from differences in neuronal or astrocytic mitochondria, or both (Oliveira and Gonçalves 2009a). Using an experimental strategy allowing in situ measurements of mitochondrial Ca^{2+} buffering capacity in intact, non-permeabilized, neurons and astrocytes in co-culture (Fig. 1*e*), we showed

that: (1) mitochondria not only in striatal neurons, but also in striatal astrocytes, buffer less Ca^{2+} before undergoing mPT in comparison with their cortical counterparts; and that (2) cyclosporin A (CsA; 1 μM) selectively increases Ca^{2+} buffering capacity in striatal astrocytes, rendering it identical to that of cortical astrocytes. Thus, the increased vulnerability of striatal vs. cortical mitochondria resides in both intact neurons and astrocytes. Moreover, mitochondria in striatal astrocytes display an increased propensity to undergo CsA-sensitive mPT, which positions the striatum at greater risk for disturbed neuron-astrocyte interactions, likely contributing for selective striatal neurodegeneration in HD. Furthermore, the selective effect of CsA over striatal astrocytes suggests that in vivo neuronal sheltering with this compound in HD models (Leventhal et al. 2000) may indirectly result from reducing the mitochondrial vulnerability of their astrocytic neighbors (Oliveira and Gonçalves 2009a).

Vulnerable astrocytes may compromise neuronal neighbors by failing to provide adequate support under pathophysiological conditions (Araque et al. 1999; Dienel and Hertz 2005; Lobsiger and Cleveland 2007). Mitochondrial impairment in astrocytes may disturb protective astrocyte-neuron lactate trafficking, and/or cause them to produce diffusible reactive oxygen species (ROS) that damage nearby neurons (Bambrick et al. 2004; Rossi et al. 2007). Also, mitochondria modulate Ca^{2+} wave propagation (Boitier et al. 1999) as well as Ca^{2+}-dependent glutamate release from astrocytes (Reyes and Parpura 2008). Thus, impairments in astrocytic mitochondrial Ca^{2+} buffering may compromise normal tripartite synapse operation, potentially decreasing the threshold for epileptic seizures and excitotoxic neurodegeneration (Reyes and Parpura 2008; Oliveira and Gonçalves 2009a) (Fig. 2). This might be particularly relevant in juvenile or infantile HD, caused by the largest mHtt polyQ expansions and frequently associated with seizures (Squitieri et al. 2006). Indeed, abnormal mitochondria were found in astrocytes from juvenile HD brain (Goebel et al. 1978), and recent evidence suggests that the length of the polyQ expansion determines the severity of glial dysfunction in transgenic HD mice (Bradford et al. 2010).

Concluding remarks

Disturbed mitochondrial bioenergetics and dynamics not only in neurons but also in glia, namely astrocytes, may play a significant role in HD pathogenesis. Impaired mitochondrial Ca^{2+} handling in striatal neurons severely compromises their ability to buffer NMDAR Ca^{2+} responses resulting from cortical glutamatergic input. Pairing with vulnerable astrocytic partners further aggravates the risk of excitotoxic injury. Dysfunctional mitochondria in HD astrocytes and cortico-striatal tripartite synapses might be particularly relevant in juvenile/infantile HD forms, associated with large polyQ expansions and reduced seizure threshold. Among emerging pharmacological strategies, histone deacetylase (HDAC) inhibitors hold great potential for a neuroprotective role (Kazantsev and Thompson 2008; Chuang et al. 2009). Significantly, HDAC inhibitors improved mitochondrial-dependent Ca^{2+} handling associated to recovery from transient NMDAR activation in HD striatal neurons (Oliveira et al. 2006). Also, the combined epigenetic mechanisms and anti-seizure activity of HDAC inhibitors compounds such as valproic acid (Monti et al. 2009) further highlights their therapeutic potential in disorders associated with disturbed tripartite-synapses and excitotoxicity.

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