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**Downstream mechanisms triggered by mitochondrial dysfunction
in the basal ganglia: from experimental models to neurodegenerative diseases**

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Abstract

Mitochondrial dysfunctions have been implicated in the cellular processes underlying several neurodegenerative disorders affecting the basal ganglia. These include Huntington's chorea and Parkinson's disease, two highly debilitating motor disorders for which recent research has also involved gene mutation linked to mitochondrial deficits. Experimental models of basal ganglia diseases have been developed by using toxins able to disrupt mitochondrial function: these molecules act by selectively inhibiting mitochondrial respiratory complexes, uncoupling cellular respiration. This in turn leads to oxidative stress and energy deficit that trigger critical downstream mechanisms, ultimately resulting in neuronal vulnerability and loss. Here we review the molecular and cellular downstream effects triggered by mitochondrial dysfunction, and the different experimental models that are obtained by the administration of selective mitochondrial toxins or by the expression of mutant genes.

1. Introduction

The basal ganglia (BG) are a richly interconnected group of brain nuclei communicating with elements of the sensory, motor, cognitive, and motivational apparatuses of the brain. In particular, BG play a key role in the subtle regulation of voluntary and purposive movements and are involved in motor learning and memory [188]. The observation of humans suffering from neurodegenerative diseases affecting the BG, such as Parkinson's disease (PD) and Huntington's disease (HD), has provided the most widely accepted view of the function of these structures, which has been further confirmed experimentally [2,55,74]. Notably, their pathophysiology includes oxidative stress and impairment of mitochondrial energy metabolism, in particular a specific dysfunction of complex I in PD and of complex II in HD [26,159,160,170]. Mitochondria provide the bulk of energy supply to the cell in the form of adenosine triphosphate (ATP). Neurons are extremely sensitive to ATP depletion due to their high energy demand and for this reason they are strongly affected by impairments of energy supply, which lead to necrotic or apoptotic cellular death [76,129]. Interestingly, although mitochondrial dysfunction is involved in both PD and HD, the pattern of neuronal loss observed in these two pathologies is different: the hallmark of PD is the loss of *substantia nigra pars compacta* (SNc) dopaminergic neurons [100,101], whereas HD is characterized by the selective degeneration of striatal medium-sized spiny neurons using γ -aminobutyric acid (GABA) as neurotransmitter and the relative sparing of cholinergic interneurons [151]. Experimental and clinical data suggest that some of the molecular mechanisms for such differential vulnerability originate from the selective mitochondrial impairment underlying PD and HD. Accordingly, chemical inhibition of either complex I or II have led to useful and relevant experimental models of, respectively, PD and HD. It should be mentioned, however, that although none of these models fully reproduces the features of the human diseases, they contributed to improve our knowledge on some of their molecular, cellular and pathophysiological mechanisms. Such mechanisms include, beside the disruption of mitochondrial energy metabolism [8], common and specific molecular cascades that ultimately lead to neuronal death. Here we will review these

models in the context of neurological diseases affecting the BG, with a particular regard to the downstream mechanisms triggered by mitochondrial dysfunction.

2. Basal ganglia in physiological and pathological condition

As mentioned above, the BG are a network of interconnected subcortical structures involved in the control of motor function and in reward and cognitive processes [2,55,74]. The striatum (caudate and putamen nuclei) is classically considered the main input nucleus of the BG, receiving abundant glutamatergic afferences from the cortex and in turn projecting to the output BG nuclei, i.e. the internal *globus pallidus* (GPi) and *substantia nigra pars reticulata* (SNr). Such projections are both direct and indirect via the external *globus pallidus* (GPe) and the subthalamic nucleus (STN). A more recent view of BG organization (Figure 1) now places both the striatum and STN as the two major input stations, the latter belonging to the "hyperdirect" cortex-STN-GPi/SNr pathway [107]. The balance between excitatory (glutamatergic) and inhibitory (GABAergic) synaptic activity within the BG is crucial for their physiological functioning and, accordingly, disruptions of this balance are believed to underlie several hypo- or hyperkinetic movement disorders, such as PD and HD [2,56,186,187]. It is thus of primary importance to further characterize their molecular and cellular pathophysiological mechanisms, in order to develop new therapeutic strategies able to slow disease progression and neuronal loss.

Parkinson's disease - PD is a debilitating neurodegenerative movement disorder with a long course and a high prevalence (1.6 over 100 individuals aged >65 years in the European Union) that increases with demographic ageing [54]. The progressive loss of dopaminergic neurons of the SNc characterizing PD leads to the disruption of the excitation/inhibition balance in the BG and to the manifestation of the main PD symptoms: postural instability, muscle rigidity, tremor and bradykinesia/akinesia. SNc neuron loss results in an excessive activity of glutamatergic pathways of

the BG, including the corticostriatal afferent pathway [86,173] and STN efferent neurons [95], both playing a key role in the expression of PD symptoms [35,79,161]. PD is also anatomopathologically characterized by cytoplasmic inclusions in dopaminergic neurons called Lewy bodies, constituted by α -synuclein-ubiquitin complexes [133]. Besides these evidences, the etiology of the disease remains largely unknown and the available treatments, such as administration of the dopamine precursor 3,4-dihydroxy-L-phenylalanine (L-DOPA) or deep-brain stimulation of BG structures, are only symptomatic. Furthermore, motor fluctuations and dyskinesia complicate L-DOPA therapy in most patients within 5-10 years of treatment initiation. The large majority of PD cases are idiopathic, but a number of specific genetic mutations and exogenous factors responsible for sporadic PD have been identified. Interestingly, current evidence suggests that dysfunction of mitochondrial complex I [52], as well as a possible aberrant interplay between dopamine and α -synuclein [190], may play a major role in PD. In this context, dysfunction of α -synuclein has been shown to indirectly but significantly impact on neuronal mitochondrial function, and there is accumulating evidence for a close relationship between α -synuclein and oxidative damage: overexpression of mutant α -synuclein sensitizes neurons to oxidative stress and damage by dopamine and complex I inhibitors, resulting in increased protein carbonylation and lipid peroxidation *in vitro* and *in vivo* [1]. However, since the function of α -synuclein is still unclear, further work is needed to understand the molecular mechanisms involving this protein and dopamine leading to the selective loss of SNc neurons characterizing PD.

Huntington's disease - HD is an autosomal dominant neurological disorder with a prevalence of 4-8 people in 100,000 in Western Europe [94]. It is characterized by the progressive loss of GABAergic medium-sized spiny neurons of the striatum, while cholinergic interneurons are spared [63]. HD symptoms include abnormal "dance-like" movements (chorea), dystonia, cognitive deficits and psychiatric disorders. Treatments that can actually prevent the physical and mental decline associated with HD are lacking, and available antichoreic drugs offer patients only a limited

respite from constant involuntary movements and have long-term side effects [18,184]. Neuronal transplantation provides some years of improvement and stability but not a permanent cure for the disease [5]. The mutated gene responsible for HD was identified in 1993 as the HD gene (IT15) by the HD Collaborative Research Group [114]. Its protein product is huntingtin (Htt) and, since its discovery, researchers are still elucidating the function of both normal and mutant Htt (mHtt). The mutation causing HD is an increased length of a CAG triplet repeat (>36) resulting in a polyglutamine (polyQ) expanded mHtt. The expression of Htt, and mHtt, is widespread in both the central and peripheral nervous systems, and the mechanisms by which mHtt induces the selective loss of striatal projection neurons are still poorly understood. In the pathophysiology of HD, excitotoxic processes linked to N-methyl-D-aspartate (NMDA) receptor activation [62], abnormal activity of corticostriatal glutamatergic pathway [81,83], and mitochondrial complex II dysfunction [24,84,112] have been hypothesized.

3. Biochemistry and physiology of the mitochondrion

Mitochondria generate most of the eukaryotic cell's supply of ATP through the process known as oxidative phosphorylation, a biochemical pathway first characterized by Mitchell and Moyle in 1967 that utilizes energy released by the catabolism of nutrients to synthesize ATP [123]. Mitochondria participate in catabolic processes by several biochemical pathways, including Krebs cycle (also known as the citric acid or tricarboxylic acid cycle), and oxidation of fatty acids and amino acids. The end result of these pathways is the production of two kinds of energy-rich electron donors: reduced nicotinamide adenine dinucleotide (NADH) and succinate. Electrons from NADH and succinate are passed to oxygen that is reduced to water through oxidative phosphorylation, a multi-step redox process occurring on the mitochondrial inner membrane through the mitochondrial electron transport chain. This chain is constituted by a set of five large monomeric (I, II and V) or dimeric (III and IV) enzyme complexes that are associated to a variety of cofactors and electron-

transferring proteins (Figure 2). Complex I or NADH-coenzyme Q oxidoreductase (also known as NADH dehydrogenase) is the first element in the electron transport chain [105]. The reaction catalyzed by complex I consists in the reduction of coenzyme Q10 (or ubiquinone) by NADH and the donation of two electrons. As the electrons pass through complex I, four protons (H^+) are pumped from the matrix into the intermembrane space. Complex II or succinate-Q oxidoreductase (also known as succinate dehydrogenase), which is also a part of the Krebs cycle, oxidizes succinate to fumarate and reduces coenzyme Q10 [36]. Complex III or Q-cytochrome *c* oxidoreductase (also known as cytochrome *c* reductase or cytochrome bc_1 complex) catalyzes the oxidation of one molecule of ubiquinol and the reduction of two molecules of cytochrome *c* through a complex reaction called Q cycle, which pumps four more H^+ in the intermembrane space [48]. Complex IV (also known as cytochrome *c* oxidase) is the last element of the electron transport chain and mediates the final reaction, consisting in the transfer of the electrons to O_2 (that is reduced to $2 H_2O$ accepting 4 electrons) while pumping two H^+ in the intermembrane space every two electrons transported [33]. The final step in the oxidative phosphorylation pathway is ATP synthesis, catalyzed by complex V or ATP synthase [21]. Interestingly, this mushroom-shaped giant protein complex is found in all forms of life and functions in the same way in both prokaryotes and eukaryotes [21]: it utilizes the energy stored in the form of the H^+ gradient (Δp) across the mitochondrial inner membrane, generated by the electron transport chain, to synthesize ATP from adenosine diphosphate (ADP) and phosphate (P_i).

Besides energy production through Krebs cycle and oxidative phosphorylation, mitochondria have some other important roles for the physiological functioning of the cell. For example, they participate to Ca^{2+} homeostasis [90,127] in association with the endoplasmic reticulum [147], contain circular DNA molecules (mitochondrial genome) encoding for tRNAs, rRNAs, and complexes I, III, IV and V [40], and participate to cellular metabolism and proliferation [115].

4. Mitochondrial downstream mechanisms in cell death

Mitochondria are essential to cell life. Accordingly, the loss of mitochondrial ATP production leading to energy metabolism deficit can kill a cell by necrosis, and lack of ATP is particularly critical for excitable cells such as neurons, in which the Na^+/K^+ -ATPase uses more than 40% of the available ATP to keep resting membrane potential at physiological values [81]. However, mitochondria also play an active role in cellular death by participating in complex phenomena leading to apoptotic processes [77]. Indeed, these organelles are involved in a number of key events in apoptosis [75,77], the most critical of which is mitochondrial outer membrane permeabilization (MOMP) occurring early during this programmed death process [76]. MOMP consists in the release of proteins that normally are found in the space between inner and outer mitochondrial membranes, such as cytochrome *c* and pro-apoptotic factors. The mechanisms responsible for MOMP may involve the opening of the mitochondrial permeability transition pore (mtPTP), leading to collapse of Δp , loss of mitochondrial membrane potential ($\Delta\Psi_m$) and matrix swelling [130], which can ultimately result in outer membrane breaking [132]. The mtPTP putatively consists of the voltage-dependent anion channel in the outer membrane, the adenine nucleotide transporter translocator in the inner membrane, cyclophilin D, and other molecules [174,175]. Other mechanisms do not involve mtPTP and seem to be mediated by apoptosis-regulating proteins of the Bcl-2 family (Bax and Bak) acting directly on the outer membrane [76]. The consequences of MOMP are dramatic: as the outer mitochondrial membrane breaks, a number of molecules are released into the cytoplasm triggering downstream processes that lead to apoptotic cell death (Figure 3). These molecules include, in particular: i) cytochrome *c*, which binds to the cytosolic protein Apaf-1 (apoptotic peptidase-activating factor 1) and to the nucleotide dATP, and then recruits and activates pro-caspase-9 into caspase-9 [108]; ii) several pro-caspases (2, 3, 8 and 9) [76] as well as apoptosis-inducing factor (AIF) [167]; iii) caspase regulators such as Smac (second mitochondria-derived activator of caspases), or its murine equivalent DIABLO (direct IAP-binding protein with low pI),

which removes IAPs (inhibitor of apoptosis proteins) from caspase-IAP complexes, abrogating caspase inhibition [64].

Another important factor contributing to mitochondrial downstream processes leading to cell death is the release of reactive oxygen species (ROS) and free radicals (Figure 3). Mitochondria are the major source of ROS, in particular superoxide anion ($\cdot\text{O}_2^-$) as a byproduct the abovementioned Q cycle at complex III level [65,93]. Other ROS include hydrogen peroxide (H_2O_2) produced from $\cdot\text{O}_2^-$ by superoxide dismutase (SOD). Uncoupling electron transfer increases ROS production, and the release of cytochrome *c* due to MOMP contributes to electron transfer uncoupling, which can result in a feedback mechanism further increasing ROS production [77]. Besides being harmful by directly damaging DNA, oxidizing fatty acids and amino acids, and inactivating enzymes by oxidation, ROS can also trigger several signaling pathways. Indeed, some of them exert a pro-survival action, such as those mediated by ERK (extracellular regulated protein), PI(3)K/Akt (phosphoinositide 3-kinase/Akt) and NF- κ B (nuclear factor κ B), whereas some others can activate p53 tumor suppressor protein transcription factor, JNK (c-Jun N-terminal kinase) and p38 protein kinases that are linked to apoptosis, contributing to the abovementioned pro-apoptotic events occurring during MOMP [65]. Moreover, glutamate transporters [50] are also vulnerable to ROS, resulting in reduced uptake function and build-up of extracellular glutamate levels that can be toxic to neurons and astrocytes [172]. Prolonged exposure to glutamate leads to hyperactivation of ionotropic glutamate receptors [13,111] and to the concomitant influx of ions and water into the cell [43]. In particular, excessive activation of NMDA receptors increases intracellular Ca^{2+} levels, which is known to promote a great number of processes contributing to cell death [60,81,88,121,128]. These processes include excitotoxicity, stimulation of phospholipase A_2 and Ca^{2+} -dependent protein kinases, and production of ROS and free radicals that may trigger a vicious cycle worsening mitochondrial dysfunction that in turn predisposes neurons to excitotoxic damage (Figure 3).

But how these mitochondrial downstream mechanisms leading to cell death are triggered? Which of them are relevant to neurodegenerative diseases? A multitude of factors have been implicated in these processes. The following sections will review the currently utilized experimental models of mitochondrial impairment that attempt to address the molecular, cellular and physiological mechanisms of BG neurodegenerative diseases.

5. Mitochondrial complex I dysfunction and Parkinson's disease

Dysfunction of mitochondrial complex I has been associated to sporadic forms of PD [52] and parkinsonism, the latter term referring to a syndrome of tremor, rigidity, bradykinesia and postural instability that, beside PD or "primary parkinsonism", includes rare secondary cases that may result from toxicity, head trauma or other medical disorders [44,52]. Complex I derangement results in an enhanced production of ROS that, in turn, further impairs mitochondrial function in a vicious cycle [170], triggering the above described downstream mechanisms leading to neuronal death. Accordingly, neuroscientists took advantage of several selective complex I inhibitors to develop experimental models relevant to PD [97].

6-hydroxydopamine (6-OHDA) - The neurotoxin 6-OHDA is a structural analog of catecholamines and has played a fundamental role in research on PD since the late 1960s [155,177], after the discovery of its lethal properties on catecholaminergic neurons [148]. Neurotoxic effects of 6-OHDA occur through a two-step mechanism involving accumulation of the toxin into catecholaminergic neurons by the dopamine and noradrenaline membrane transporters, followed by mitochondrial complex I inhibition [17]. The action mechanism by which 6-OHDA reversibly inhibits complex I still needs to be better characterized. However, it seems that this toxin does not compete with NADH for its binding site on the enzyme, but rather it binds to the enzyme-substrate

complex and the resulting enzyme-substrate-inhibitor complex has a reduced catalytic activity [72]. 6-OHDA is toxic at both central and peripheral level. However, since this molecule is unable to cross the blood-brain barrier, it is toxic only when directly injected into the brain by stereotaxic procedures. The selectivity of 6-OHDA for SNc dopaminergic neurons is thus achieved by directly targeting this structure, in association or not with a treatment blocking noradrenalin uptake such as desipramine. 6-OHDA injection in the SNc or in the medial forebrain bundle produces a massive (>90%) degeneration of dopaminergic neurons and specific motor deficits, some of which mimicking those of PD. 6-OHDA is classically utilized in rats, but an increasingly number of papers now reports this technique also in mice. However, 6-OHDA dopaminergic denervation in rodents lacks key neuropathological and clinical features of PD: Lewy bodies do not appear in neurons and tremor is absent. Usually, 6-OHDA is injected unilaterally, and this is for two main reasons: first, the massive bilateral dopamine depletion provoked by 6-OHDA is easily lethal in rodents, which moreover need to be force-fed; second, animals with unilateral 6-OHDA lesion have unilateral motor impairments that can be efficiently detected and measured, such as ipsilateral akinesia and contralateral rotations following administration of dopaminergic agents [37,86,178]. Some scientists perform partial bilateral lesions (~60% of dopaminergic loss) by injecting 6-OHDA in the dorsal striatum to target dopaminergic terminals, thus representing an "early stage" model of PD that can also allow to study more complex behavioral changes such as reaction time [3], a parameter also measured in humans [70]. The 6-OHDA PD model has provided a considerable amount of information at cellular and molecular level. For example, glutamatergic transmission in the BG is significantly affected in rats with massive dopamine denervation [162]: *in vitro* electrophysiological studies show an enhanced glutamatergic activity of striatal medium-sized spiny neurons [31,87] that is supported by *in vivo* data [173]. Moreover, the two forms of synaptic plasticity described at corticostriatal synapse, long-term depression (LTD) and long-term potentiation (LTP), are lost in parkinsonian rats [28], as well as depotentiation of LTP [28,142]. Another interesting feature of the 6-OHDA model of PD is that it can be associated to

antiparkinsonian therapies, such as L-DOPA and chronic high-frequency stimulation of the STN [89], with some interesting outcomes [29]. For example, L-DOPA treatment has been shown to either ameliorate hypokinetic motor deficits [113] or to induce dyskinesia in hemiparkinsonian rats [143] depending on the administration mode. Moreover, L-DOPA treatment is also capable to reverse some cellular and molecular changes observed at striatal level after unilateral 6-OHDA lesion in rats, in particular glutamatergic hyperactivity at corticostriatal synapses [113], hypersensitization of striatal D2-like dopamine receptors [4,144] and of group II metabotropic glutamate receptors [146], as well as endocannabinoid system abnormalities [87,113]. Similarly, STN stimulation is also effective in progressively reducing akinesia and reversing glutamatergic hyperactivity at corticostriatal synapses [86].

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) - This neurotoxin was discovered when drug-addicted Californians accidentally injected with "synthetic heroin" (1-methyl-4-phenyl-4-propionoxypiperidine, MPPP or desmethylprodine) contaminated with MPTP and suddenly developed parkinsonian symptoms [7,102]. MPTP is a highly lipophilic mitochondrial complex I inhibitor that easily crosses the blood-brain barrier and can thus be administrated systemically to obtain experimental PD models [149]. It becomes toxic through its transformation to 1-methyl-4-phenyl-2,3-dihydropyridinium (MPDP⁺) by the enzyme monoamine oxidase B (MAO-B) expressed by glia and serotonergic neurons [99], and its subsequent spontaneous oxidation to 1-methyl-4-phenylpyridinium (MPP⁺) [165,171]. The dopamine transporter (DAT) then carries MPP⁺ into dopaminergic neurons, where it is accumulated into synaptic vesicles by the vesicular monoamine transporter (VMAT). Once in the cell, it can enter mitochondria using $\Delta\Psi_m$ driving force where it blocks complex I [131,152,164,166]. Various protocols of MPTP administration in mice (acute vs. chronic; repeated injections of low doses vs. a single high dose, etc.) exist in the literature [120], while the rat appears to be refractory to this toxin. Chronic intoxication with low doses of MPTP is also used in monkeys, in which it mimics at best the motor symptoms of PD including tremor,

bradykinesia, muscle rigidity and postural instability, as well as some neuropathological features including the massive loss of SNc neurons. [97,124,166]. On the other hand, degeneration of nigrostriatal dopamine neurons is not accompanied by the more general loss of monoaminergic cells and the presence of Lewy bodies observed in PD, at least in non-chronic models [67]. The MPTP model has been particularly useful for studying the functional changes occurring in the primate BG after dopamine depletion, providing insights into the pathophysiology of PD. For example, STN neurons recorded from awake monkeys showed an increased rate of spontaneous firing activity with periodic oscillations at low β -frequency, which in humans is highly correlated with tremor [12,15,117], confirming that this structure becomes hyperactive in PD and contributes to the disruption of BG functioning. Moreover, the rationale for targeting STN by deep-brain stimulation arose from the primate MPTP model. Recently, some studies using chronic MPTP intoxication in mice showed inhibition of the ubiquitin-proteasome system paralleled by the apparition of neuronal inclusions containing ubiquitin and α -synuclein [66,119]. Interestingly, such alterations were reduced in mice lacking α -synuclein [66], further supporting the involvement of this protein in the pathophysiological processes of PD [190]. In MPTP-treated mice some interesting motor impairments relevant to PD can be detected by a number of behavioral tests that are sensitive to subtle changes in dopamine levels [118].

Concerning the mechanisms of MPTP toxicity, NMDA receptor and inducible nitric oxide synthase (iNOS) have also been implicated in MPTP-mediated loss of SNc neurons, because such neurodegeneration was reduced by NMDA receptor antagonists [176], in mice lacking iNOS [109] or by an anti-inflammatory compound (of the diindolylmethane class) suppressing NF- κ B-dependent expression of iNOS [34]. Other reports have suggested a neurodegenerative mechanism based on mitochondrial downstream apoptotic processes [17], which involve up-regulation of JNK [157] and Bax [180], release of cytochrome *c* and activation of caspase-3 and -9 [183]. Accordingly, systemic pharmacological blockade of JNK or p53 (which regulates Bax) resulted in reduced damage to nigrostriatal neurons after MPTP intoxication [59,157].

Rotenone - Rotenone is a complex I inhibitor used in broad spectrum insecticides, piscicides and pesticides. It can naturally be found in the roots and stems of several tropical plants such as the jícama vine plant (*Pachyrhizus erosus*). Rotenone is highly lipophilic and, unlike MPP^+ , it does not need to be actively transported. Thus, it can quickly and easily accumulate in tissues and then reach the brain [78]. Systemic (intravenous) administration of rotenone in rats induces a highly selective degeneration of SNc neurons paralleled with hypokinesia and rigidity, and cytoplasmatic inclusions containing ubiquitin and α -synuclein [14,27]. The administration route of this inhibitor seems essential for the set up of experimental PD models, since chronic intranasal inoculation failed to induce dopamine neuron degeneration [154]. The action mechanisms of rotenone are still poorly characterized, but evidence from experimental *in vitro* and *in vivo* PD models by chronic intoxication suggest that this toxin causes a delayed depletion of glutathione, which is accompanied by oxidative damage to proteins and DNA [78]. Electrophysiological data show that rotenone acute application on corticostriatal slices induces an early hyperpolarization of striatal cholinergic interneurons, followed by a late depolarizing response that seems linked, respectively, to activation of ATP-sensitive K^+ channels and decreased Na^+/K^+ -ATPase function [19]. More recent electrophysiological studies targeted striatal spiny neurons, which respond by a marked membrane depolarization resulting in the loss of field potential, coupled to an increased release of excitatory amino acids and dopamine. Interestingly, these effects were not attenuated by acting on glutamatergic synaptic transmission [39,47] but they were reduced by antagonists of dopamine D2-like [47] or adenosine A2A receptors [11], suggesting that monoaminergic and purinergic systems may play a role in the selective toxicity of rotenone.

Paraquat - Paraquat is the trade name for 1,1'-dimethyl-4,4'-bipyridinium dichloride, a structural analog of MPP^+ and one of the most widely used pesticides and herbicides in the world. It is lipophilic and enters easily into the cell where is taken up across the mitochondrial inner membrane

by a carrier-mediated and $\Delta\Psi_m$ -dependent process, and once in the matrix it is reduced by complex I. The reduced form of paraquat then reacts with oxygen to form ROS (in particular $\cdot\text{O}_2^-$) and cause mitochondrial oxidative stress leading to neuronal death [45]. Accordingly, synthetic SOD/catalase mimetics can protect dopaminergic neurons both in cell lines and in mice treated with this toxin [140]. An epidemiological association has been found between the use of paraquat in agriculture and the incidence of parkinsonism [57], and this toxin is now used to model PD. Some mechanisms underlying acute paraquat neurotoxicity begin now to be elucidated. At molecular level, they seem to involve sequential phosphorylation of JNK and c-Jun, and the activation of caspase-3, leading to apoptosis [139]. At the cellular level, paraquat inhibits AMPA receptors on dopaminergic neurons, suggesting that a decreased excitability and function of these cells in the SNc may contribute to the pathogenesis of PD [104]. Long-term paraquat administration (up to 24 weeks) has been shown to produce a slowly progressing degeneration of dopaminergic neurons of the SNc [135] and the ventral tegmental area (a source of the mesocortical dopaminergic projection) [134], leading to delayed deficits in dopaminergic transmission that resemble early presymptomatic stages of PD. However, another study with chronic exposure to paraquat (intranasal inoculation) in mice did not report dopaminergic loss, but this toxin induced severe hypokinesia and vestibular damage [154],

Manganese - Although manganese intoxication is not properly an experimental model of PD, its action mechanisms are relevant to this disease because they involve mitochondrial complex I inhibition. Manganese (Mn) is an essential trace element and is required for several physiological enzymatic reactions. Exposure to excessive levels of this metal through inhalation of manganese-containing dust can evoke a syndrome with psychiatric disturbances and severe extrapyramidal motor dysfunctions closely resembling those of PD, called manganism. Clinical manifestations of manganism arise from focal injury to the BG due to the fact that these nuclei accumulate most of the excess manganese compared with other brain regions. The mechanisms of the cellular damage caused by this element have been attributed to its capacity to produce toxic levels of free radicals

and induce mitochondrial dysfunction, thus enhancing ROS production [58,91]. Manganese is sequestered by brain and liver mitochondria via the mitochondrial Ca^{2+} uniporter [71], and its toxicity seems to result from a direct and/or indirect inhibitory effect of Mn(III) and Mn(II) on complex I leading to oxidative stress [42,68]. Moreover, Mn(II) catalyzes the auto oxidation of dopamine to dihydrobenzothiazine (DHBT) and then to 7-(2-aminoethyl)-3,4-dihydro-5-hydroxy-2H-1,4-benzothiazine-3-carboxylic acid (DHBT-1), which is a complex I inhibitor [163]. We have shown that chronic manganese intoxication in rats (MnCl_2 dissolved in drinking water) induces a hyperactivation of corticostriatal glutamatergic afferences [38], reminding that observed after 6-OHDA lesion, paralleled by an enhanced sensitivity of presynaptic D2-like dopamine receptors [32].

6. Gene mutation, mitochondria and Parkinson's disease

Around 5% of PD cases are familial forms that have an earlier onset (~45 years) [53] and that arise from the mutation of several identified genes, in particular *parkin* and *PINK1* [phosphatase and tensin homologue (PTEN)-induced putative kinase 1] [1,192]. *Parkin* and *PINK1* are involved in autosomal recessive PD (ARPD), and they are particularly interesting here because both clinical and experimental evidence have associated their mutation with mitochondrial dysfunction.

Parkin - Parkin is a 465 amino acid E2-dependent E3 ubiquitin ligase involved in protein ubiquitination and removal of damaged proteins. A large variety of homozygous and heterozygous mutations of parkin gene have been reported, all of them abolishing or reducing parkin activity and representing ~50% of ARPD cases [1]. Severe mitochondrial pathology and increased vulnerability to oxidative stress have been shown in *Drosophila* null mutants [80]. In mice, deletion of *parkin* exon 3 resulted in reduced excitability of striatal medium-sized spiny neurons and behavioral deficits sensitive to nigrostriatal pathway dysfunction [73]. Moreover, these animals had an impaired

mitochondrial function paralleled by a decreased expression of complex I and IV subunits [136]. On the other hand, deletion of either exon 2 or 3 did not cause dopaminergic neuron degeneration [73,96], thus questioning the validity of *parkin* mutant mice as a PD model [141].

The mechanisms by which parkin is linked to mitochondrial function are not yet completely elucidated. In ceramide-mediated apoptosis, parkin seems to promote the degradation of protein substrates localized in or around mitochondria, delaying MOMP and subsequent cytochrome *c* release and caspase-3 activation [51]. Other indirect actions of parkin on mitochondria have been hypothesized, all linked to its role in protein ubiquitination and degradation [1]. Recently, it has been shown that a wide array of complex I inhibitors, including 6-OHDA, MPP⁺, paraquat, NO and iron, as well as dopamine, all altered parkin solubility, leading to parkin aggregation and thus compromising its neuroprotective function [185]. Overall, parkin seems to play an important role in cell protection against oxidative stress, and its mutation disrupts this function as well as its role as ubiquitin ligase, leading to an impaired removal of mitochondrion-associated damaged proteins and thus to mitochondrial damage [192]. Interestingly, dopamine covalently modifies parkin, which results in the loss of its activity [103] and suggests a possible mechanism for the selective loss of dopaminergic neurons in ARPD as well as in idiopathic PD. Furthermore, it has been shown that overexpression of Pael-R (parkin associated-endothelin-receptor-like receptor, an endoplasmic reticulum-associated substrate of parkin) leads to Pael-R aggregation, which in turn results in endoplasmic reticulum stress and dopaminergic neuron death. This effect was alleviated by parkin overexpression as well as dopamine synthesis inhibition, implicating dopamine in such toxic aggregation process [192].

PINK1 - Mutations in *PINK1* have been identified in three large consanguineous families, two Italian and one Spanish, in which several cases of ARPD were reported [179]. PINK1 is a 581 amino acid protein expressed ubiquitously, whose transcription is activated by PTEN and whose N-terminal kinase domain targets mitochondria. *PINK1* mutations have been reported to be distributed

mostly in the region coding for this domain. In particular, the G→A amino acid substitution in exon 4 (G309D) found in the Spanish family, when overexpressed in a neuroblastoma cell line, impaired PINK1 kinase activity, its interaction with mitochondria and its import inside these organelles, resulting in the reduction of $\Delta\Psi_m$. Accordingly, overexpression of wild-type PINK1 decreased the release of cytochrome *c* from mitochondria both in control conditions and in staurosporine-induced stress [179]. However, the direct link between PINK1 and mitochondria still needs to be elucidated. Interestingly, data arising from *Drosophila* suggest that PINK1 and parkin may act in a common pathway in the pathogenesis of PD, because PINK1 loss of function mutation could be rescued by using transgenic expression of parkin [168,191]. Moreover, recent evidence in *Drosophila* and mammalian cell culture showed that parkin phosphorylation by PINK1 promotes its mitochondrial translocation, further suggesting a possible pathological mechanism for PD associating these two proteins [98].

7. Mitochondrial complex II dysfunction and Huntington's disease

Complex II or succinate dehydrogenase (succinate-Q oxidoreductase) participates not only to oxidative phosphorylation, but also to the Krebs cycle. Its experimental or accidental blockade thus results in a dramatic ATP depletion and, interestingly, in a neurodegenerative pattern and motor symptoms closely resembling those of HD. Several toxins have been discovered being able to selectively block this enzyme, and scientists took advantage of this for developing models relevant to HD. In particular, 3-nitropropionic acid (3-NP), which was identified in the 1950s in a toxic tropical flowering plant belonging to the *Fabaceae* family, but is also present in other plants and fungi. It has been suggested that 3-NP is dehydrogenated by complex II to 3-nitroacrylate, which then irreversibly inhibits the enzyme [46]. Accidental exposure to 3-NP mimics the pathophysiology of HD in humans and, accordingly, chronic 3-NP administration has long been used as an experimental HD model both in rodents and monkeys [22-25,112,137,189]. 3-NP

produces brain lesions that are mostly confined to the striatum, with selective loss of medium-sized spiny neurons and relative sparing of interneurons [10,137]. Other complex II blockers also used in research include malonic acid (MA) [82] and the more cell-permeable methylmalonic acid (MMA) [116], two synthetic dicarboxylic acids. They compete with succinate but do not react with the enzyme, thus their inhibitory effect is reversible, and chronic MA treatment produces lesions similar to those observed with 3-NP [9,82].

At functional level, rats with systemic subacute 3-NP administration develop motor symptoms resembling striatal-induced dystonia [10,92], impaired responses in the cross-maze task and perseverative behavior [145]. Motor troubles relevant to HD have been observed in tufted capuchin monkeys (*Cebus apella*), which showed spontaneous and apomorphine-induced dystonia and dyskinesia after two months of chronic 3-NP treatment [153]. Spontaneous and apomorphine-induced dystonias of foot and leg, and some dyskinesias of the hindlimb were also reported in baboons, which also showed significant impairments in a test designed to assess the functional integrity of the frontostriatal pathway in both humans and nonhuman primates [137].

The mechanisms of action underlying the selective toxicity of 3-NP, MA and MMA have been in part elucidated. The strong impairment in energy metabolism due to succinate dehydrogenase inhibition depletes ATP levels, leading to Na^+/K^+ -ATPase dysfunction and subsequent neuronal membrane depolarization, followed by de-inactivation of NMDA receptors by relieving voltage-dependent Mg^{2+} block [81,83]. In line with these findings, it has been shown that acute slice application of 3-NP or MMA on brain slices induces a potentiation of NMDA receptor-mediated synaptic excitation, resulting in a LTP of corticostriatal synaptic transmission [30], as well as other alterations of striatal synaptic plasticity [145]. Interestingly, NMDA receptor-mediated potentiation is observed in medium-sized spiny neurons but not in cholinergic interneurons [30], is promoted by NR2B containing NMDA receptors [39], is induced by the activation of the metabotropic glutamate subtype 1 (mGlu1) receptor/protein kinase C pathway as well as dopamine D2-like receptors, and is negatively regulated by D1-like receptors [85]. Accordingly, acute 3-NP

treatment modulates the expression of several genes involved in glutamatergic and dopaminergic signaling [125]. These evidences suggest that NMDA receptor-dependent mechanisms may underlie the vulnerability of striatal medium-sized spiny neurons to complex II dysfunction, and that the selective loss might be attributed to the high contents of dopamine in the striatum. Another striking difference in the reactivity to complex II blockade between striatal medium-sized spiny neurons and cholinergic interneurons is that the former respond to the toxin by a depolarization, while the latter by a hyperpolarization that might represent a neuroprotective mechanism [158]. The excitotoxic dopamine-mediated hyperactivation of NMDA receptors confined to striatal spiny neurons, combined with energy failure, could converge into a common downstream mechanism leading to the selective neuronal loss observed during HD. Such neurodegeneration seems also to involve acute inflammatory processes, as suggested by the activation of NF- κ B by *in vitro* 3-NP application [126]. Moreover, an apoptotic mechanism might also participate, as suggested by the progressive activation of p53 and JNK, followed by c-Jun activation and expression, induced in the striatum of 3-NP intoxicated rats and in cultured neurons treated with this toxin [69]. Moreover, enhanced expression of the apoptosis markers Bcl-2 and caspase-3 has also been detected in the caudate nucleus of HD patients [181], as well as in the striatum of rats intoxicated with 3-NP [182].

The 3-NP model of HD has also been successfully used to test neuroprotective strategies that might be helpful in slowing the progression of the disease. For example, it has been shown that the uncompetitive NMDA receptor antagonist memantine, which is currently used for Alzheimer's disease treatment, can reduce the irreversible loss of corticostriatal field potential caused by acute 3-NP application [169]. Another study showed that striatal *in situ* continuous delivery of CNTF (ciliary neurotrophic factor), an endogenous astrocyte activator, could reduce striatal neurodegeneration, cognitive impairment and motor symptoms in primates chronically treated with 3-NP [16]. Interestingly, CNTF-mediated neuroprotection seems to occur by improving glutamate handling [61], in particular by enhancing the function of glutamate transporters (unpublished

observation), further suggesting a role of glutamate-mediated toxicity in the progressive loss of striatal medium-sized spiny neurons in HD.

8. Mutant huntingtin, mitochondria and Huntington's disease

There is increasing evidence suggesting that mitochondrial dysfunction characterizing HD is associated to the expression of mHtt [20]. For example, it has been shown that mitochondrial impairment occurs even in asymptomatic carriers expressing mHtt with 41-47 CAG repeats [156], and mitochondria isolated from early HD patients and YAC72 transgenic mice have reduced Ca^{2+} buffering capacity [138]. Furthermore, culture cells expressing mHtt showed impairment of mitochondrial respiration and ATP synthesis [122]. But which is the molecular link between mHtt and mitochondria leading to the dysfunction of these organelles? One candidate is p53: mHtt has been shown to bind to p53, up-regulating its transcriptional activity and increasing its levels in PC12 cells, primary neuronal cultures, mHtt-Tg mice and *post-mortem* brains of HD patients [6]. This, in turn, increases the expression of PUMA (p53 up-regulated modulator of apoptosis) and BAX (Bcl-2-associated X protein), two pro-apoptotic members of the Bcl-2 family, leading to mitochondrial damage and cell death [6]. Another possible molecular link between mHtt and mitochondrial dysfunction is PGC-1 α [peroxisome proliferator-activated receptor (PPAR)- γ coactivator 1 α]. PGC-1 α is a key element that co-regulates the transcription of some cellular programs modulating mitochondrial respiration, oxidative stress defense and adaptive thermogenesis. In particular, PGC-1 α binds to nuclear transcription factors regulating nuclear-encoded mitochondrial genes such as cytochrome *c*, mitochondrial transcription factor A and complex I-V components [150]. At molecular level, mHtt interferes with the PGC-1 α promoter leading to reduced PGC-1 α transcription [49]. Interestingly, PGC-1 α knock-out mice showed microvacuolation of neuropil and neurons in the striatum, as well as an abnormal energy metabolic phenotype [106,110]. Furthermore, PGC-1 α is a potent suppressor of ROS production, suggesting

that reduced expression or loss of this transcriptional co-regulator might sensitize neurons to oxidative stress [20]. Accordingly, PGC-1 α is decreased in striatal medium-sized spiny neurons, but not in interneurons, of R6/2 HD mice, suggesting a possible mechanism for the selective neuronal loss characterizing HD [49]. Finally, beside these indirect molecular links, mHtt binds directly to the mitochondrial outer membrane, forming large clusters that can impair mitochondrial function, change their morphology and interfere with their intracellular movement [20,41].

9. Conclusions

The experimental use of selective mitochondrial toxins allowed the development of animal models of neurodegenerative diseases involving the BG. Uncoupling complex I or II leads to physiological, behavioral and anatomopathological alterations in rodents and primates that mimic those observed, respectively, in PD and HD. The major contribute of these experimental models, however, is that they provided precious insights to understand the mechanisms underlying PD and HD onset and progression. In particular, elucidation of the events occurring at the cellular level leading to neuronal death offered us several clues on possible new treatments for PD and HD targeting specific molecules that participate to the pathogenic cascade triggered by mitochondrial dysfunction.

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ACCEPTED MANUSCRIPT

Reference List

1. P.M. Abou-Sleiman, M.M. Muqit, N.W. Wood, Expanding insights of mitochondrial dysfunction in Parkinson's disease, *Nat. Rev. Neurosci.* 7 (2006) 207-219.
2. R.L. Albin, A.B. Young, J.B. Penney, The functional anatomy of basal ganglia disorders, *Trends Neurosci.* 12 (1989) 366-375.
3. M. Amalric, G.F. Koob, Depletion of dopamine in the caudate nucleus but not in nucleus accumbens impairs reaction-time performance in rats, *J Neurosci.* 7 (1987) 2129-2134.
4. J. Arnt, J. Hyttel, Differential involvement of dopamine D-1 and D-2 receptors in the circling behaviour induced by apomorphine, SK & F 38393, pergolide and LY 171555 in 6-hydroxydopamine-lesioned rats, *Psychopharmacology (Berl)* 85 (1985) 346-352.
5. A.C. Bachoud-Levi, V. Gaura, P. Brugieres, J.P. Lefaucheur, M.F. Boisse, P. Maison, S. Baudic, M.J. Ribeiro, C. Bourdet, P. Remy, P. Cesaro, P. Hantraye, M. Peschanski, Effect of fetal neural transplants in patients with Huntington's disease 6 years after surgery: a long-term follow-up study, *Lancet Neurol* 5 (2006) 303-309.
6. B.I. Bae, H. Xu, S. Igarashi, M. Fujimuro, N. Agrawal, Y. Taya, S.D. Hayward, T.H. Moran, C. Montell, C.A. Ross, S.H. Snyder, A. Sawa, p53 mediates cellular dysfunction and behavioral abnormalities in Huntington's disease, *Neuron* 47 (2005) 29-41.
7. P.A. Ballard, J.W. Tetrad, J.W. Langston, Permanent human parkinsonism due to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): seven cases, *Neurology* 35 (1985) 949-956.
8. M.F. Beal, Mitochondria take center stage in aging and neurodegeneration, *Ann. Neurol* 58 (2005) 495-505.
9. M.F. Beal, E. Brouillet, B. Jenkins, R. Henshaw, B. Rosen, B.T. Hyman, Age-dependent striatal excitotoxic lesions produced by the endogenous mitochondrial inhibitor malonate, *J Neurochem.* 61 (1993) 1147-1150.
10. M.F. Beal, E. Brouillet, B.G. Jenkins, R.J. Ferrante, N.W. Kowall, J.M. Miller, E. Storey, R. Srivastava, B.R. Rosen, B.T. Hyman, Neurochemical and histologic characterization of striatal excitotoxic lesions produced by the mitochondrial toxin 3-nitropropionic acid, *J Neurosci.* 13 (1993) 4181-4192.
11. V. Belcastro, A. Tozzi, M. Tantucci, C. Costa, F.M. Di, A. Autuori, B. Picconi, S. Siliquini, E. Luchetti, F. Borsini, P. Calabresi, A2A adenosine receptor antagonists protect the striatum against rotenone-induced neurotoxicity, *Exp. Neurol* 217 (2009) 231-234.
12. H. Bergman, T. Wichmann, B. Karmon, M.R. DeLong, The primate subthalamic nucleus. II. Neuronal activity in the MPTP model of parkinsonism, *J. Neurophysiol.* 72 (1994) 507-520.
13. L. Berliocchi, D. Bano, P. Nicotera, Ca²⁺ signals and death programmes in neurons, *Philos. Trans. R. Soc Lond B Biol Sci.* 360 (2005) 2255-2258.
14. R. Betarbet, T.B. Sherer, G. MacKenzie, M. Garcia-Osuna, A.V. Panov, J.T. Greenamyre, Chronic systemic pesticide exposure reproduces features of Parkinson's disease, *Nat. Neurosci.* 3 (2000) 1301-1306.

15. E. Bezard, T. Boraud, B. Bioulac, C.E. Gross, Involvement of the subthalamic nucleus in glutamatergic compensatory mechanisms, *Eur. J. Neurosci.* 11 (1999) 2167-2170.
16. J. Bloch, A.C. Bachoud-Levi, N. Deglon, J.P. Lefaucheur, L. Winkel, S. Palfi, J.P. Nguyen, C. Bourdet, V. Gaura, P. Remy, P. Brugieres, M.F. Boisse, S. Baudic, P. Cesaro, P. Hantraye, P. Aebischer, M. Peschanski, Neuroprotective gene therapy for Huntington's disease, using polymer-encapsulated cells engineered to secrete human ciliary neurotrophic factor: results of a phase I study, *Hum. Gene Ther.* 15 (2004) 968-975.
17. D. Blum, S. Torch, N. Lambeng, M. Nissou, A.L. Benabid, R. Sadoul, J.M. Verna, Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: contribution to the apoptotic theory in Parkinson's disease, *Prog Neurobiol.* 65 (2001) 135-172.
18. R.M. Bonelli, G.K. Wenning, Pharmacological management of Huntington's disease: an evidence-based review, *Curr. Pharm. Des* 12 (2006) 2701-2720.
19. P. Bonsi, P. Calabresi, P.C. De, M. Papa, D. Centonze, G. Bernardi, A. Pisani, Early ionic and membrane potential changes caused by the pesticide rotenone in striatal cholinergic interneurons, *Exp. Neurol* 185 (2004) 169-181.
20. E. Bossy-Wetzel, A. Petrilli, A.B. Knott, Mutant huntingtin and mitochondrial dysfunction, *Trends Neurosci.* 31 (2008) 609-616.
21. P.D. Boyer, The ATP synthase--a splendid molecular machine, *Annu. Rev. Biochem.* 66 (1997) 717-749.
22. E. Brouillet, F. Conde, M.F. Beal, P. Hantraye, Replicating Huntington's disease phenotype in experimental animals, *Prog. Neurobiol.* 59 (1999) 427-468.
23. E. Brouillet, P. Hantraye, Effects of chronic MPTP and 3-nitropropionic acid in nonhuman primates, *Curr. Opin. Neurol* 8 (1995) 469-473.
24. E. Brouillet, P. Hantraye, R.J. Ferrante, R. Dolan, A. Leroy-Willig, N.W. Kowall, M.F. Beal, Chronic mitochondrial energy impairment produces selective striatal degeneration and abnormal choreiform movements in primates, *Proc. Natl. Acad. Sci. U. S. A* 92 (1995) 7105-7109.
25. E. Brouillet, C. Jacquard, N. Bizat, D. Blum, 3-Nitropropionic acid: a mitochondrial toxin to uncover physiopathological mechanisms underlying striatal degeneration in Huntington's disease, *J Neurochem.* 95 (2005) 1521-1540.
26. S.E. Browne, M.F. Beal, The energetics of Huntington's disease, *Neurochem. Res.* 29 (2004) 531-546.
27. P. Caboni, T.B. Sherer, N. Zhang, G. Taylor, H.M. Na, J.T. Greenamyre, J.E. Casida, Rotenone, deguelin, their metabolites, and the rat model of Parkinson's disease, *Chem. Res. Toxicol.* 17 (2004) 1540-1548.
28. P. Calabresi, D. Centonze, G. Bernardi, Electrophysiology of dopamine in normal and denervated striatal neurons, *Trends Neurosci.* 23 (2000) S57-S63.
29. P. Calabresi, D. Centonze, G. Bernardi, Electrophysiology of dopamine in normal and denervated striatal neurons, *Trends Neurosci.* 23 (2000) S57-S63.

30. P. Calabresi, P. Gubellini, B. Picconi, D. Centonze, A. Pisani, P. Bonsi, P. Greengard, R.A. Hipskind, E. Borrelli, G. Bernardi, Inhibition of mitochondrial complex II induces a long-term potentiation of NMDA-mediated synaptic excitation in the striatum requiring endogenous dopamine, *J. Neurosci.* 21 (2001) 5110-5120.
31. P. Calabresi, N.B. Mercuri, G. Sancesario, G. Bernardi, Electrophysiology of dopamine-denervated striatal neurons. Implications for Parkinson's disease, *Brain* 116 (Pt 2) (1993) 433-452.
32. P. Calabresi, M. mmassari-Teule, P. Gubellini, G. Sancesario, M. Morello, D. Centonze, G.A. Marfia, E. Saulle, E. Passino, B. Picconi, G. Bernardi, A synaptic mechanism underlying the behavioral abnormalities induced by manganese intoxication, *Neurobiol. Dis.* 8 (2001) 419-432.
33. M.W. Calhoun, J.W. Thomas, R.B. Gennis, The cytochrome oxidase superfamily of redox-driven proton pumps, *Trends Biochem. Sci.* 19 (1994) 325-330.
34. D.L. Carbone, K.A. Popichak, J.A. Moreno, S. Safe, R.B. Tjalkens, Suppression of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced nitric-oxide synthase 2 expression in astrocytes by a novel diindolylmethane analog protects striatal neurons against apoptosis, *Mol. Pharmacol.* 75 (2009) 35-43.
35. M. Carlsson, A. Carlsson, Interactions between glutamatergic and monoaminergic systems within the basal ganglia--implications for schizophrenia and Parkinson's disease, *Trends Neurosci.* 13 (1990) 272-276.
36. G. Cecchini, Function and structure of complex II of the respiratory chain, *Annu. Rev. Biochem.* 72 (2003) 77-109.
37. M.A. Cenci, I.Q. Wishaw, T. Schallert, Animal models of neurological deficits: how relevant is the rat?, *Nat. Rev. Neurosci.* 3 (2002) 574-579.
38. D. Centonze, P. Gubellini, G. Bernardi, P. Calabresi, Impaired excitatory transmission in the striatum of rats chronically intoxicated with manganese, *Exp. Neurol.* 172 (2001) 469-476.
39. D. Centonze, C. Prosperetti, I. Barone, S. Rossi, B. Picconi, A. Tscherter, C. De, V, G. Bernardi, P. Calabresi, NR2B-containing NMDA receptors promote the neurotoxic effects of 3-nitropropionic acid but not of rotenone in the striatum, *Exp. Neurol* 202 (2006) 470-479.
40. D.C. Chan, Mitochondria: dynamic organelles in disease, aging, and development, *Cell* 125 (2006) 1241-1252.
41. D.T. Chang, G.L. Rintoul, S. Pandipati, I.J. Reynolds, Mutant huntingtin aggregates impair mitochondrial movement and trafficking in cortical neurons, *Neurobiol. Dis.* 22 (2006) 388-400.
42. J.Y. Chen, G.C. Tsao, Q. Zhao, W. Zheng, Differential cytotoxicity of Mn(II) and Mn(III): special reference to mitochondrial [Fe-S] containing enzymes, *Toxicol. Appl. Pharmacol.* 175 (2001) 160-168.
43. D.W. Choi, Excitotoxic cell death, *J Neurobiol.* 23 (1992) 1261-1276.

44. C.W. Christine, M.J. Aminoff, Clinical differentiation of parkinsonian syndromes: prognostic and therapeutic relevance, *Am. J Med.* 117 (2004) 412-419.
45. H.M. Cocheme, M.P. Murphy, Complex I is the major site of mitochondrial superoxide production by paraquat, *J Biol Chem.* 283 (2008) 1786-1798.
46. C.J. Coles, D.E. Edmondson, T.P. Singer, Inactivation of succinate dehydrogenase by 3-nitropropionate, *J Biol Chem.* 254 (1979) 5161-5167.
47. C. Costa, V. Belcastro, A. Tozzi, F.M. Di, M. Tantucci, S. Siliquini, A. Autuori, B. Picconi, M.G. Spillantini, E. Fedele, A. Pittaluga, M. Raiteri, P. Calabresi, Electrophysiology and pharmacology of striatal neuronal dysfunction induced by mitochondrial complex I inhibition, *J Neurosci.* 28 (2008) 8040-8052.
48. A.R. Crofts, The cytochrome bc1 complex: function in the context of structure, *Annu. Rev. Physiol* 66 (2004) 689-733.
49. L. Cui, H. Jeong, F. Borovecki, C.N. Parkhurst, N. Tanese, D. Krainc, Transcriptional repression of PGC-1alpha by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration, *Cell* 127 (2006) 59-69.
50. N.C. Danbolt, Glutamate uptake, *Prog. Neurobiol.* 65 (2001) 1-105.
51. F. Darios, O. Corti, C.B. Lucking, C. Hampe, M.P. Muriel, N. Abbas, W.J. Gu, E.C. Hirsch, T. Rooney, M. Ruberg, A. Brice, Parkin prevents mitochondrial swelling and cytochrome c release in mitochondria-dependent cell death, *Hum. Mol. Genet.* 12 (2003) 517-526.
52. T.M. Dawson, V.L. Dawson, Molecular pathways of neurodegeneration in Parkinson's disease, *Science* 302 (2003) 819-822.
53. T.M. Dawson, V.L. Dawson, Rare genetic mutations shed light on the pathogenesis of Parkinson disease, *J Clin. Invest* 111 (2003) 145-151.
54. M.C. de Rijk, C. Tzourio, M.M. Breteler, J.F. Dartigues, L. Amaducci, S. Lopez-Pousa, J.M. Manubens-Bertran, A. Alperovitch, W.A. Rocca, Prevalence of parkinsonism and Parkinson's disease in Europe: the EUROPARKINSON Collaborative Study. European Community Concerted Action on the Epidemiology of Parkinson's disease, *J Neurol Neurosurg. Psychiatry* 62 (1997) 10-15.
55. M.R. DeLong, Primate models of movement disorders of basal ganglia origin, *Trends Neurosci.* 13 (1990) 281-285.
56. M.R. DeLong, T. Wichmann, Circuits and circuit disorders of the basal ganglia, *Arch Neurol* 64 (2007) 20-24.
57. R.J. Dinis-Oliveira, F. Remiao, H. Carmo, J.A. Duarte, A.S. Navarro, M.L. Bastos, F. Carvalho, Paraquat exposure as an etiological factor of Parkinson's disease, *Neurotoxicology* 27 (2006) 1110-1122.
58. A.W. Dobson, K.M. Erikson, M. Aschner, Manganese neurotoxicity, *Ann. N. Y. Acad. Sci.* 1012 (2004) 115-128.

59. W. Duan, X. Zhu, B. Ladenheim, Q.S. Yu, Z. Guo, J. Oyler, R.G. Cutler, J.L. Cadet, N.H. Greig, M.P. Mattson, p53 inhibitors preserve dopamine neurons and motor function in experimental parkinsonism, *Ann. Neurol* 52 (2002) 597-606.
60. Y. Duan, R.A. Gross, S.S. Sheu, Ca²⁺-dependent generation of mitochondrial reactive oxygen species serves as a signal for poly(ADP-ribose) polymerase-1 activation during glutamate excitotoxicity, *J Physiol* 585 (2007) 741-758.
61. C. Escartin, E. Brouillet, P. Gubellini, Y. Trioulier, C. Jacquard, C. Smadja, G.W. Knott, G.L. Kerkerian-Le, N. Deglon, P. Hantraye, G. Bonvento, Ciliary neurotrophic factor activates astrocytes, redistributes their glutamate transporters GLAST and GLT-1 to raft microdomains, and improves glutamate handling in vivo, *J. Neurosci.* 26 (2006) 5978-5989.
62. M.M. Fan, L.A. Raymond, N-methyl-D-aspartate (NMDA) receptor function and excitotoxicity in Huntington's disease, *Prog. Neurobiol.* 81 (2007) 272-293.
63. R.J. Ferrante, N.W. Kowall, M.F. Beal, E.P. Richardson, Jr., E.D. Bird, J.B. Martin, Selective sparing of a class of striatal neurons in Huntington's disease, *Science* 230 (1985) 561-563.
64. S.W. Fesik, Y. Shi, Structural biology. Controlling the caspases, *Science* 294 (2001) 1477-1478.
65. T. Finkel, N.J. Holbrook, Oxidants, oxidative stress and the biology of ageing, *Nature* 408 (2000) 239-247.
66. F. Fornai, O.M. Schluter, P. Lenzi, M. Gesi, R. Ruffoli, M. Ferrucci, G. Lazzeri, C.L. Busceti, F. Pontarelli, G. Battaglia, A. Pellegrini, F. Nicoletti, S. Ruggieri, A. Paparelli, T.C. Sudhof, Parkinson-like syndrome induced by continuous MPTP infusion: convergent roles of the ubiquitin-proteasome system and alpha-synuclein, *Proc. Natl. Acad. Sci. U. S. A* 102 (2005) 3413-3418.
67. L.S. Forno, L.E. DeLanney, I. Irwin, J.W. Langston, Similarities and differences between MPTP-induced parkinsonism and Parkinson's disease. Neuropathologic considerations, *Adv. Neurol* 60 (1993) 600-608.
68. P. Galvani, P. Fumagalli, A. Santagostino, Vulnerability of mitochondrial complex I in PC12 cells exposed to manganese, *Eur. J Pharmacol.* 293 (1995) 377-383.
69. M. Garcia, P. Vanhoutte, C. Pages, M.J. Besson, E. Brouillet, J. Caboche, The mitochondrial toxin 3-nitropropionic acid induces striatal neurodegeneration via a c-Jun N-terminal kinase/c-Jun module, *J Neurosci.* 22 (2002) 2174-2184.
70. J. Gauntlett-Gilbert, V.J. Brown, Reaction time deficits and Parkinson's disease, *Neurosci. Biobehav. Rev.* 22 (1998) 865-881.
71. C.E. Gavin, K.K. Gunter, T.E. Gunter, Manganese and calcium transport in mitochondria: implications for manganese toxicity, *Neurotoxicology* 20 (1999) 445-453.
72. Y. Glinka, K.F. Tipton, M.B. Youdim, Mechanism of inhibition of mitochondrial respiratory complex I by 6-hydroxydopamine and its prevention by desferrioxamine, *Eur. J Pharmacol.* 351 (1998) 121-129.

73. M.S. Goldberg, S.M. Fleming, J.J. Palacino, C. Cepeda, H.A. Lam, A. Bhatnagar, E.G. Meloni, N. Wu, L.C. Ackerson, G.J. Klapstein, M. Gajendiran, B.L. Roth, M.F. Chesselet, N.T. Maidment, M.S. Levine, J. Shen, Parkin-deficient mice exhibit nigrostriatal deficits but not loss of dopaminergic neurons, *J Biol Chem.* 278 (2003) 43628-43635.
74. A.M. Graybiel, T. Aosaki, A.W. Flaherty, M. Kimura, The basal ganglia and adaptive motor control, *Science* 265 (1994) 1826-1831.
75. D.R. Green, Apoptotic pathways: the roads to ruin, *Cell* 94 (1998) 695-698.
76. D.R. Green, G. Kroemer, The pathophysiology of mitochondrial cell death, *Science* 305 (2004) 626-629.
77. D.R. Green, J.C. Reed, Mitochondria and apoptosis, *Science* 281 (1998) 1309-1312.
78. J.T. Greenamyre, R. Betarbet, T.B. Sherer, The rotenone model of Parkinson's disease: genes, environment and mitochondria, *Parkinsonism. Relat Disord.* 9 Suppl 2 (2003) S59-S64.
79. J.T. Greenamyre, C.F. O'Brien, N-methyl-D-aspartate antagonists in the treatment of Parkinson's disease, *Arch. Neurol.* 48 (1991) 977-981.
80. J.C. Greene, A.J. Whitworth, I. Kuo, L.A. Andrews, M.B. Feany, L.J. Pallanck, Mitochondrial pathology and apoptotic muscle degeneration in *Drosophila parkin* mutants, *Proc. Natl. Acad. Sci. U. S. A* 100 (2003) 4078-4083.
81. J.G. Greene, J.T. Greenamyre, Bioenergetics and glutamate excitotoxicity., *Prog Neurobiol.* 48 (1996) 613-634.
82. J.G. Greene, R.H. Porter, R.V. Eller, J.T. Greenamyre, Inhibition of succinate dehydrogenase by malonic acid produces an "excitotoxic" lesion in rat striatum, *J Neurochem.* 61 (1993) 1151-1154.
83. J.G. Greene, S.S. Sheu, R.A. Gross, J.T. Greenamyre, 3-Nitropropionic acid exacerbates N-methyl-D-aspartate toxicity in striatal culture by multiple mechanisms, *Neuroscience* 84 (1998) 503-510.
84. M. Gu, M.T. Gash, V.M. Mann, F. Javoy-Agid, J.M. Cooper, A.H. Schapira, Mitochondrial defect in Huntington's disease caudate nucleus, *Ann. Neurol* 39 (1996) 385-389.
85. P. Gubellini, D. Centonze, D. Tropepi, G. Bernardi, P. Calabresi, Induction of corticostriatal LTP by 3-nitropropionic acid requires the activation of mGluR1/PKC pathway, *Neuropharmacology* 46 (2004) 761-769.
86. P. Gubellini, A. Eusebio, A. Oueslati, C. Melon, G.L. Kerkerian-Le, P. Salin, Chronic high-frequency stimulation of the subthalamic nucleus and L-DOPA treatment in experimental parkinsonism: effects on motor behaviour and striatal glutamate transmission, *Eur. J. Neurosci.* 24 (2006) 1802-1814.
87. P. Gubellini, B. Picconi, M. Bari, N. Battista, P. Calabresi, D. Centonze, G. Bernardi, A. Finazzi-Agro, M. Maccarrone, Experimental parkinsonism alters endocannabinoid degradation: implications for striatal glutamatergic transmission, *J. Neurosci.* 22 (2002) 6900-6907.

88. P. Gubellini, A. Pisani, D. Centonze, G. Bernardi, P. Calabresi, Metabotropic glutamate receptors and striatal synaptic plasticity: implications for neurological diseases, *Prog. Neurobiol.* 74 (2004) 271-300.
89. P. Gubellini, P. Salin, L. Kerkerian-Le Goff, C. Baunez, Deep brain stimulation in neurological diseases and experimental models: From molecule to complex behavior, *Prog Neurobiol.* (2009).
90. T.E. Gunter, K.K. Gunter, Uptake of calcium by mitochondria: transport and possible function, *IUBMB. Life* 52 (2001) 197-204.
91. D. HaMai, S.C. Bondy, Oxidative basis of manganese neurotoxicity, *Ann. N. Y. Acad. Sci.* 1012 (2004) 129-141.
92. B.F. Hamilton, D.H. Gould, Nature and distribution of brain lesions in rats intoxicated with 3-nitropropionic acid: a type of hypoxic (energy deficient) brain damage, *Acta Neuropathol.* 72 (1987) 286-297.
93. D. Han, E. Williams, E. Cadenas, Mitochondrial respiratory chain-dependent generation of superoxide anion and its release into the intermembrane space, *Biochem. J* 353 (2001) 411-416.
94. P.S. Harper, The epidemiology of Huntington's disease, *Hum. Genet.* 89 (1992) 365-376.
95. E.C. Hirsch, C. Perier, G. Orieux, C. Francois, J. Feger, J. Yelnik, M. Vila, R. Levy, E.S. Tolosa, C. Marin, H.M. Trinidad, J.A. Obeso, Y. Agid, Metabolic effects of nigrostriatal denervation in basal ganglia, *Trends Neurosci.* 23 (2000) S78-S85.
96. J.M. Itier, P. Ibanez, M.A. Mena, N. Abbas, C. Cohen-Salmon, G.A. Bohme, M. Laville, J. Pratt, O. Corti, L. Pradier, G. Ret, C. Joubert, M. Periquet, F. Araujo, J. Negroni, M.J. Casarejos, S. Canals, R. Solano, A. Serrano, E. Gallego, M. Sanchez, P. Deneffe, J. Benavides, G. Tremp, T.A. Rooney, A. Brice, Y.J. Garcia de, Parkin gene inactivation alters behaviour and dopamine neurotransmission in the mouse, *Hum. Mol. Genet.* 12 (2003) 2277-2291.
97. P. Jenner, Functional models of Parkinson's disease: a valuable tool in the development of novel therapies, *Ann. Neurol* 64 Suppl 2 (2008) S16-S29.
98. Y. Kim, J. Park, S. Kim, S. Song, S.K. Kwon, S.H. Lee, T. Kitada, J.M. Kim, J. Chung, PINK1 controls mitochondrial localization of Parkin through direct phosphorylation, *Biochem. Biophys. Res. Commun.* 377 (2008) 975-980.
99. K. Kitahama, T. Maeda, R.M. Denney, M. Jouvett, Monoamine oxidase: distribution in the cat brain studied by enzyme- and immunohistochemistry: recent progress, *Prog Neurobiol.* 42 (1994) 53-78.
100. A.E. Lang, A.M. Lozano, Parkinson's disease. First of two parts, *N. Engl. J Med.* 339 (1998) 1044-1053.
101. A.E. Lang, A.M. Lozano, Parkinson's disease. Second of two parts, *N. Engl. J Med.* 339 (1998) 1130-1143.
102. J.W. Langston, P. Ballard, J.W. Tetrud, I. Irwin, Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis, *Science* 219 (1983) 979-980.

103. M.J. LaVoie, B.L. Ostaszewski, A. Weihofen, M.G. Schlossmacher, D.J. Selkoe, Dopamine covalently modifies and functionally inactivates parkin, *Nat. Med.* 11 (2005) 1214-1221.
104. C.Y. Lee, C.H. Lee, C.C. Shih, H.H. Liou, Paraquat inhibits postsynaptic AMPA receptors on dopaminergic neurons in the substantia nigra pars compacta, *Biochem. Pharmacol.* 76 (2008) 1155-1164.
105. G. Lenaz, R. Fato, M.L. Genova, C. Bergamini, C. Bianchi, A. Biondi, Mitochondrial Complex I: structural and functional aspects, *Biochim. Biophys. Acta* 1757 (2006) 1406-1420.
106. T.C. Leone, J.J. Lehman, B.N. Finck, P.J. Schaeffer, A.R. Wende, S. Boudina, M. Courtois, D.F. Wozniak, N. Sambandam, C. Bernal-Mizrachi, Z. Chen, J.O. Holloszy, D.M. Medeiros, R.E. Schmidt, J.E. Saffitz, E.D. Abel, C.F. Semenkovich, D.P. Kelly, PGC-1alpha deficiency causes multi-system energy metabolic derangements: muscle dysfunction, abnormal weight control and hepatic steatosis, *PLoS Biol* 3 (2005) e101.
107. R. Levy, L.N. Hazrati, M.T. Herrero, M. Vila, O.K. Hassani, M. Mouroux, M. Ruberg, H. Asensi, Y. Agid, J. Feger, J.A. Obeso, A. Parent, E.C. Hirsch, Re-evaluation of the functional anatomy of the basal ganglia in normal and Parkinsonian states, *Neuroscience* 76 (1997) 335-343.
108. P. Li, D. Nijhawan, I. Budihardjo, S.M. Srinivasula, M. Ahmad, E.S. Alnemri, X. Wang, Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade, *Cell* 91 (1997) 479-489.
109. G.T. Liberatore, V. Jackson-Lewis, S. Vukosavic, A.S. Mandir, M. Vila, W.G. McAuliffe, V.L. Dawson, T.M. Dawson, S. Przedborski, Inducible nitric oxide synthase stimulates dopaminergic neurodegeneration in the MPTP model of Parkinson disease, *Nat. Med.* 5 (1999) 1403-1409.
110. J. Lin, P.H. Wu, P.T. Tarr, K.S. Lindenberg, J. St-Pierre, C.Y. Zhang, V.K. Mootha, S. Jager, C.R. Vianna, R.M. Reznick, L. Cui, M. Manieri, M.X. Donovan, Z. Wu, M.P. Cooper, M.C. Fan, L.M. Rohas, A.M. Zavacki, S. Cinti, G.I. Shulman, B.B. Lowell, D. Krainc, B.M. Spiegelman, Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1alpha null mice, *Cell* 119 (2004) 121-135.
111. S.A. Lipton, P.A. Rosenberg, Excitatory amino acids as a final common pathway for neurologic disorders, *N. Engl. J Med.* 330 (1994) 613-622.
112. A.C. Ludolph, F. He, P.S. Spencer, J. Hammerstad, M. Sabri, 3-Nitropropionic acid-exogenous animal neurotoxin and possible human striatal toxin, *Can. J Neurol Sci.* 18 (1991) 492-498.
113. M. Maccarrone, P. Gubellini, M. Bari, B. Picconi, N. Battista, D. Centonze, G. Bernardi, A. Finazzi-Agro, P. Calabresi, Levodopa treatment reverses endocannabinoid system abnormalities in experimental parkinsonism, *J. Neurochem.* 85 (2003) 1018-1025.
114. M. MacDonald, C.M. Ambrose, M.P. Duyao, et al., A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes, *Cell* 72 (1993) 971-983.
115. H.M. McBride, M. Neuspiel, S. Wasiak, Mitochondria: more than just a powerhouse, *Curr. Biol* 16 (2006) R551-R560.

116. B.A. McLaughlin, D. Nelson, I.A. Silver, M. Erecinska, M.F. Chesselet, Methylmalonate toxicity in primary neuronal cultures, *Neuroscience* 86 (1998) 279-290.
117. W. Meissner, A. Leblois, D. Hansel, B. Bioulac, C.E. Gross, A. Benazzouz, T. Boraud, Subthalamic high frequency stimulation resets subthalamic firing and reduces abnormal oscillations, *Brain* 128 (2005) 2372-2382.
118. G.E. Meredith, U.J. Kang, Behavioral models of Parkinson's disease in rodents: a new look at an old problem, *Mov Disord.* 21 (2006) 1595-1606.
119. G.E. Meredith, S. Totterdell, E. Petroske, C.K. Santa, R.C. Callison, Jr., Y.S. Lau, Lysosomal malfunction accompanies alpha-synuclein aggregation in a progressive mouse model of Parkinson's disease, *Brain Res.* 956 (2002) 156-165.
120. G.E. Meredith, S. Totterdell, J.A. Potashkin, D.J. Surmeier, Modeling PD pathogenesis in mice: advantages of a chronic MPTP protocol, *Parkinsonism. Relat Disord.* 14 Suppl 2 (2008) S112-S115.
121. E.K. Michaelis, Molecular biology of glutamate receptors in the central nervous system and their role in excitotoxicity, oxidative stress and aging, *Prog. Neurobiol.* 54 (1998) 369-415.
122. T. Milakovic, G.V. Johnson, Mitochondrial respiration and ATP production are significantly impaired in striatal cells expressing mutant huntingtin, *J Biol Chem.* 280 (2005) 30773-30782.
123. P. Mitchell, J. Moyle, Chemiosmotic hypothesis of oxidative phosphorylation, *Nature* 213 (1967) 137-139.
124. R. Moratalla, B. Quinn, L.E. DeLanney, I. Irwin, J.W. Langston, A.M. Graybiel, Differential vulnerability of primate caudate-putamen and striosome-matrix dopamine systems to the neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, *Proc. Natl. Acad. Sci. U. S. A* 89 (1992) 3859-3863.
125. M. Napolitano, D. Centonze, P. Gubellini, S. Rossi, S. Spiezia, G. Bernardi, A. Gulino, P. Calabresi, Inhibition of mitochondrial complex II alters striatal expression of genes involved in glutamatergic and dopaminergic signaling: possible implications for Huntington's disease, *Neurobiol. Dis.* 15 (2004) 407-414.
126. M. Napolitano, D. Zei, D. Centonze, R. Palermo, G. Bernardi, A. Vacca, P. Calabresi, A. Gulino, NF- κ B/NOS cross-talk induced by mitochondrial complex II inhibition: implications for Huntington's disease, *Neurosci. Lett.* 434 (2008) 241-246.
127. D. Nicholls, K. Akerman, Mitochondrial calcium transport, *Biochim. Biophys. Acta* 683 (1982) 57-88.
128. D.G. Nicholls, S.L. Budd, Mitochondria and neuronal glutamate excitotoxicity, *Biochim. Biophys. Acta* 1366 (1998) 97-112.
129. D.G. Nicholls, S.L. Budd, Mitochondria and neuronal survival, *Physiol Rev.* 80 (2000) 315-360.
130. D.G. Nicholls, M.W. Ward, Mitochondrial membrane potential and neuronal glutamate excitotoxicity: mortality and millivolts, *Trends Neurosci.* 23 (2000) 166-174.

131. W.J. Nicklas, I. Vyas, R.E. Heikkila, Inhibition of NADH-linked oxidation in brain mitochondria by 1-methyl-4-phenyl-pyridine, a metabolite of the neurotoxin, 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine, *Life Sci.* 36 (1985) 2503-2508.
132. M.D. Norenberg, K.V. Rama Rao, The mitochondrial permeability transition in neurologic disease, *Neurochem. Int.* 50 (2007) 983-997.
133. C.W. Olanow, The pathogenesis of cell death in Parkinson's disease--2007, *Mov Disord.* 22 Suppl 17 (2007) S335-S342.
134. K. Ossowska, M. Smialowska, K. Kuter, J. Wieronska, B. Zieba, J. Wardas, P. Nowak, J. Dabrowska, A. Bortel, I. Biedka, G. Schulze, H. Rommelspacher, Degeneration of dopaminergic mesocortical neurons and activation of compensatory processes induced by a long-term paraquat administration in rats: implications for Parkinson's disease, *Neuroscience* 141 (2006) 2155-2165.
135. K. Ossowska, J. Wardas, M. Smialowska, K. Kuter, T. Lenda, J.M. Wieronska, B. Zieba, P. Nowak, J. Dabrowska, A. Bortel, A. Kwiecinski, S. Wolfarth, A slowly developing dysfunction of dopaminergic nigrostriatal neurons induced by long-term paraquat administration in rats: an animal model of preclinical stages of Parkinson's disease?, *Eur. J Neurosci.* 22 (2005) 1294-1304.
136. J.J. Palacino, D. Sagi, M.S. Goldberg, S. Krauss, C. Motz, M. Wacker, J. Klose, J. Shen, Mitochondrial dysfunction and oxidative damage in parkin-deficient mice, *J Biol Chem.* 279 (2004) 18614-18622.
137. S. Palfi, R.J. Ferrante, E. Brouillet, M.F. Beal, R. Dolan, M.C. Guyot, M. Peschanski, P. Hantraye, Chronic 3-nitropropionic acid treatment in baboons replicates the cognitive and motor deficits of Huntington's disease, *J Neurosci.* 16 (1996) 3019-3025.
138. A.V. Panov, C.A. Gutekunst, B.R. Leavitt, M.R. Hayden, J.R. Burke, W.J. Strittmatter, J.T. Greenamyre, Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines, *Nat. Neurosci.* 5 (2002) 731-736.
139. J. Peng, X.O. Mao, F.F. Stevenson, M. Hsu, J.K. Andersen, The herbicide paraquat induces dopaminergic nigral apoptosis through sustained activation of the JNK pathway, *J Biol Chem.* 279 (2004) 32626-32632.
140. J. Peng, F.F. Stevenson, S.R. Doctrow, J.K. Andersen, Superoxide dismutase/catalase mimetics are neuroprotective against selective paraquat-mediated dopaminergic neuron death in the substantia nigra: implications for Parkinson disease, *J Biol Chem.* 280 (2005) 29194-29198.
141. F.A. Perez, R.D. Palmiter, Parkin-deficient mice are not a robust model of parkinsonism, *Proc. Natl. Acad. Sci. U. S. A.* 102 (2005) 2174-2179.
142. B. Picconi, D. Centonze, K. Hakansson, G. Bernardi, P. Greengard, G. Fisone, M.A. Cenci, P. Calabresi, Loss of bidirectional striatal synaptic plasticity in L-DOPA-induced dyskinesia, *Nat. Neurosci.* 6 (2003) 501-506.
143. B. Picconi, D. Centonze, K. Hakansson, G. Bernardi, P. Greengard, G. Fisone, M.A. Cenci, P. Calabresi, Loss of bidirectional striatal synaptic plasticity in L-DOPA-induced dyskinesia, *Nat. Neurosci.* 6 (2003) 501-506.

144. B. Picconi, D. Centonze, S. Rossi, G. Bernardi, P. Calabresi, Therapeutic doses of L-dopa reverse hypersensitivity of corticostriatal D2-dopamine receptors and glutamatergic overactivity in experimental parkinsonism, *Brain* 127 (2004) 1661-1669.
145. B. Picconi, E. Passino, C. Sgobio, P. Bonsi, I. Barone, V. Ghiglieri, A. Pisani, G. Bernardi, M. mmassari-Teule, P. Calabresi, Plastic and behavioral abnormalities in experimental Huntington's disease: a crucial role for cholinergic interneurons, *Neurobiol. Dis.* 22 (2006) 143-152.
146. B. Picconi, A. Pisani, D. Centonze, G. Battaglia, M. Storto, F. Nicoletti, G. Bernardi, P. Calabresi, Striatal metabotropic glutamate receptor function following experimental parkinsonism and chronic levodopa treatment, *Brain* 125 (2002) 2635-2645.
147. P. Pizzo, T. Pozzan, Mitochondria-endoplasmic reticulum choreography: structure and signaling dynamics, *Trends Cell Biol* 17 (2007) 511-517.
148. C.C. Porter, J.A. Totaro, C.A. Stone, Effect of 6-hydroxydopamine and some other compounds on the concentration of norepinephrine in the hearts of mice, *J Pharmacol. Exp. Ther.* 140 (1963) 308-316.
149. S. Przedborski, M. Vila, The 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model: a tool to explore the pathogenesis of Parkinson's disease, *Ann. N. Y. Acad. Sci.* 991 (2003) 189-198.
150. P. Puigserver, B.M. Spiegelman, Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator, *Endocr. Rev.* 24 (2003) 78-90.
151. W.A. Pulsinelli, Selective neuronal vulnerability: morphological and molecular characteristics, *Prog. Brain Res.* 63 (1985) 29-37.
152. R.R. Ramsay, T.P. Singer, Energy-dependent uptake of N-methyl-4-phenylpyridinium, the neurotoxic metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, by mitochondria, *J Biol Chem.* 261 (1986) 7585-7587.
153. B.Z. Roitberg, M.E. Emborg, J.G. Sramek, S. Palfi, J.H. Kordower, Behavioral and morphological comparison of two nonhuman primate models of Huntington's disease, *Neurosurgery* 50 (2002) 137-145.
154. A.I. Rojo, C. Cavada, M.R. de Sagarra, A. Cuadrado, Chronic inhalation of rotenone or paraquat does not induce Parkinson's disease symptoms in mice or rats, *Exp. Neurol* 208 (2007) 120-126.
155. C. Sachs, G. Jonsson, Mechanisms of action of 6-hydroxydopamine, *Biochem. Pharmacol.* 24 (1975) 1-8.
156. C. Saft, J. Zange, J. Andrich, K. Muller, K. Lindenberg, B. Landwehrmeyer, M. Vorgerd, P.H. Kraus, H. Przuntek, L. Schols, Mitochondrial impairment in patients and asymptomatic mutation carriers of Huntington's disease, *Mov Disord.* 20 (2005) 674-679.
157. M.S. Saporito, E.M. Brown, M.S. Miller, S. Carswell, CEP-1347/KT-7515, an inhibitor of c-jun N-terminal kinase activation, attenuates the 1-methyl-4-phenyl tetrahydropyridine-mediated loss of nigrostriatal dopaminergic neurons *In vivo*, *J Pharmacol. Exp. Ther.* 288 (1999) 421-427.

158. E. Saulle, P. Gubellini, B. Picconi, D. Centonze, D. Tropepi, A. Pisani, M. Morari, M. Marti, L. Rossi, M. Papa, G. Bernardi, P. Calabresi, Neuronal vulnerability following inhibition of mitochondrial complex II: a possible ionic mechanism for Huntington's disease, *Mol. Cell Neurosci.* 25 (2004) 9-20.
159. A.H. Schapira, J.M. Cooper, D. Dexter, J.B. Clark, P. Jenner, C.D. Marsden, Mitochondrial complex I deficiency in Parkinson's disease, *J Neurochem.* 54 (1990) 823-827.
160. A.H. Schapira, V.M. Mann, J.M. Cooper, D. Dexter, S.E. Daniel, P. Jenner, J.B. Clark, C.D. Marsden, Anatomic and disease specificity of NADH CoQ1 reductase (complex I) deficiency in Parkinson's disease, *J Neurochem.* 55 (1990) 2142-2145.
161. W.J. Schmidt, Dopamine-glutamate interactions in the basal ganglia, *Amino. Acids* 14 (1998) 5-10.
162. R.K. Schwarting, J.P. Huston, Unilateral 6-hydroxydopamine lesions of meso-striatal dopamine neurons and their physiological sequelae, *Prog Neurobiol.* 49 (1996) 215-266.
163. X.M. Shen, G. Dryhurst, Iron- and manganese-catalyzed autoxidation of dopamine in the presence of L-cysteine: possible insights into iron- and manganese-mediated dopaminergic neurotoxicity, *Chem. Res. Toxicol.* 11 (1998) 824-837.
164. T.P. Singer, N. Castagnoli, Jr., R.R. Ramsay, A.J. Trevor, Biochemical events in the development of parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, *J Neurochem.* 49 (1987) 1-8.
165. T.P. Singer, R.R. Ramsay, K. McKeown, A. Trevor, N.E. Castagnoli, Jr., Mechanism of the neurotoxicity of 1-methyl-4-phenylpyridinium (MPP⁺), the toxic bioactivation product of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), *Toxicology* 49 (1988) 17-23.
166. R.J. Smeyne, V. Jackson-Lewis, The MPTP model of Parkinson's disease, *Brain Res. Mol. Brain Res.* 134 (2005) 57-66.
167. S.A. Susin, H.K. Lorenzo, N. Zamzami, I. Marzo, B.E. Snow, G.M. Brothers, J. Mangion, E. Jacotot, P. Costantini, M. Loeffler, N. Larochette, D.R. Goodlett, R. Aebersold, D.P. Siderovski, J.M. Penninger, G. Kroemer, Molecular characterization of mitochondrial apoptosis-inducing factor, *Nature* 397 (1999) 441-446.
168. J.M. Tan, T.M. Dawson, Parkin blushed by PINK1, *Neuron* 50 (2006) 527-529.
169. A. Tozzi, C. Costa, F.M. Di, M. Tantucci, S. Siliquini, V. Belcastro, L. Parnetti, B. Picconi, P. Calabresi, Memantine reduces neuronal dysfunctions triggered by in vitro ischemia and 3-nitropropionic acid, *Exp. Neurol* 207 (2007) 218-226.
170. L. Tretter, I. Sipos, V. dam-Vizi, Initiation of neuronal damage by complex I deficiency and oxidative stress in Parkinson's disease, *Neurochem. Res.* 29 (2004) 569-577.
171. A.J. Trevor, N. Castagnoli, T.P. Singer, The formation of reactive intermediates in the MAO-catalyzed oxidation of the nigrostriatal toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), *Toxicology* 49 (1988) 513-519.
172. D. Trotti, N.C. Danbolt, A. Volterra, Glutamate transporters are oxidant-vulnerable: a molecular link between oxidative and excitotoxic neurodegeneration?, *Trends Pharmacol. Sci.* 19 (1998) 328-334.

173. K.Y. Tseng, F. Kasanetz, L. Kargieman, L.A. Riquelme, M.G. Murer, Cortical slow oscillatory activity is reflected in the membrane potential and spike trains of striatal neurons in rats with chronic nigrostriatal lesions, *J. Neurosci.* 21 (2001) 6430-6439.
174. Y. Tsujimoto, T. Nakagawa, S. Shimizu, Mitochondrial membrane permeability transition and cell death, *Biochim. Biophys. Acta* 1757 (2006) 1297-1300.
175. Y. Tsujimoto, S. Shimizu, Role of the mitochondrial membrane permeability transition in cell death, *Apoptosis*. 12 (2007) 835-840.
176. L. Turski, K. Bressler, K.J. Rettig, P.A. Loschmann, H. Wachtel, Protection of substantia nigra from MPP⁺ neurotoxicity by N-methyl-D-aspartate antagonists, *Nature* 349 (1991) 414-418.
177. U. Ungerstedt, 6-Hydroxy-dopamine induced degeneration of central monoamine neurons, *Eur. J Pharmacol.* 5 (1968) 107-110.
178. U. Ungerstedt, G.W. Arbuthnott, Quantitative recording of rotational behavior in rats after 6-hydroxy-dopamine lesions of the nigrostriatal dopamine system, *Brain Res.* 24 (1970) 485-493.
179. E.M. Valente, P.M. bou-Sleiman, V. Caputo, M.M. Muqit, K. Harvey, S. Gispert, Z. Ali, T.D. Del, A.R. Bentivoglio, D.G. Healy, A. Albanese, R. Nussbaum, R. Gonzalez-Maldonado, T. Deller, S. Salvi, P. Cortelli, W.P. Gilks, D.S. Latchman, R.J. Harvey, B. Dallapiccola, G. Auburger, N.W. Wood, Hereditary early-onset Parkinson's disease caused by mutations in PINK1, *Science* 304 (2004) 1158-1160.
180. M. Vila, V. Jackson-Lewis, S. Vukosavic, R. Djaldetti, G. Liberatore, D. Offen, S.J. Korsmeyer, S. Przedborski, Bax ablation prevents dopaminergic neurodegeneration in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease, *Proc. Natl. Acad. Sci. U. S. A* 98 (2001) 2837-2842.
181. J.C. Vis, E. Schipper, de Boer-van Huizen RT, M.M. Verbeek, R.M. de Waal, P. Wesseling, H.J. ten Donkelaar, B. Kremer, Expression pattern of apoptosis-related markers in Huntington's disease, *Acta Neuropathol.* 109 (2005) 321-328.
182. J.C. Vis, M.M. Verbeek, R.M. de Waal, H.J. ten Donkelaar, B. Kremer, The mitochondrial toxin 3-nitropropionic acid induces differential expression patterns of apoptosis-related markers in rat striatum, *Neuropathol. Appl. Neurobiol.* 27 (2001) 68-76.
183. V. Viswanath, Y. Wu, R. Boonplueang, S. Chen, F.F. Stevenson, F. Yantiri, L. Yang, M.F. Beal, J.K. Andersen, Caspase-9 activation results in downstream caspase-8 activation and bid cleavage in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinson's disease, *J Neurosci.* 21 (2001) 9519-9528.
184. F.O. Walker, Huntington's disease, *Lancet* 369 (2007) 218-228.
185. C. Wang, H.S. Ko, B. Thomas, F. Tsang, K.C. Chew, S.P. Tay, M.W. Ho, T.M. Lim, T.W. Soong, O. Pletnikova, J. Troncoso, V.L. Dawson, T.M. Dawson, K.L. Lim, Stress-induced alterations in parkin solubility promote parkin aggregation and compromise parkin's protective function, *Hum. Mol. Genet.* 14 (2005) 3885-3897.
186. T. Wichmann, M.R. DeLong, Functional and pathophysiological models of the basal ganglia, *Curr. Opin. Neurobiol.* 6 (1996) 751-758.

187. T. Wichmann, M.R. DeLong, Models of basal ganglia function and pathophysiology of movement disorders, *Neurosurg. Clin. N. Am.* 9 (1998) 223-236.
188. R.A. Wise, Dopamine, learning and motivation, *Nat. Rev. Neurosci.* 5 (2004) 483-494.
189. U. Wullner, A.B. Young, J.B. Penney, M.F. Beal, 3-Nitropropionic acid toxicity in the striatum, *J Neurochem.* 63 (1994) 1772-1781.
190. J. Xu, S.Y. Kao, F.J. Lee, W. Song, L.W. Jin, B.A. Yankner, Dopamine-dependent neurotoxicity of alpha-synuclein: a mechanism for selective neurodegeneration in Parkinson disease, *Nat. Med.* 8 (2002) 600-606.
191. Y. Yang, S. Gehrke, Y. Imai, Z. Huang, Y. Ouyang, J.W. Wang, L. Yang, M.F. Beal, H. Vogel, B. Lu, Mitochondrial pathology and muscle and dopaminergic neuron degeneration caused by inactivation of *Drosophila* Pink1 is rescued by Parkin, *Proc. Natl. Acad. Sci. U. S. A* 103 (2006) 10793-10798.
192. Y.X. Yang, N.W. Wood, D.S. Latchman, Molecular basis of Parkinson's disease, *Neuroreport* 20 (2009) 150-156.

Figure legends

Figure 1. Schematic representation of the basal ganglia circuit. The structures and circuitry of the BG are represented here, along with their connections to the cerebral cortex, the thalamus and the brain stem/spinal cord. Note the three projection pathways to the output BG structures (GPi/SNr): the "direct" one from the striatum, the "indirect" from the striatum through GPe and STN, and the "hyperdirect" from the cortex through the STN. The neurotransmitters utilized in the described projections are illustrated in different colors. Abbreviations: GABA, γ -aminobutyric acid; GPe/i, *globus pallidus pars externa/interna*; SNc/r, *substantia nigra pars compacta/reticulata*; STN, subthalamic nucleus.

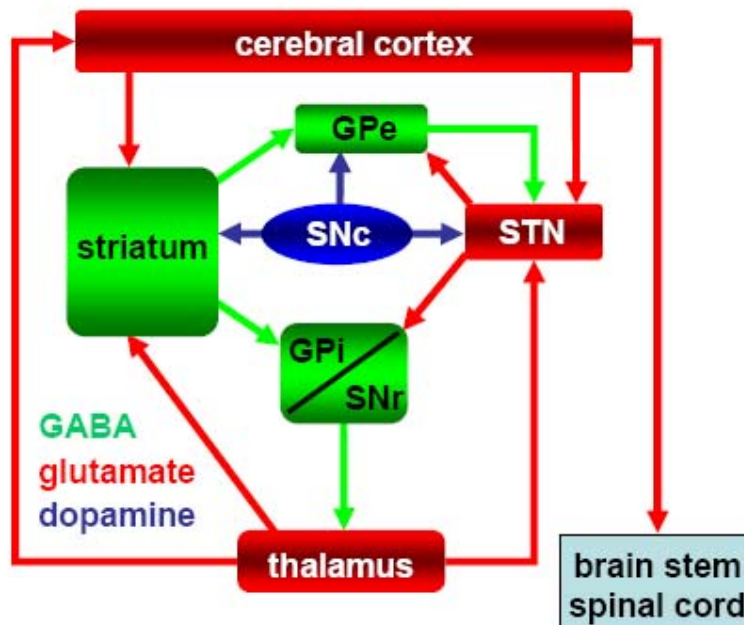


Figure 2. The mitochondrial respiratory chain and the targets of mitochondrial toxins.

Complex I to IV of the mitochondrial respiratory chain catalyzing oxidative phosphorylation are in green. The two electrons ($2 e^-$) donated during the reduction of ubiquinone (Q) by NADH are transported through complexes I, II, III and cytochrome c (Cyt c) to complex IV, which transfers them to O_2 to generate water. During this process ten protons (H^+) for each $2 e^-$ are pumped from the matrix to the intermembrane space by complexes I, II and IV, generating a H^+ gradient (Δp) across the inner membrane contributing to mitochondrial membrane potential ($\Delta\Psi_m$). This gradient is utilized by ATP synthase (in red), also called complex V, to synthesize ATP from ADP and P_i . The toxins utilized in experimental models of BG diseases target either complex I (for PD) or II (for HD). Note that complex II blockade also affects Krebs cycle, having a greater impact on cellular energy production.

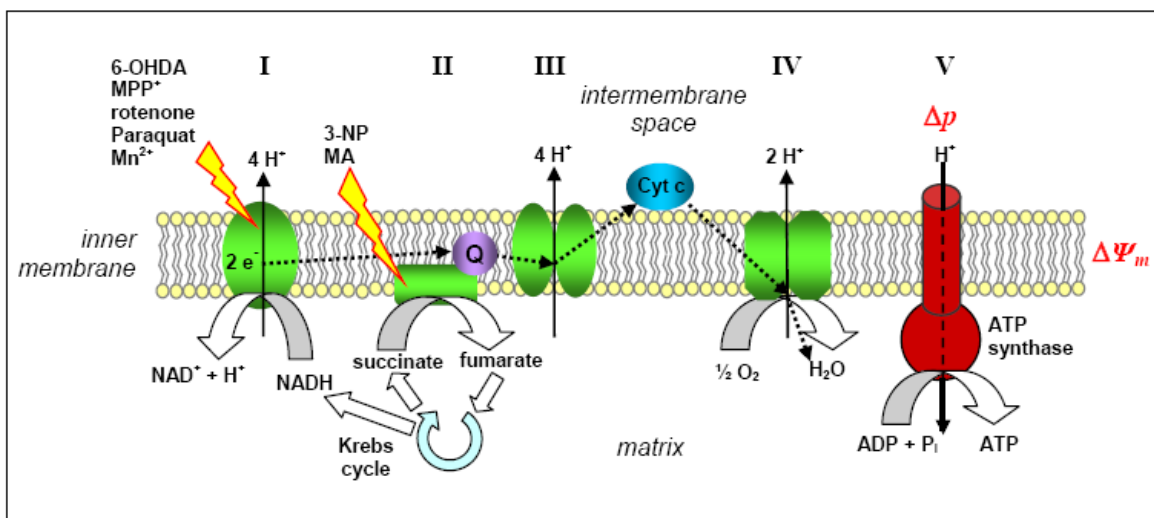
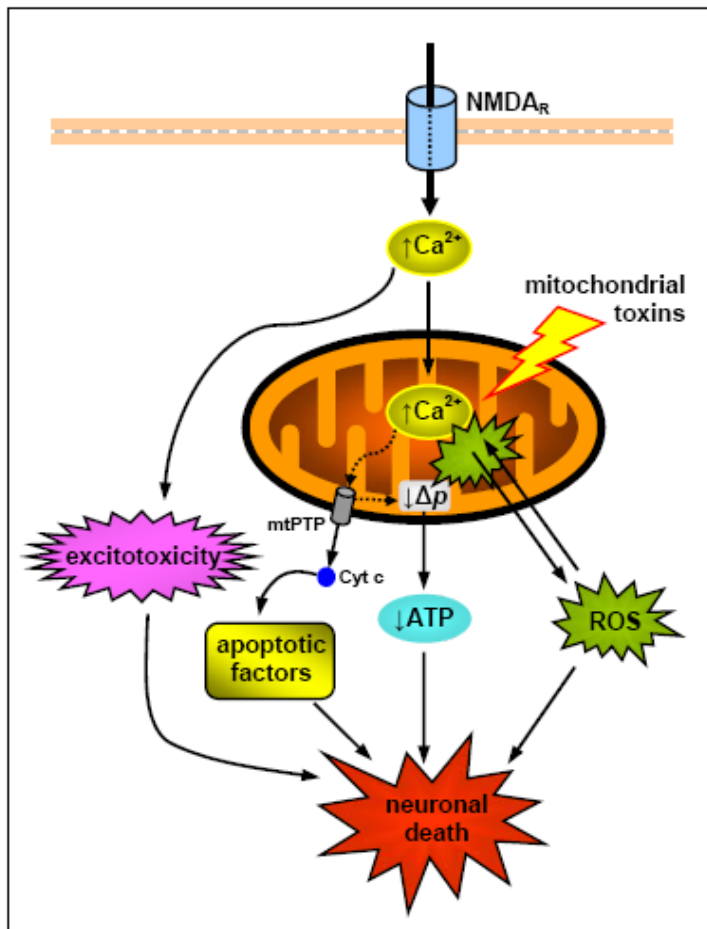


Figure 3. Downstream mechanisms triggered by mitochondrial dysfunction: converging towards neuronal death. Mitochondrial damage and respiratory chain uncoupling by mitochondrial toxins results in the decrease of ATP synthesis and in the increase in ROS production, the former leading to energy failure promoting cell death, the latter further contributing to cellular and mitochondrial damage. This can result in an impaired Ca^{2+} buffering that, together with excessive Ca^{2+} entering through NMDA receptors, leads to increased Ca^{2+} levels both in the cytoplasm and in the mitochondria. Elevated mitochondrial Ca^{2+} concentration triggers mitochondrial outer membrane permeabilization (MOMP) by opening of the mitochondrial permeability transition pore (mtPTP), resulting in the collapse of Δp and $\Delta\Psi_m$, release of cytochrome c and other apoptosis-promoting factors, and increased ROS production and Ca^{2+} accumulation. This downstream effects, in turn, will further contribute to cellular and mitochondrial damage in a deadly feedback cycle. Impaired ATP synthesis, excessive ROS production, Ca^{2+} -mediated excitotoxic processes, and release of pro-apoptotic factors all converge towards neuronal death.



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