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MINIREVIEW

# The hallmarks of Parkinson's disease

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Since the discovery of dopamine as a neurotransmitter in the 1950s, Parkinson's disease (PD) research has generated a rich and complex body of knowledge, revealing PD to be an age-related multifactorial disease, influenced by both genetic and environmental factors. The tremendous complexity of the disease is increased by a nonlinear progression of the pathogenesis between molecular, cellular and organic systems. In this minireview, we explore the complexity of PD and propose a systems-based approach, organizing the available information around cellular disease hallmarks. We encourage our peers to adopt this cell-based view with the aim of improving communication in interdisciplinary research endeavors targeting the molecular events, modulatory cell-to-cell signaling pathways and emerging clinical phenotypes related to PD.

## Introduction

Idiopathic Parkinson's disease (IPD) is the second most common neurodegenerative disease after Alzheimer's disease, and affects populations worldwide. The clinical syndrome of IPD is characterized by bradykinesia, resting tremor, rigidity, and postural instability. Bradykinesia is considered to be the main feature of and the necessary condition for a diagnosis of IPD [1]. In addition to these motor symptoms, many patients experience a wide range of nonmotor symptoms that sometimes even precede the typical movement disorder, such as hyposmia, sleep disturbances (i.e. rapid eye movement sleep behavior disorder), depression, constipation, and other dysautonomic symptoms [2–4]. In particular, hyposmia and rapid eye movement sleep behavior disorder often start prior to the onset of the core motor syndrome [5]. In this review, we discuss Parkinson's disease in the global sense of the term (PD), including IPD and genetically or environmentally induced forms of parkinsonism. Indeed, many cellular hallmarks have been found to occur in both PD and IPD. The motor symptoms of PD are thought to arise primarily from the loss of dopaminergic (DA) neurons within the substantia nigra (SN), although other neurotransmitter systems (i.e. glutamatergic, cholinergic, tryptaminergic, noradrenergic, adrenergic, serotoninergic, and peptidergic) also appear to be affected. Treatment of PD is currently symptomatic, and essentially involves substituting dopamine, or suppressing pathological neuronal oscillations via deep brain stimulation.

It is tempting to speculate that the search for the origin and effective treatment of this disease will con-

**Abbreviations**
ATP13A2, ATPase type 13A2; CNS, central nervous system; DA, dopaminergic; IPD, idiopathic Parkinson's disease; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson's disease; ROS, reactive oxygen species; SN, substantia nigra.

tinue to add complexity to the scientific literature. However, appreciating the progress in cancer research [6,7], we anticipate otherwise: the new generation of PD researchers will be practicing a dramatically different type of science than PD researchers from the old school, which deserves full credit for developments such as dopamine substitution and deep brain stimulation. The approaches that have driven the development of these treatments have been practice-oriented and often pragmatic. The fundamental conceptual change required to progress efficiently from this point will be to develop PD research into a more exact science in terms of mathematical concepts. The complex relationships between molecular, cellular and clinical traits will need to be explained in an intelligible way, in terms of a small number of underlying principles. In this review, we focus on the cellular hallmarks of PD, and discuss strategies for understanding the relationships between these cellular traits, molecular factors, and clinical traits.

## Age as a dominant risk factor

IPD is an age-related disorder. Population-based prevalence and incidence studies show a strong correlation with age [8], with ~ 1% of people older than 60 years [9] being affected. At the age of 80 years, the prevalence rises to 3%. In familial forms of PD, an earlier onset is possible, but, with increasing age, the risk of disease onset rises as well [10]. Indeed, to date, aging represents the most significant risk factor for developing PD [11].

## Genetic risk factors

In 5–10% of cases, PD presents as a Mendelian form with autosomal dominant or recessive inheritance. The genetic contribution to PD had been discounted until the early 1990s; however, 15 years after the identification of the first gene related to an autosomal dominant form of PD, we know of ~ 28 distinct chromosomal regions that are related to PD [12]. For only six of these regions have the underlying genes that cause common monogenic forms of PD been identified, namely *SNCA (α-synuclein)* and *LRRK2* for autosomal dominant PD, and *PINK1*, *PARK7 (DJ-1)*, *ATPase type 13A2 (ATP13A2)*, and *PARK2* (*Parkin*) for autosomal recessive PD. It is noteworthy that the clinical features of the early-onset PD subtypes associated with *PINK1*, *Parkin*, and *DJ-1*, which are all either directly or indirectly involved in oxidative stress mechanisms, are indistinguishable. The SN shows neuronal loss and gliosis, but Lewy bodies are typically lacking in affected carriers of mutations in the *Parkin* gene. In contrast, the forms of PD associated with other common monogenic factors are clearly distinguishable. Mutations of *α-synuclein* are fully penetrant, *and* typically cause fast-progressing early-onset to late-onset PD, with widespread and abundant Lewy body formation as a pathophysiological hallmark. In contrast, *LRRK2* mutations show variable penetrance, approximately 30–70% at age 80 years, and typically cause late-onset PD, mostly without dementia, and in most cases typical Lewy body pathology [13–15]. Finally, mutations in *ATP13A2* cause an atypical form of PD with dementia, named Kufor–Rakeb syndrome [16].

Among all of the suspected chromosomal regions, 18 have by now gained the status of 'PARK' loci (Table 1). However, many of those have been identified only on the basis of chromosomal linkage studies and causality, and confirmation is still pending. Incomplete penetrance, variable expression and phenocopies often pose problems in assessing whether PD is caused solely by genetic susceptibility or is modified by environmental factors.

## Environmental risk factors

Many reports have shown that exposure to environmental toxins is associated with an increased risk of PD. Proof of principle that an environmental toxin could lead to PD was the observation that a side product produced in the synthesis of the narcotic drug meperidine, namely 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), caused irreversible parkinsonism, with all of the clinical features of PD [17]. As MPTP is an inhibitor of complex I of the mitochondrial electron transport chain [18], this observation was instrumental in identifying the key role of mitochondria in the pathogenesis of PD. Later, it was recognized that various insecticides, such as paraquat and rotenone, as well as solvents such as trichloroethylene and perchloroethylene, also cause mitochondrial dysfunction [19–24]. PD has also been linked to living in a rural environment, gardening, farming, and occupational exposure to agricultural chemicals [25]. The exact role of environmental factors in the pathogenesis of PD remains elusive. However, it is well established that both environmental and genetic factors correlate with cellular phenotypes that can be considered to be cellular hallmarks of PD (Fig. 1).

## α-Synuclein aggregation

Up until the 1990s, hypotheses regarding genetic causes of PD had not been confirmed. This situation changed

**Table 1.** Genetic factors with *PARK* status. Among all the genes that have been proposed as potential PD-related genetic factors, only a subgroup, listed here, have gained the official status of *PARK* loci. The column 'Inheritance' summarizes the modes of inheritance, namely, autosomal dominant (AD), autosomal recessive (AR), and unknown modes of inheritance (not available, NA). The column 'Association with PD' summarizes the population-based frequency of genetic association with PD. In contrast to 'rare' and 'common' genetic factors, 'risk factors' are not sufficient to cause the disease, but are only associated with the risk of disease onset

| Locus | Gene | Inheritance | Association with PD | Chromosome | Gene product description |
|---|---|---|---|---|---|
| *PARK1* | *SNCA* | AD | Common | 4q21 | Four point mutations of α-synuclein |
| *PARK2* | *Parkin* | AR | Common | 6q25.2–27 | E3 ubiquitin protein ligase; > 150 mutations, including point mutations, deletions, and insertions |
| *PARK3* | Unknown | AD | Rare | 2p13 | NA |
| *PARK4* | *SNCA* | AD | Common | 4p15 | Duplication or triplication of α-synuclein |
| *PARK5* | *UCHL1* | AD | Rare | 4p14 | Ubiquitin C-terminal hydrolase L1; single point mutation |
| *PARK6* | *PINK1* | AR | Common | 1p35–36 | PTEN-induced mitochondrial serine/threonine kinase; > 60 mutations, including point mutations (most), rarely deletions, and insertions |
| *PARK7* | *DJ-1* | AR | Common | 1p36 | Redox-dependent molecular chaperone; > 10 mutations, including point mutations, deletions, and duplications |
| *PARK8* | *LRRK2* | AD | Common | 12q12 | Leucine-rich repeat-containing kinase; point mutations as the most frequent cause for AD PD |
| *PARK9* | *ATP13A2* | AR | Common | 1p36 | Neuronal P-type ATPase, atypical PD |
| *PARK10* | Unknown | NA | Risk factor | 1p32 | NA/identified by linkage analysis |
| *PARK11* | '*GIGYF2*'? | AD | Rare or risk | 2q36–37 | The gene *GIGYF2* remains unconfirmed |
| *PARK12* | Unknown | NA | Risk factor | Xq21–25 | NA/identified by linkage analysis |
| *PARK13* | *Omi/HTRA2* | AD? | Rare or risk | 2p12 | Mitochondrial serine protease, point mutations |
| *PARK14* | *PLA2G6* | AR | Rare | 22q13.1 | Phospholipase A2, atypical PD |
| *PARK15* | *FBXO7* | NA | Risk factor | 22q12–13 | E3 ubiquitin protein ligase, atypical PD |
| *PARK16* | Unknown | NA | Risk factor | 1q32 | NA/identified by linkage analysis |
| *PARK17* | *VPS35* | AD | Rare | 16q11.2 | Vacuolar protein sorting 35, point mutations |
| *PARK18* | *EIF4G1* | AD | Rare | 3q27.1 | Eukaryotic translation initiation factor 4 gamma 1, point mutations |

when PD-causative dominant mutations in the α-*synuclein* gene were identified. α-Synuclein is the major protein component of Lewy bodies, the pathological hallmark not only in the brains of mutation carriers, but also in the common sporadic form of the disease. Indeed, genome-wide association studies revealed that genetic variants in the α-*synuclein* gene represent the most consistent genetic risk factor for PD across different populations [26,27]. Abnormal protein structure resulting from pathological amino acid substitutions, or overexpression of physiological α-synuclein owing to gene dose effects, can lead to oligomerization, fibrillization, and aggregation, and to subsequent neurodegeneration. Aggregated α-synuclein (in the form of Lewy bodies or Lewy neurites) interferes with the mechanisms of microtubule-based subcellular transport, thus causing synaptic dysfunction and other disruptions to neuronal homeostasis [28]. In addition to functional genetic variants in regulatory regions of the α-*synuclein* gene and duplications or triplications that promote the aggregation of α-synuclein, other factors can contribute to this process. A well-validated and common risk factor that influences the aggregation of α-synuclein is the lysosomal enzyme β-glucocerebrosidase. Whereas homozygous mutations in the gene encoding this enzyme cause Gaucher disease, a lysosomal storage disorder, heterozygous mutations lead to a five-fold increased risk for PD, and functional loss of the enzyme, leading to an accumulation of glucocerebroside. This, in turn, influences the aggregation of α-synuclein by stabilizing oligomeric intermediates and interfering with lysosomal clearance [29].

The consideration of α-synuclein aggregation as a major pathophysiological hallmark of PD led Braak and others to formulate and refine the ascending spread hypothesis. This hypothesis states that PD could have its origin in the bulbus olfactorius, in the motor nucleus of the vagal nerve, or at a strictly peripheral site. One idea is that the spread of PD pathogenesis may start in the gastrointestinal tract and propagate cell to cell, spreading from the enteric nervous system all the way up to the brainstem, midbrain, and other parts of the central nervous system (CNS), finally resulting in disease staging, as proposed by

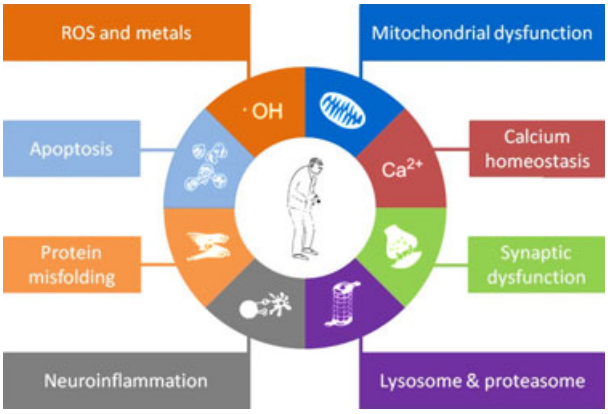


**Fig. 1.** Cellular hallmarks of PD. All subtypes of PD may share common cellular phenotypes. Correlations between the represented cellular phenotypes and disease progression are well established. In contrast, the chronology and mechanisms of cell type-specific and PD subtype-specific cellular dysfunction remain to be elucidated. Although PD pathogenesis has been associated with different cellular dysfunctions, it is likely that the respective initial tipping point of disease progression is caused by a PD subtype-specific process. Finally, it is also likely that multiple hits on different cellular targets may hasten the emergence of higher-scale organic dysfunction.

Braak *et al.* [30]. However, it remains to be seen whether this ascending spread of the disease is related to α-synuclein or other factors. As recently as 2009, Braak *et al.* revisited and strengthened the 'dual hit' theory, which proposes that an unknown neurotropic pathogen, possibly a virus, could gain access to the brain via the nose and/or the gut [31]. Another idea is that misfolded α-synuclein spreads in a prion-like manner [32]. In wild-type mice, it has been demonstrated that intrastriatal inoculation of synthetic α-synuclein leads to the cell-to-cell transmission of PD-like Lewy pathology in anatomically interconnected brain regions [33]. Desplats *et al.* demonstrated α-synuclein propagation between neuronal cells in culture, and the propagation of α-synuclein to transplanted stem cells in mice [34]. There is also increasing evidence, going beyond cell culture and mouse brain models, to support the idea of α-synuclein as a key factor in the ascending spread of disease. First, as mentioned above, the acquisition of exogenous α-synuclein fibrils triggers the formation of PD-like Lewy bodies and Lewy neurites [35]. Second, evidence is accumulating that cell-to-cell transmission of toxic α-synuclein oligomers occurs both in experimental models and in patients [34,36–38]. Third, autopsy studies have shown that healthy grafted embryonic neurons of mesencephalic origin rapidly acquire Lewy body-like inclusions after being placed in the host brain of a patient with PD [36,37], suggesting the spread of pathology from host tissues to the grafts. Finally, Pan Montojo *et al.* modeled environmental causes for the ascending spread of α-synuclein accumulation through intragastric administration of the pesticide rotenone in mice [39]. Resection of the autonomic nerves in these mice stops the spread of the PD-specific pathology [38].

## Lysosomal and proteasomal dysfunction

Dysfunction of molecular and organelle degradation pathways is a further hallmark of PD, and increasing evidence indicates functional interactions between the ubiquitin–proteasome system and autophagy [40,41]. Whereas both degradation systems are involved in the clearance of misfolded proteins, a special form of autophagy, also known as mitophagy, removes defective mitochondria from cells [42]. This clearance process is controlled via PINK1 and Parkin [43]. In PD, the dysfunction of these clearance systems facilitates the accumulation of α-synuclein and defective mitochondria. A positive feedback loop, which turns seemingly mild dysfunctions in misfolded protein handling into a self-potentiating cycle, is provided by the finding that mutated forms of α-synuclein may inhibit their own degradation via chaperone-mediated autophagy [44].

Further insights into lysosomal dysfunction in PD come from studies investigating the lysosomal enzyme β-glucocerebrosidase, as described above, and from studies on the transmembrane lysosomal P5-type ATPase named ATP13A2. In cell culture models, a loss of function of this PD-associated protein is correlated with impaired lysosomal acidification, decreased proteolytic processing of lysosomal enzymes, reduced degradation of lysosomal substrates, and diminished lysosome-mediated clearance of autophagosomes. In addition to these findings, Dehay *et al.* [45] have shown that DA SN neurons from patients with sporadic PD show decreased levels of ATP13A2.

## Mitochondrial dysfunction, reactive oxygen species (ROS), and $Ca^{2+}$

Evidence for the involvement of mitochondrial dysfunction in the pathogenesis of PD comes primarily from two sources. As described above, some toxins that can act as complex I inhibitors cause parkinsonism. For example, rotenone and paraquat lead to DA cell death in the SN and the induction of PD-like symptoms. Even more compelling is the observation that most of the genes known to cause familial PD are also involved in some aspect of mitochondrial

function. These include the genes encoding Parkin, PINK1, and DJ-1. PINK1 and Parkin are important for maintaining the delicate orchestration of mitochondrial turnover [46–50]. DJ-1 is involved in oxidative stress responses. The level of DJ-1 increases when cellular levels of ROS increase [51,52]. The oxidized form of DJ-1 translocates to the mitochondrial outer membrane and protects neurons from cell death, by an as yet unknown mechanism [53].

Similar to the loss of function of PINK1, mutations in DJ-1 also lead to a fragmentation of mitochondria in mammalian models of acute PD, a phenotypic response that is often associated with a loss of the mitochondrial membrane potential, and dysfunction of this organelle. Fusion and fission of mitochondria, two processes that actively control the level of mitochondrial fragmentation, are pivotal for quality control and turnover of mitochondria, and are highly relevant to PD pathogenesis [54].

Despite all the progress made in understanding mitochondrial biology and its role in controlling apoptosis, it is not well understood why mitochondrial dysfunction takes such a center stage in PD [55,56]. Bolam *et al*. suggest that this is a result of the specific neuroanatomical features of DA neurons and their projections into the striatum. DA neurons have extremely long projections from the SN into the striatum, are unmyelinated, and are characterized by a high degree of arborization and a high number of synapses [57,58]. Moreover, DA neurons of the SN show reduced mitochondrial mass as compared with other neuronal subpopulations in the same area, at least in mice [59]. However, a significant number of non-DA central and peripheral neurons are also subject to degeneration in PD. Interestingly, the at-risk populations of neurons share a number of features with DA neurons, including highly branched axons, pacemaker activity, elevated oxidative stress, and $Ca^{2+}$ buffering stress. It has been speculated that these physiological cellular characteristics could potentially explain the selective local degeneration of both DA and non-DA cells in PD [60,61].

In general, the brain in its resting state consumes ~ 20% of the total body energy, while accounting for only 2% of the total body mass. Striatal DA neurons, because of their autonomously generated activity (pacemaker function) and their above-mentioned physiological properties, are among the highest-energy-consuming and, therefore, the most vulnerable cells of all the neurons in the CNS [61]. The maintenance of a balanced energy budget might therefore be more difficult for DA neurons than for other neuronal cell types. Moreover, Yo *et al*. [62] demonstrated that PINK1 deficiency leads to a very different response in neurons than in myocytes with respect to changes in mitochondrial membrane potential, mitochondrial $Ca^{2+}$ buffering capacity, and cell survival. It has also been suggested that DA neurons are particularly sensitive to repetitive cycles of energetic stress triggered by oscillatory increases in $Ca^{2+}$ [63].

Mitochondrial dysfunction not only leads to a deficit in energy supply, but is also an important factor in the generation of oxidative stress. During the transfer of electrons through the electron transfer chain, single electrons can escape and lead to the reduction of molecular oxygen to form superoxide anions ($O_2^-$). Under normal circumstances, the amount of ROS produced can reach up to 1% of all the oxygen consumed. In cases of mitochondrial dysfunction, increased production of ROS is observed, which can have detrimental consequences such as oxidative DNA damage, lipid peroxidation, or protein oxidation [64]. ROS also have a physiological role as signaling molecules, with abnormal levels therefore disrupting physiological signaling cascades [65]. There is ample evidence that oxidative stress plays an important role in the pathogenesis of PD. A substantial proportion of patients with sporadic PD have reduced levels of complex I in the brain, and the levels of many markers of oxidative damage are increased during the clinical syndrome [66–68]. Findings from phosphorus and proton magnetic resonance spectroscopy in the mesostriatal region of patients with PD support this concept by revealing the bilateral reduction of high-energy phosphates such as ATP [69]. At the same time, antioxidative defense mechanisms are activated to compensate for the deranged redox homeostasis. One of the key components in this process is the Nrf2–Keap1 pathway [70,71]. In response to a changed redox status, Nrf2 activates an antioxidative cascade of cytoprotective and anti-inflammatory genes, counteracting the increased load of ROS and cellular damage [72,73].

In addition to their pivotal roles in energy metabolism and $Ca^{2+}$ homeostasis, mitochondria are important in many other processes, such as programmed cell death and inflammatory reactions [55]. It is likely that many of these processes are directly or indirectly involved in PD pathogenesis, even though it is not clear whether they are causative or consequential.

It is important to keep in mind that mitochondria are evolutionarily of bacterial origin, and have undergone endosymbiosis. This evolutionary history is reflected in the fact that mitochondria still have their own genome. It consists of 13 protein-coding genes and 22 rRNA genes that are contained in the mitochondrial DNA, the majority of mitochondrial genes being in the nucleus. Many efforts have been made to identify mitochondrial DNA mutations as a potential causal factor

in PD. Thus far, these efforts have not yielded clear answers, but it has been observed that neurons of patients with PD have an unusually high rate of mitochondrial DNA deletions [74]. In the early stages of PD, these mutations are seen exclusively in neurons of the basal ganglia, but not in glial cells – a finding indicating a specific susceptibility to mitochondrial damage in this cell type. Indeed, the number of mitochondrial DNA deletions is closely correlated with the aging process [75]. One of the biggest challenges in the analysis of mitochondrial DNA mutations is heteroplasmy – the fact that each individual cell can contain hundreds of mitochondria, and not all of them will carry the same mutation. Similarly, even if all mitochondria within a cell are mutated, not all cells within an organ necessarily carry mutated mitochondria.

## Iron and other metals

In addition to a wide spectrum of genetic and environmental factors, PD is also significantly linked to a disturbance of iron metabolism [76]. In fact, apoptotic cell death in DA neurons correlates with iron-mediated hydroxyl radical formation. This is mainly because both superoxide and peroxide, which are mitochondrial byproducts, can react with iron, through the Fenton and Haber–Weiss reactions [77,78]. In turn, iron has a catalytic function in producing hydroxyl radicals, which are the most damaging of the ROS. It is of note that the SN is the brain region with the highest iron content [79,80], an additional feature that adds to our understanding of its high degree of vulnerability in PD. Neuromelanin efficiently binds iron and thereby contributes to the high iron load in the SN [81]. Importantly, abnormally increased SN iron contents have been described in both genetic and idiopathic forms of PD [82]. This increased iron content is, however, more pronounced in idiopathic forms of PD than in familial forms. Furthermore, iron levels in a chronic MPTP mouse model have been shown to correlate with the selective degeneration of DA neurons in the SN [83]. The two main types of organelle involved in iron accumulation in the brain are lysosomes and mitochondria [84]. Two genetic PD factors that are clearly correlated with iron accumulation are *PLA2G6* and *ATP13A2* [85,86]. Their precise mechanisms of action remain to be understood.

Mitochondria usually constitute the main source of cellular ROS, in the form of peroxide and superoxide. The release of these comparatively mild ROS in the presence of free iron can lead to the formation of highly reactive hydroxyl radicals and initiate a positive feedback loop that triggers the release of additional iron from mitochondrial iron–sulfur centers [87]. Interestingly, not only iron but also other redox metals, such as copper, and nonredox metals, such as zinc, have also been reported to be involved in oxidative stress in the context of age-related neurodegenerative diseases [88]. Potential chelation therapies are currently under development [89].

## Synaptic dysfunction

Whereas the main function of neurons, namely communication with neighboring neurons, requires synaptic activity, synaptic function requires tight control of intracellular processes such as neurotransmitter packaging, energetic homoeostasis, and $Ca^{2+}$ buffering. SN DA neurons have a very complex morphology, with ~ 150 000 presynaptic terminals per neuron in the striatum [90]. It is likely that the high number and the widespread distribution of synapses, with the accompanying high local demands on energy and $Ca^{2+}$ buffering resources, correlate with decreased robustness of SN DA neurons against mitochondrial dysfunction and axonal transport defects [54]. Furthermore, local dysfunctions in protein degradation and turnover may affect synaptic function [91].

In cell cultures, α-synuclein associates with presynaptic vesicles prior to neurotransmitter release, upon which it rapidly redistributes into the cytosol [92]. It has been shown that modest overexpression is sufficient to markedly inhibit neurotransmitter release [93]. Most likely, this functional relationship between α-synuclein and synaptic function is attributable to the ability of α-synuclein to bind membranes, synaptic proteins, and actin filaments, and to cause changes in membrane dynamics [94].

## Neuroinflammation – cause or consequence?

Neither the complex etiology of PD nor its relationship with neuroinflammation are yet fully understood (Fig. 2). Research on neuroinflammation in PD is mainly focused on the innate immune system and, in particular, on the role of microglia, the most abundant resting macrophage population in the CNS [95].

Resting microglia monitor the local environment [96] and control the response of the immune system. Under minor stress, microglia release anti-inflammatory cytokines and growth factors. Under more significant stress, microglia release proinflammatory cytokines to recruit systemic immune cells and toxic factors to kill the pathogen. The interpretation by microglia of a given environmental state makes the difference between beneficial and detrimental outcomes of the immune response.

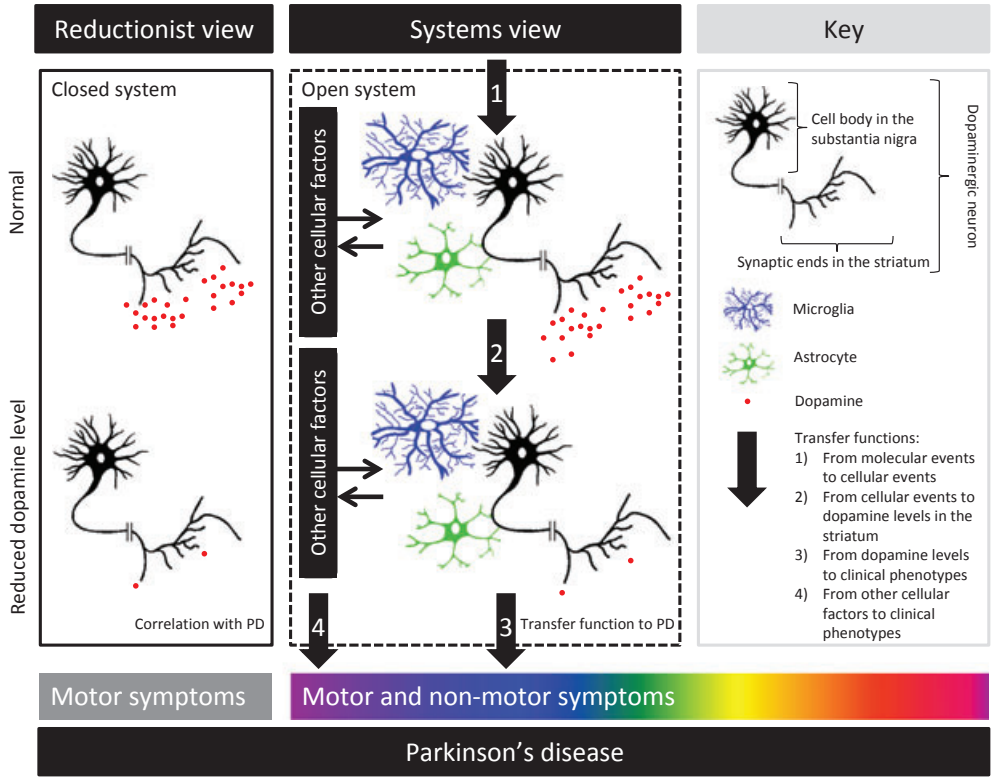


**Fig. 2.** Reductionist and holistic views of PD. Since the discovery of dopamine as a neurotransmitter, a reductionist view of depletion of dopamine in the striatum has been the driving force for translational research in PD (left panel). Looking forward, we believe that important new developments will come from more holistic, systems-based approaches that aim to understand the transfer functions between molecular, cellular, intercellular and high-level pathogenic events. For example, the control of neuroinflammation, which involves intercellular interactions between astrocytes, microglia, and DA neurons, could potentially influence emerging clinical symptoms. An oversimplified reductionist model that ignores these intercellular interactions could serve to complicate rather than facilitate the development of disease-modifying therapies. It is important to understand that the onset of motor symptoms does not correlate in a linear manner with the decrease in the number of DA neurons. Instead, the motor symptoms start only after the loss of the majority of DA neurons projecting from the SN into the striatum. In addition, a nonreductionist view needs to integrate other neurotransmitter systems, as well as nonmotor symptoms and their underlying cell populations in the CNS or in autonomic ganglia. Finally, we stress that the heterogeneous disease progression observed from patient to patient cannot be understood with oversimplified reductionist views. The challenge is to understand the key factors in each case, and to bring this concept to the level of personalized medicine.

It remains unclear, however, whether neuroinflammation is purely a side effect of PD, or whether it plays a more causal role in the pathogenesis. It has been demonstrated that neurons can exert feedback control on microglia. Many neuronal products, including membrane glycoproteins such as CD22, CD47, and CD200, and neuronal cell adhesion molecules, bind microglia receptors and inhibit microglial activation [97]. In the majority of experimental approaches, neuroinflammation is a secondary detrimental effect triggered by PD-related chemical or genetic stress factors [98]. For example, microglia are able to detect misfolded α-synuclein and increase neurotoxicity through the production of ROS and proinflammatory cytokines [99,100].

Astrocytes modulate the microglial response and play a supportive role for brain neurons [101,102]. Recent *in vitro* data suggest that DJ-1 deficiency in astrocytes might deregulate their neuroinflammatory response, thereby contributing to neurodegeneration [103]. The proportion of astrocytes in the SN is the lowest of any brain area [104]. The high number of microglia promoting neuroinflammation, opposing a low number of regulatory astrocytes, appears to provide a plausible explanation for the elevated risk of neuroinflammation in this brain region. Furthermore, the pigment neuromelanin, which is present in substantia nigra pars compacta neurons, has been shown to activate microglia [105], which further increases the susceptibility of this brain area to neuroinflammation.

Finally, there is evidence supporting the idea that the adaptive immune system, as well as the innate immune system, might be involved in PD. For example, a genetic association has been found between a region of the human leukocyte antigen and PD [106]. Responses of the adaptive immune system have been reported near both activated microglia and degenerating neurons, in particular in the substantia nigra pars compacta [107]. Importantly, regulatory T cells have been shown to attenuate nigrostriatal DA neurodegeneration in an MPTP mouse model [108]. Given this plethora of supporting evidence, we believe that neuroinflammation is at least a modulator of disease progression, and that further basic research in this field is needed to form the basis for future developments that target inflammatory pathways for disease-modifying therapies [109].

## Systems approaches to PD – a roadmap for translational research

Historically, PD research has been driven by medical knowledge. However, neuroscience research based on molecular and cellular biology has added further layers of complexity to our understanding of PD. In turn, increased efforts are required to coordinate and integrate interdisciplinary PD research. The ultimate aim in systems PD biomedicine is to translate mechanistic insights into clinical applications, and to use these to improve patient quality of life [110]. One important challenge is the need to develop theoretical models that are able to accurately represent the pathogenesis of PD. We believe that such models will help interdisciplinary cooperation by helping to structure both the interdisciplinary learning process and the formulation of testable key hypotheses. However, a multifactorial disease such as PD, whose progression is believed to be influenced by the collective action of several genes and environmental factors, cannot be adequately represented by a simple model. The multifactorial nature renders making predictions related to onset and progression a very challenging task. The progression of the syndrome emerges from the flow of spatiotemporal information between molecular and organic scales. Adequate multiple-scale models, aiming to bring PD research to a more integrated level, should therefore aim to understand the subsystem properties that connect the scales and to incorporate information about as many manageable factors as possible, including environmental factors, genetics, proteomics, metabolomics, cell biology, higher-level physiology, and patient quality of life. Graphical network modeling can provide an adequate view of a dynamic system such as PD, which is defined by the coordinated action of several factors and their local dynamics [111].

Wellstead *et al.* recently described a systems-control approach in which disturbances in energy metabolism act as a driving core module, and serve as an analytical and modeling framework with which to study the pathogenesis of PD [112,113]. It is important to keep in mind that energy metabolism is highly integrated with complex cell-to-cell interactions. Astrocytes, for example, are closely coupled to neurons, and play a key role in energy metabolism, neurotransmission, and many other neuronal functions [114–116]. This is reflected in the concept of the tripartite synapse, describing the importance of bidirectional communication between synapses formed by presynaptic and postsynaptic neurons and astrocytes [117].

A major challenge in the experimental analysis of multifactorial diseases is the extensive phenotypic buffering driven by network robustness. In turn, well-chosen predictions of phenotypic changes need to consider the network context and prior knowledge. Detailed knowledge of both biology and systems theory are prerequisites for meaningfully furthering our understanding of PD. The main challenge will be to understand the transfer functions that connect the multiple scales involved in PD pathogenesis. We believe that our understanding of the signaling pathways connecting molecular events to clinical traits will have made significant progress in a decade from now. In addition to an appropriate analytical framework, it will be crucial to develop noninvasive experimental approaches for time-resolved analyses in experimental models. Because of the multitude of relevant cellular phenotypes in PD, and the need to understand causality in cellular pathogenesis, we believe that light microscopy applications in systems biology will be a major source of data in time-resolved multifactorial single-cell analysis [118]. We also think that the integration of information from different, coordinated experimental approaches, combined with appropriate modeling strategies, will greatly enhance our understanding of PD pathogenesis, as well as the predictive power for early PD diagnosis and personalized identification of underlying disease factors. Currently, genetic testing of patients with PD is already possible, and is acting as a driving force for translational research; the current lack of PD subtype-specific therapies, however, makes the use of such genetic testing disputable [12,119]. Preparing for an integrated systems-level understanding of PD is now within reach, but remains an extremely ambitious goal. We believe that this major endeavor will pave the way for a new era of improved personalized medicine.

More short-term goals include improved human cell culture models for genetic and pharmacological screening approaches, and the development of more efficient

differentiation methods for patient-derived induced pluripotent stem cells [120], as well as improved animal models for translational research [98]. In ongoing projects, the integration of high-dimensional and nonlinear data is already creating an interesting challenge for data interpretation, and stresses the urgent need to extend the application of systems theory to PD research. Although regenerative medicine aiming to restore dopamine levels in the striatum offers transient relief from at least some clinical symptoms, we believe that systems approaches will allow for the development of increasingly sustainable and personalized disease-modifying therapies.

## Concluding remarks and outlook

Two centuries after the first detailed clinical description of PD by James Parkinson, and more than a decade after the first identification of genetic factors in this disease, PD research has evolved into a very mature research field, which has developed an arsenal of successfully applied symptomatic treatments. However, causative treatment approaches for PD have not yet become available, and both motor and nonmotor symptoms continue to interfere with patient quality of life. This fact highlights the complexity of this disease, and stresses the need for an intelligible integration of findings from 200 years of PD research.

Only a few research fields in translational medicine are more advanced than PD research and hence can serve as role models. Cancer research is certainly one of those. There are two main take-home messages [6,7]: first, it is crucial to define the signals exchanged between various cell types involved in the disease, and it is important to understand the effects of those signals on the integrated circuits of each of those cell types. Second, to develop accurate disease prognosis and treatment paradigms, it will be crucial to reach a level of mathematical clarity with regard to pathogenesis mechanisms, integrating the conceptual structure of systems theory and the logical coherence of neuroscience.

We foresee that PD research will evolve to be an increasingly exact science, in which the growing myriad of measurable phenotypic traits will be understood as manifestations of a small set of key principles.

## References

1 Ling H, Massey L, Lees AJ, Brown P & Day BL (2012) Hypokinesia without decrement distinguishes progressive supranuclear palsy from Parkinson's disease. *Brain* **135**, 1141–1153.
2 Gallagher D, Lees AJ & Schrag A (2010) What are the most important nonmotor symptoms in patients with Parkinson's disease and are we missing them? *Mov Disord* **25**, 2493–2500.
3 Park A & Stacy M (2009) Non-motor symptoms in Parkinson's disease. *J Neurol* **256** (Suppl), 293–298.
4 Simuni T & Sethi K (2008) Nonmotor manifestations of Parkinson's disease. *Ann Neurol* **64** (Suppl 2), S65–S80.
5 Postuma RB, Aarsland D, Barone P, Burn DJ, Hawkes CH, Oertel W & Ziemssen T (2012) Identifying prodromal Parkinson's disease: pre-motor disorders in Parkinson's disease. *Mov Disord* **27**, 617–626.
6 Hanahan D & Weinberg RA (2000) The hallmarks of cancer. *Cell* **100**, 57–70.
7 Hanahan D & Weinberg RA (2011) Hallmarks of cancer: the next generation. *Cell* **144**, 646–674.
8 De Lau LML & Breteler MMB (2006) Epidemiology of Parkinson's disease. *Lancet Neurol* **5**, 525–535.
9 Nussbaum RL & Ellis CE (2003) Alzheimer's disease and Parkinson's disease. *N Engl J Med* **348**, 1356–1364.
10 Crosiers D, Theuns J, Cras P & Van Broeckhoven C (2011) Parkinson disease: insights in clinical, genetic and pathological features of monogenic disease subtypes. *J Chem Neuroanat* **42**, 131–141.
11 Hindle JV (2010) Ageing, neurodegeneration and Parkinson's disease. *Age Ageing* **39**, 156–161.
12 Klein C & Westenberger A (2012) Genetics of Parkinson's disease. *Cold Spring Harb Perspect Med* **2**, 1–15.
13 Singleton AB, Farrer MJ & Bonifati V (2013) The genetics of Parkinson's disease: progress and therapeutic implications. *Mov Disord* **28**, 14–23.
14 Ozelius LJ, Senthil G, Saunders-Pullman R, Ohmann E, Deligtisch A, Tagliati M, Hunt AL, Klein C, Henick B, Hailpern SM *et al.* (2006) LRRK2 G2019S as a cause of Parkinson's disease in Ashkenazi Jews. *N Engl J Med* **354**, 424–425.
15 Goldwurm S, Zini M, Mariani L, Tesei S, Miceli R, Sironi F, Clementi M, Bonifati V & Pezzoli G (2007) Evaluation of LRRK2 G2019S penetrance: relevance for genetic counseling in Parkinson disease. *Neurology* **68**, 1141–1143.
16 Ramirez A, Heimbach A, Gründemann J, Stiller B, Hampshire D, Cid LP, Goebel I, Mubaidin AF, Wriekat A-L, Roeper J *et al.* (2006) Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase. *Nat Genet* **38**, 1184–1191.
17 Langston JW & Ballard PA (1983) Parkinson's disease in a chemist working with 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. *N Engl J Med* **309**, 310.
18 Mizuno Y, Suzuki K, Sone N & Saitoh T (1988) Inhibition of mitochondrial respiration by

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mouse brain in vivo. *Neurosci Lett* **91**, 349–353.
19 Thiruchelvam M, Richfield EK, Baggs RB, Tank AW & Cory-Slechta DA (2000) The nigrostriatal dopaminergic system as a preferential target of repeated exposures to combined paraquat and maneb: implications for Parkinson's disease. *J Neurosci* **20**, 9207–9214.
20 Priyadarshi A, Khuder S, Schaub E & Priyadarshi SS (2001) Environmental risk factors and Parkinson's disease: a metaanalysis. *Environ Res* **86**, 122–127.
21 Uversky VN (2004) Neurotoxicant-induced animal models of Parkinson's disease: understanding the role of rotenone, maneb and paraquat in neurodegeneration. *Cell Tissue Res* **318**, 225–241.
22 Kieburtz K & Wunderle KB (2013) Parkinson's disease: evidence for environmental risk factors. *Mov Disord* **28**, 8–13.
23 Gash DM, Rutland K, Hudson NL, Sullivan PG, Bing G, Cass WA, Pandya JD, Liu M, Choi D-Y, Hunter RL *et al.* (2008) Trichloroethylene: parkinsonism and complex 1 mitochondrial neurotoxicity. *Ann Neurol* **63**, 184–192.
24 Goldman SM, Quinlan PJ, Ross GW, Marras C, Meng C, Bhudhikanok GS, Comyns K, Korell M, Chade AR, Kasten M *et al.* (2012) Solvent exposures and Parkinson disease risk in twins. *Ann Neurol* **71**, 776–784.
25 Wirdefeldt K, Adami H-O, Cole P, Trichopoulos D & Mandel J (2011) Epidemiology and etiology of Parkinson's disease: a review of the evidence. *Eur J Epidemiol* **26** (Suppl 1), S1–S58.
26 Simón-Sánchez J, Schulte C, Bras JM, Sharma M, Gibbs JR, Berg D, Paisan-Ruiz C, Lichtner P, Scholz SW, Hernandez DG *et al.* (2009) Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nat Genet* **41**, 1308–1312.
27 Satake W, Nakabayashi Y, Mizuta I, Hirota Y, Ito C, Kubo M, Kawaguchi T, Tsunoda T, Watanabe M, Takeda A *et al.* (2009) Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. *Nat Genet* **41**, 1303–1307.
28 Sheng Z-H & Cai Q (2012) Mitochondrial transport in neurons: impact on synaptic homeostasis and neurodegeneration. *Nat Rev Neurosci* **13**, 77–93.
29 Mazzulli JR, Xu Y-H, Sun Y, Knight AL, McLean PJ, Caldwell G, Sidransky E, Grabowski G & Krainc D (2011) Gaucher disease glucocerebrosidase and α-synuclein form a bidirectional pathogenic loop in synucleinopathies. *Cell* **146**, 37–52.
30 Braak H, Del Tredici K, Rüb U, De Vos RAI, Jansen Steur ENH & Braak E (2003) Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging* **24**, 197–211.
31 Hawkes CH, Del Tredici K & Braak H (2009) Parkinson's disease: the dual hit theory revisited. *Ann N Y Acad Sci* **1170**, 615–622.
32 Olanow CW & Prusiner SB (2009) Is Parkinson's disease a prion disorder? *Proc Natl Acad Sci USA* **106**, 12571–12572.
33 Luk KC, Kehm V, Carroll J, Zhang B, O'Brien P, Trojanowski JQ & Lee VM-Y (2012) Pathological α-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. *Science* **338**, 949–953.
34 Desplats P, Lee H-J, Bae E, Patrick C, Rockenstein E, Crews L, Spencer B, Masliah E & Lee S-J (2009) Inclusion formation and neuronal cell death through neuron-to-neuron transmission of alpha-synuclein. *Proc Natl Acad Sci USA* **106**, 13010–13015.
35 Volpicelli-Daley LA, Luk KC, Patel TP, Tanik SA, Riddle DM, Stieber A, Meaney DF, Trojanowski JQ & Lee VM-Y (2011) Exogenous α-synuclein fibrils induce Lewy body pathology leading to synaptic dysfunction and neuron death. *Neuron* **72**, 57–71.
36 Kordower JH, Chu Y, Hauser RA, Freeman TB & Olanow CW (2008) Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. *Nat Med* **14**, 504–506.
37 Li J-Y, Englund E, Holton JL, Soulet D, Hagell P, Lees AJ, Lashley T, Quinn NP, Rehncrona S, Björklund A *et al.* (2008) Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. *Nat Med* **14**, 501–503.
38 Pan-Montojo F, Schwarz M, Winkler C, Arnhold M, O'Sullivan G, Pal A, Said J, Marsico G, Verbavatz J-M, Rodrigo-Angulo M *et al.* (2012) Environmental toxins trigger PD-like progression via increased alpha-synuclein release from enteric neurons in mice. *Sci Rep* **2**, 1–12.
39 Pan-Montojo F, Anichtchik O, Dening Y, Knels L, Pursche S, Jung R, Jackson S, Gille G, Spillantini MG, Reichmann H *et al.* (2010) Progression of Parkinson's disease pathology is reproduced by intragastric administration of rotenone in mice. *PLoS ONE* **5**, 1–10.
40 Korolchuk VI, Menzies FM & Rubinsztein DC (2010) Mechanisms of cross-talk between the ubiquitin–proteasome and autophagy–lysosome systems. *FEBS Lett* **584**, 1393–1398.
41 Chu Y, Dodiya H, Aebischer P, Olanow CW & Kordower JH (2009) Alterations in lysosomal and proteasomal markers in Parkinson's disease: relationship to alpha-synuclein inclusions. *Neurobiol Dis* **35**, 385–398.
42 Lee J, Giordano S & Zhang J (2012) Autophagy, mitochondria and oxidative stress: cross-talk and redox signalling. *Biochem J* **441**, 523–540.

43 Shiba-Fukushima K, Imai Y, Yoshida S, Ishihama Y, Kanao T, Sato S & Hattori N (2012) PINK1-mediated phosphorylation of the Parkin ubiquitin-like domain primes mitochondrial translocation of Parkin and regulates mitophagy. *Sci Rep* **2**, 1002.
44 Martinez-Vicente M, Talloczy Z, Kaushik S, Massey AC, Mazzulli JR, Mosharov EV, Hodara R, Fredenburg R, Wu D-C, Follenzi A *et al.* (2008) Dopamine-modified alpha-synuclein blocks chaperone-mediated autophagy. *J Clin Invest* **118**, 777–788.
45 Dehay B, Martinez-Vicente M, Ramirez A, Perier C, Klein C, Vila M & Bezard E (2012) Lysosomal dysfunction in Parkinson disease: ATP13A2 gets into the groove. *Autophagy* **8**, 1389–1391.
46 Clark IE, Dodson MW, Jiang C, Cao JH, Huh JR, Seol JH, Yoo SJ, Hay BA & Guo M (2006) Drosophila pink1 is required for mitochondrial function and interacts genetically with parkin. *Nature* **441**, 1162–1166.
47 Exner N, Treske B, Paquet D, Holmström K, Schiesling C, Gispert S, Carballo-Carbajal I, Berg D, Hoepken H-H, Gasser T *et al.* (2007) Loss-of-function of human PINK1 results in mitochondrial pathology and can be rescued by parkin. *J Neurosci* **27**, 12413–12418.
48 Gegg ME, Cooper JM, Schapira AHV & Taanman J-W (2009) Silencing of PINK1 expression affects mitochondrial DNA and oxidative phosphorylation in dopaminergic cells. *PLoS ONE* **4**, 1–10.
49 Geisler S, Holmström KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ & Springer W (2010) PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. *Nat Cell Biol* **12**, 119–131.
50 Abramov AY, Gegg M, Grunewald A, Wood NW, Klein C & Schapira AHV (2011) Bioenergetic consequences of PINK1 mutations in Parkinson disease. *PLoS ONE* **6**, 1–9.
51 Lev N, Ickowicz D, Melamed E & Offen D (2008) Oxidative insults induce DJ-1 upregulation and redistribution: implications for neuroprotection. *Neurotoxicology* **29**, 397–405.
52 Yanagida T, Tsushima J, Kitamura Y, Yanagisawa D, Takata K, Shibaike T, Yamamoto A, Taniguchi T, Yasui H, Taira T *et al.* (2009) Oxidative stress induction of DJ-1 protein in reactive astrocytes scavenges free radicals and reduces cell injury. *Oxid Med Cell Longev* **2**, 36–42.
53 Canet-Avilés RM, Wilson MA, Miller DW, Ahmad R, McLendon C, Bandyopadhyay S, Baptista MJ, Ringe D, Petsko GA & Cookson MR (2004) The Parkinson's disease protein DJ-1 is neuroprotective due to cysteine-sulfinic acid-driven mitochondrial localization. *Proc Natl Acad Sci USA* **101**, 9103–9108.
54 Perier C & Vila M (2012) Mitochondrial biology and Parkinson's disease. *Cold Spring Harb Perspect Med* **2**, 1–19.
55 Koopman WJH, Willems PHGM & Smeitink JAM (2012) Monogenic mitochondrial disorders. *N Engl J Med* **366**, 1132–1141.
56 Exner N, Lutz AK, Haass C & Winklhofer KF (2012) Mitochondrial dysfunction in Parkinson's disease: molecular mechanisms and pathophysiological consequences. *EMBO J* **31**, 3038–3062.
57 Matsuda W, Furuta T, Nakamura KC, Hioki H, Fujiyama F, Arai R & Kaneko T (2009) Single nigrostriatal dopaminergic neurons form widely spread and highly dense axonal arborizations in the neostriatum. *J Neurosci* **29**, 444–453.
58 Bolam JP & Pissadaki EK (2012) Living on the edge with too many mouths to feed: why dopamine neurons die. *Mov Disord* **27**, 1478–1483.
59 Liang C-L, Wang TT, Luby-Phelps K & German DC (2007) Mitochondria mass is low in mouse substantia nigra dopamine neurons: implications for Parkinson's disease. *Exp Neurol* **203**, 370–380.
60 Sulzer D & Surmeier DJ (2013) Neuronal vulnerability, pathogenesis, and Parkinson's disease. *Mov Disord* **28**, 41–50.
61 Surmeier DJ & Sulzer D (2013) The pathology roadmap in Parkinson disease. *Prion* **7**, 85–91.
62 Yao Z, Gandhi S, Burchell VS, Plun-Favreau H, Wood NW & Abramov AY (2011) Cell metabolism affects selective vulnerability in PINK1-associated Parkinson's disease. *J Cell Sci* **124**, 4194–4202.
63 Chan CS, Gertler TS & Surmeier DJ (2010) A molecular basis for the increased vulnerability of substantia nigra dopamine neurons in aging and Parkinson's disease. *Mov Disord* **25** (Suppl 1), S63–S70.
64 Orhan H, Van Holland B, Krab B, Moeken J, Vermeulen NPE, Hollander P & Meerman JHN (2004) Evaluation of a multi-parameter biomarker set for oxidative damage in man: increased urinary excretion of lipid, protein and DNA oxidation products after one hour of exercise. *Free Radic Res* **38**, 1269–1279.
65 Turrens JF (2003) Mitochondrial formation of reactive oxygen species. *J Physiol* **552**, 335–344.
66 Parker WD, Boyson SJ & Parks JK (1989) Abnormalities of the electron transport chain in idiopathic Parkinson's disease. *Ann Neurol* **26**, 719–723.
67 Schapira AH, Cooper JM, Dexter D, Clark JB, Jenner P & Marsden CD (1990) Mitochondrial complex I deficiency in Parkinson's disease. *J Neurochem* **54**, 823–827.
68 Jenner P (2003) Oxidative stress in Parkinson's disease. *Ann Neurol* **53** (Suppl 3), S26–S36.
69 Hattingen E, Magerkurth J, Pilatus U, Mozer A, Seifried C, Steinmetz H, Zanella F & Hilker R (2009) Phosphorus and proton magnetic resonance spectroscopy demonstrates mitochondrial dysfunction

in early and advanced Parkinson's disease. *Brain* **132**, 3285–3297.
70 Lo S-C, Li X, Henzl MT, Beamer LJ & Hannink M (2006) Structure of the Keap1:Nrf2 interface provides mechanistic insight into Nrf2 signaling. *EMBO J* **25**, 3605–3617.
71 Satoh T, Harada N, Hosoya T, Tohyama K, Yamamoto M & Itoh K (2009) Keap1/Nrf2 system regulates neuronal survival as revealed through study of keap1 gene-knockout mice. *Biochem Biophys Res Commun* **380**, 298–302.
72 Tufekci KU, Civi Bayin E, Genc S & Genc K (2011) The Nrf2/ARE pathway: a promising target to counteract mitochondrial dysfunction in Parkinson's disease. *Parkinson's Dis* **2011**, 1–14.
73 Zhang DD (2010) The Nrf2-Keap1-ARE signaling pathway: the regulation and dual function of Nrf2 in cancer. *Antioxid Redox Signal* **13**, 1623–1626.
74 Lin MT, Cantuti-Castelvetri I, Zheng K, Jackson KE, Tan YB, Arzberger T, Lees AJ, Betensky R, Beal MF & Simon DK (2012) Somatic mitochondrial DNA mutations in early Parkinson and incidental Lewy body disease. *Ann Neurol* **71**, 850–854.
75 Bender A, Krishnan KJ, Morris CM, Taylor G, Reeve AK, Perry RH, Jaros E, Hersheson JS, Betts J, Klopstock T *et al.* (2006) High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. *Nat Genet* **38**, 515–517.
76 Funke C, Schneider S, Berg D & Kell DB (2013) Genetics and iron in the systems biology of Parkinson's disease and some related disorders. *Neurochem Int* **62**, 637–652.
77 Wardman P & Candeias LP (1996) Fenton chemistry: an introduction. *Radiat Res* **145**, 523–531.
78 Kehrer JP (2000) The Haber–Weiss reaction and mechanisms of toxicity. *Toxicology* **149**, 43–50.
79 Hallgren B & Sourander P (1958) The effect of age on the non-haemin iron in the human brain. *J Neurochem* **3**, 41–51.
80 Riederer P, Dirr A, Goetz M, Sofic E, Jellinger K & Youdim MB (1992) Distribution of iron in different brain regions and subcellular compartments in Parkinson's disease. *Ann Neurol* **32** (Suppl), S101–S104.
81 Ben-Shachar D, Riederer P & Youdim MB (1991) Iron–melanin interaction and lipid peroxidation: implications for Parkinson's disease. *J Neurochem* **57**, 1609–1614.
82 Schweitzer KJ, Brüssel T, Leitner P, Krüger R, Bauer P, Woitalla D, Tomiuk J, Gasser T & Berg D (2007) Transcranial ultrasound in different monogenetic subtypes of Parkinson's disease. *J Neurol* **254**, 613–616.
83 Lv Z, Jiang H, Xu H, Song N & Xie J (2011) Increased iron levels correlate with the selective nigral dopaminergic neuron degeneration in Parkinson's disease. *J Neural Transm* **118**, 361–369.
84 Dusek P, Jankovic J & Le W (2012) Iron dysregulation in movement disorders. *Neurobiol Dis* **46**, 1–18.
85 Schneider S, Paisan-Ruiz C, Quinn NP, Lees AJ, Houlden H, Hardy J & Bhatia KP (2010) ATP13A2 mutations (PARK9) cause neurodegeneration with brain iron accumulation. *Mov Disord* **25**, 979–984.
86 McNeill A (2012) PLA2G6 mutations and other rare causes of neurodegeneration with brain iron accumulation. *Curr Drug Targets* **13**, 1204–1206.
87 Kell DB (2010) Towards a unifying, systems biology understanding of large-scale cellular death and destruction caused by poorly liganded iron: Parkinson's, Huntington's, Alzheimer's, prions, bactericides, chemical toxicology and others as examples. *Arch Toxicol* **84**, 825–889.
88 Jomova K, Vondrakova D, Lawson M & Valko M (2010) Metals, oxidative stress and neurodegenerative disorders. *Mol Cell Biochem* **345**, 91–104.
89 Ward RJ, Dexter DT & Crichton RR (2012) Chelating agents for neurodegenerative diseases. *Curr Med Chem* **19**, 2760–2772.
90 Sulzer D (2007) Multiple hit hypotheses for dopamine neuron loss in Parkinson's disease. *Trends Neurosci* **30**, 244–250.
91 Tai H-C & Schuman EM (2008) Ubiquitin, the proteasome and protein degradation in neuronal function and dysfunction. *Nat Rev Neurosci* **9**, 826–838.
92 Fortin DL, Nemani VM, Voglmaier SM, Anthony MD, Ryan TA & Edwards RH (2005) Neural activity controls the synaptic accumulation of alpha-synuclein. *J Neurosci* **25**, 10913–10921.
93 Nemani VM, Lu W, Berge V, Nakamura K, Onoa B, Lee MK, Chaudhry FA, Nicoll RA & Edwards RH (2010) Increased expression of alpha-synuclein reduces neurotransmitter release by inhibiting synaptic vesicle reclustering after endocytosis. *Neuron* **65**, 66–79.
94 Bellucci A, Zaltieri M, Navarria L, Grigoletto J, Missale C & Spano P (2012) From α-synuclein to synaptic dysfunctions: new insights into the pathophysiology of Parkinson's disease. *Brain Res* **1476**, 183–202.
95 Lucin KM & Wyss-Coray T (2009) Immune activation in brain aging and neurodegeneration: too much or too little? *Neuron* **64**, 110–122.
96 Nimmerjahn A, Kirchhoff F & Helmchen F (2005) Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science* **308**, 1314–1318.
97 Tian L, Rauvala H & Gahmberg CG (2009) Neuronal regulation of immune responses in the central nervous system. *Trends Immunol* **30**, 91–99.
98 Antony PM, Diederich NJ & Balling R (2011) Parkinson's disease mouse models in translational research. *Mamm Genome* **22**, 401–419.

99 Zhang W, Wang T, Pei Z, Miller DS, Wu X, Block ML, Wilson B, Zhang W, Zhou Y, Hong J-S *et al.* (2005) Aggregated alpha-synuclein activates microglia: a process leading to disease progression in Parkinson's disease. *FASEB J* **19**, 533–542.
100 Alvarez-Erviti L, Couch Y, Richardson J, Cooper JM & Wood MJA (2011) Alpha-synuclein release by neurons activates the inflammatory response in a microglial cell line. *Neurosci Res* **69**, 337–342.
101 Glass CK, Saijo K, Winner B, Marchetto MC & Gage FH (2010) Mechanisms underlying inflammation in neurodegeneration. *Cell* **140**, 918–934.
102 Rocha SM, Cristovão AC, Campos FL, Fonseca CP & Baltazar G (2012) Astrocyte-derived GDNF is a potent inhibitor of microglial activation. *Neurobiol Dis* **47**, 407–415.
103 Waak J, Weber SS, Waldenmaier A, Görner K, Alunni-Fabbroni M, Schell H, Vogt-Weisenhorn D, Pham T-T, Reumers V, Baekelandt V *et al.* (2009) Regulation of astrocyte inflammatory responses by the Parkinson's disease-associated gene DJ-1. *FASEB J* **23**, 2478–2489.
104 Mena M & García de Yébenes J (2008) Glial cells as players in parkinsonism: the 'good', the 'bad', and the 'mysterious' glia. *Neuroscientist* **14**, 544–560.
105 Zecca L, Wilms H, Geick S, Claasen J-H, Brandenburg L-O, Holzknecht C, Panizza ML, Zucca F, Deuschl G, Sievers J *et al.* (2008) Human neuromelanin induces neuroinflammation and neurodegeneration in the rat substantia nigra: implications for Parkinson's disease. *Acta Neuropathol* **116**, 47–55.
106 Hamza TH, Zabetian CP, Tenesa A, Laederach A, Montimurro J, Yearout D, Kay DM, Doheny KF, Paschall J, Pugh E *et al.* (2010) Common genetic variation in the HLA region is associated with late-onset sporadic Parkinson's disease. *Nat Genet* **42**, 781–785.
107 Brochard V, Combadière B, Prigent A, Laouar Y, Perrin A, Beray-Berthat V, Bonduelle O, Alvarez-Fischer D, Callebert J, Launay J *et al.* (2009) Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. *J Clin Invest* **119**, 182–192.
108 Reynolds AD, Stone DK, Hutter JAL, Benner EJ, Mosley RL & Gendelman HE (2010) Regulatory T cells attenuate Th17 cell-mediated nigrostriatal dopaminergic neurodegeneration in a model of Parkinson's disease. *J Immunol* **184**, 2261–2271.
109 Hirsch EC, Vyas S & Hunot S (2012) Neuroinflammation in Parkinson's disease. *Parkinsonism Relat Disord* **18** (Suppl 1), S210–S212.
110 Antony PMA, Balling R & Vlassis N (2012) From systems biology to systems biomedicine. *Curr Opin Biotechnol* **23**, 604–608.
111 Ma'ayan A (2011) Introduction to network analysis in systems biology. *Sci Signal* **4**, tr5
112 Cloutier M & Wellstead P (2010) The control systems structures of energy metabolism. *J R Soc* **7**, 651–665.
113 Wellstead P & Cloutier M (2011) An energy systems approach to Parkinson's disease. *Wiley Interdiscip Rev Syst Biol Med* **3**, 1–6.
114 Magistretti PJ (2011) Neuron–glia metabolic coupling and plasticity. *Exp Physiol* **96**, 407–410.
115 Wade JJ, McDaid LJ, Harkin J, Crunelli V & Kelso JAS (2011) Bidirectional coupling between astrocytes and neurons mediates learning and dynamic coordination in the brain: a multiple modeling approach. *PLoS ONE* **6**, 1–24.
116 Aubert A, Costalat R, Magistretti PJ & Pellerin L (2005) Brain lactate kinetics: modeling evidence for neuronal lactate uptake upon activation. *Proc Natl Acad Sci USA* **102**, 16448–16453.
117 Perea G, Navarrete M & Araque A (2009) Tripartite synapses: astrocytes process and control synaptic information. *Trends Neurosci* **32**, 421–431.
118 Antony PMA, Trefois C, Stojanovic A, Baumuratov AS & Kozak K (2013) Light microscopy applications in systems biology: opportunities and challenges. *Cell Commun Signal* **11**, 1–19.
119 Jacobs H, Latza U, Vieregge A & Vieregge P (2001) Attitudes of young patients with Parkinson's disease towards possible presymptomatic and prenatal genetic testing. *Genet Couns* **12**, 55–67.
120 Song M, Paul S, Lim H, Dayem AA & Cho S-G (2012) Induced pluripotent stem cell research: a revolutionary approach to face the challenges in drug screening. *Arch Pharm Res* **35**, 245–260.