Journal of Neurochemistry

JOURNAL OF NEUROCHEMISTRY | 2011 | 118 | 939–957

doi: 10.1111/j.1471-4159.2010.07132.x

REVIEW

The relevance of iron in the pathogenesis of Parkinson's disease

Jeswinder Sian-Hülsmann,* Silvia Mandel,† Moussa B. H.Youdim†,‡ and Peter Riederer*

*Clinical Neurochemistry, National Parkinson Foundation Centre of Excellence Laboratories, Clinic and Polyclinic for Psychiatry, Psychosomatic and Psychotherapy, Medical School, University of Würzburg, Würzburg, Germany

†Eve Topf Centre of Excellence for Neurodegenerative diseases, Technion-Rappaport Faculty of Medicine, Haifa, Israel

‡Department of Biology, World Central Yonsei University, Seoul, South Korea

Abstract

Alterations of iron levels in the brain has been observed and documented in a number of neurodegenerative disorders including Parkinson's disease (PD). The elevated nigral iron levels observed in PD may reflect a dysfunction of brain iron homeostasis. Under normal physiological conditions excess iron can be sequestrated in ferritin and neuromelanin. Alternatively, the excess iron may represent a component of brain iron deposition associated with ageing. The aetiology of idiopathic PD largely remains an enigma. However, intensive investigations have provided a host of putative mechanisms that might contribute to the pathogenesis underlying the characteristic degeneration of the dopaminergic neurons in the substantia nigra (SN). The mechanisms proposed include oxidative (and nitrative) stress, inflammation, excitotoxicity, mitochondrial dysfunction, altered proteolysis and finally apoptotic induced cell death. Iron-mediated cellular destruction is mediated primarily via reactive oxygen or/and nitrogen species induced oxidative stress. Furthermore, these pathogenic mechanisms appear to be closely interlinked to the cascade of events leading to cellular death. There are conflicting reports about the stage during disease progression at which nigral iron change occurs in PD. Some have found that there are no changes in iron content SN in asymptomatic incidental Lewy body disease, suggesting it may represent a secondary event in the cascade of neuronal degeneration. In contrast, others have found an elevation of iron in SN in preclinical stages. These discrepancies may be attributed to the occurrence of different sub-groups of the disease. This concurs with the notion that PD represents a group of related diseases with a number of potential pathogenic pathways.

Keywords: ferritin, iron, iron-related disorders, neurodegenerative diseases, neuromelanin, Parkinsonism subtypes.

J. Neurochem. (2011) **118**, 939–957.

Iron plays a pivotal role in many physiological functions (Schmidt 1940) including neuronal metabolism, a component of proteins involved in cellular processes such as DNA synthesis, oxygen transport and mitochondrial respiration. It is also fundamental in the brain processes such as neurotransmission and myelination. For instance, iron serves as a co-factor for the enzyme tyrosine hydroxylase that is involved in the dopamine synthesis pathway. As such, *in vitro* studies show that tyrosine hydroxylase activity is stimulated dosis-dependently by iron (Rausch *et al.* 1988). The versatility of iron is related to its ability to serve as either an electron donor or acceptor. In living organisms, it occurs either in its reduced Fe^{2+} or oxidised Fe^{3+} state.

Iron toxicity ensues in the presence of unbound or free iron in the cell. This occurs when the iron concentrations exceed the binding capacities of transferrin. It is therefore generally sequestered in the cells. Thus, iron overload can elicit a cascade of cellular deleterious events. The oxidising

Received October 21, 2010; accepted November 16, 2010.

Address correspondence and reprint requests to Prof. Dr. Dr. h.c. Peter Riederer, Clinic and Policlinic of Psychiatry, Psychosomatic and Psychotherapy, Clinical Neurochemistry, Fuechsleinstr. 15, 97080 Würzburg, Germany. E-mail: peter.riederer@mail.uni-wuerzburg.de

Abbreviations used: 6-OHDA, 6-hydroxydopamine; BBB, blood–brain barrier; DCT1, divalent cation metal transporter; FDRI, field-dependent relaxation rate; HNE, 4-hydroxynonenal; LB, Lewy bodies; LC, locus coeruleus; MSA, multiple systems atrophy; NM, neuromelanin; PD, Parkinson's disease; SN, substantia nigra; SNc, SN zona compacta; TCS, transcranial sonography; UPS, ubiquitin-proteasome system.

agent, hydrogen peroxide is generated during normal metabolism, for instance, during electron transport in mitochondria. This can be subsequently converted to the extremely toxic hydroxyl radical in the presence of iron via the Fenton reaction and Haber-Weiss reaction (Youdim and Riederer 1993). Subsequently, these free radicals may elicit cellular damage, lipid peroxidation and eventually cytotoxic processes such as apoptosis evoking cellular destruction (Gutteridge 1994).

Iron in neurodegenerative disorders: Parkinson's disease

Reduced iron content in substantia nigra (SN) has been linked to the neurological disease, such as restless legs syndrome (Connor *et al.* 2004). Severe iron deficiency may halt growth and induce cell death. Additionally, an iron deficit during development may cause irreversible motor and cognitive dysfunction (Beard and Connor 2003).

Conversely, brain iron accumulation has been implicated in a host of chronic neurological diseases (Table 1).This notion is supported by its distribution in the brain regions that exhibit pathology characteristic of the relevant disorder. Elevated brain iron levels are associated to neurodegenerative disorders that can be broadly divided into two categories. Firstly, it is associated to specific regional brain iron accumulation, although it is unclear whether this represents a cause or a result of neuronal destruction. Secondly, a consequence of malfunction in iron homeostasis, that is the primary cause of the disease (Berg *et al.* 2002a,b; Zecca *et al.* 2004).

Table 1 Chronic neurodegenerative disorders exhibiting changes in brain iron levels

Parkinson's disease
Alzheimer's disease
Multiple sclerosis
Amyotrophic lateral sclerosis
Friedreich's ataxia
Aceruloplasminemia
Prion's disease

(Modified and adapted from Youdim and Riederer 2004).

The iron within the normal brain exhibits a heterogeneous pattern of distribution both regionally and cell-type dependent. The rank order of total iron distribution in normal brain tissue was globus pallidus > putamen > substantia nigra > caudate nucleus > cerebral cortex = cerebellum (Dexter *et al.* 1993). It is most abundant in areas that are rich in dopaminergic neurons namely, the globus pallidus, putamen and SN of the basal ganglia (Dexter *et al.* 1993; Youdim and Riederer 2004). Thus, iron concentration may confer a predilection to neuronal degeneration and subsequently precipitate basal ganglia movement disorders (Table 2). This concept is demonstrated and supported by the cognitive decline and movement difficulties observed in neuroferritinopathy (Table 2), a genetic disease characterised by abnormal deposition of iron in the basal ganglia (Wills *et al.* 2002). Basal ganglia disorders account for a significant proportion of motor disability arising from neurological disorders.

Dysregulations of brain iron due to a genetic component have been reported in a number of movement disorders (Table 2, Gerlach *et al.* 2006). Genetic mutation(s) may alter the expression and thus function of proteins involved in iron homeostasis and /or its metabolism resulting in its abnormal accumulation in the brain. Changes in brain iron and the iron storage protein ferritin have been found in a prevalent motor disease, Parkinson's disease (PD) (Riederer *et al.* 1985; Dexter *et al.* 1987). The presence of iron deposition and increased free iron concentration in the basal ganglia appears to play a pivotal role in the manifestation of Parkinsonian-like features or Parkinsonism caused by dopamine deficiency.

Table 2 Basal ganglia diseases: iron accumulation in brain and Parkinsonism

Disease	Neuropathology, principle location of iron deposition	Clinical symptoms
Parkinson's disease	Substantia nigra	I° Parkinsonism
Multiple systems atropy	Substantia nigra, caudate-putamen	2° Parkinsonism
Progressive supranuclear palsy	Substantia nigra, putamen	2° Parkinsonism
Hallervorden-Spatz syndrome[a]	Basal ganglia	2° Parkinsonism
Pantothenate kinase-associated neurodegenerarion[a]	Basal ganglia	2° Parkinsonism
Neuroferritinopathy[a]	Basal ganglia	2° Parkinsonism
Hypoprebetalipoproteinemia acanthocytosis and retinitis pigmentosa with pallidal degeneration	Basal ganglia	2° Parkinsonisni

Modified from Gerlach *et al.* 2006.

I°, primary; 2°, secondary.

[a]Diseases exhibiting genetic abnonnalities in iron regulation.

Indeed, this phenomenon is demonstrated by a diverse spectrum of motor disorders (Table 2), that all exhibit two common features abnormal iron deposition in this area of the brain and parkinsonism (Gerlach *et al.* 2006).

Interestingly, it appears that elevation of total nigral iron is greater in progressive supranuclear palsy and multiple systems atrophy (MSA) (70% and 59% respectively, Dexter *et al.* 1993), compared with PD (35%). This may be related to shorter disease duration although this might be associated with other factors independent from nigral cell loss. However, the higher iron deposition in the substantia nigra may represent a greater vulnerability to iron-related cell destruction. Therefore, the increase in iron content may exacerbate the neuronal destruction cascade and/or may indirectly contribute to the average duration of the disease. Thus, the brain iron deposition may be inversely related to the duration of the disease. Indeed, progressive supranuclear palsy (a phenotype called Richardson syndrome has a duration of 4 years) has a shortest duration compared to multiple systems atrophy (average duration of 7 ± 0.9 years) or PD (mean duration of 14 years). However, in Huntington disease, the substantia nigra is spared from the impact of the disease, and instead there is marked pathology and iron deposition in the caudate nucleus and putamen and no Parkinsonian-like clinical features (Dexter *et al.* 1993), therefore cementing the involvement of the nigral iron overload and the clinical manifestation of PD.

Parkinson's disease was first described as *scelotyrbe festinans* (abnormal gait characterised by quick short steps) by Boissier de Sauvages (1768). Subsequently, James Parkinson (1817) identified the illness as *paralysis agitans* (shaking palsy). PD is a chronic progressive neurodegenerative disorder, which begins insidiously and gradually worsens in severity. Majority of PD cases are idiopathic with a sporadic occurrence. This is often referred to as primary PD. In contrast, Parkinsonism (Fahn and Sulzer 2004) is a secondary phenomenon to other syndromes (Table 2), such as post-encephalitic, drug-induced, Parkinson-plus MSA subtypes and heredodegenerative diseases. There are rare occurrences of familial PD, where genetic factors are known to be associated with the cause of the disease. Twin studies assessing the concordance rates in monozygotic and dizygotic twins, suggest that a genetic component may serve a vital role in younger onset patients (< 50 years of age) as opposed to older onset ones (Tanner *et al.* 1999). Thus, it is highly likely that a number of factors may contribute to the neuronal destruction observed in PD.

A host of mechanisms have been suggested in the pathogenesis of PD including oxidative (nitrative) stress, inflammation, altered proteolysis, mitochondrial dysfunction. The loss of greater than 50–60% of the neuromelanin (NM)-containing dopaminergic neurons primarily in the lateral ventral tier of the SN zona compacta (SNc), results in a marked reduction in dopamine concentrations (70–80%) in the striatum (mainly in the putamen). This results in the clinical manifestation of the disease. Indeed, the reduction of SNc neurons reduces the nigrostriatal dopaminergic projections and decreases the 'direct' inhibitory pathway of the basal ganglia motor circuit resulting in the over activity of the 'indirect' pathway and the subsequent development of Parkinsonian motor signs (Wichmann and DeLong 1993). Also, the reduction in NM-containing neurons produces the characteristic 'bleached' appearance of the SN in PD. The presence of eosinophilic cytoplasmic inclusions, Lewy bodies (LB), in the remaining surviving neurons are the pathological hallmark of the disease, although LB are not exclusive to the disorder.

Alterations in iron levels in PD (Table 3) have been demonstrated using both quantitative (biochemical analysis using postmortem brain tissue) and semi-quantitative [such as histochemical methods, transcranial sonography (TCS), magnetic resonance imaging (MRI) techniques] methods.

Changes in iron content (Table 3) in the basal ganglia (globus pallidus) in PD were first demonstrated in 1924 (Lhermitte *et al.* 1924). Subsequent studies employing different techniques demonstrated an increase in total iron and non-heme ferric iron (Fe^{3+}) in SN (Earle 1968; Riederer *et al.* 1985; Dexter *et al.* 1991). These findings were further endorsed by histopathological and *in vivo* brain imaging techniques. More importantly, the biochemical analysis of iron deposition in PD has been found be mainly in the SNc (31%, Table 3) compared with the total SN (35%, Table 3; Dexter *et al.* 1991). This suggests an important role of the iron changes in PD because the SNc bears the brunt of the pathology of the disease. Indeed, studies using highly specific particle-induced X-ray emission have shown that the iron content in the SN zona reticulata is greater than the SNc in control subjects, but this pattern is reversed in PD (Morawski *et al.* 2005). Elevated iron levels and reduced antioxidants may compromise the cellular defence mechanisms ensuing in free radical-mediated neuronal destruction.

The iron changes observed in SN in PD produces a shift of the Fe^{3+} : Fe^{2+} from 2 : 1 in normal subjects to 1 : 2 in patients with PD (Riederer *et al.* 1988; Sofic *et al.* 1988). This shift to the more toxic iron form may result in the production of hydrogen peroxide-derived reactive hydroxyl radicals (via Fenton reaction and enhanced Haber-Weiss cycle). Lipids with unsaturated fatty acids in cell membranes can donate electrons to hydroxyl radicals causing oxidative destruction of membranal lipids. This notion is demonstrated by the increase in markers for lipid peroxidation that is an elevation in basal levels of malondialdehyde and lipid peroxides (Dexter *et al.* 1989a).

In contrast, studies employing Mössbauer spectroscopy (investigates the electronic structure of iron complexes), atomic absorption spectroscopy or colorimetry analysis, reported no significant changes in iron content in the SN (Uitti *et al.* 1989; Loeffler *et al.* 1995; Galazka-Friedman

Table 3 Semi-quantitative and quantitative analysis of changes in iron levels in basal ganglia in PD

Analysis	SN	Putamen	Globus pallidus	Authority
Semiquantitative				
Histological staining; X-ray fluor. spectroscopy		General ↑	In basal ganglia ↑	Lhermitte *et al.* 1924; Earle 1968
Energy disperse X-ray analysis	↑			Jellinger *et al.* 1992
Magnetic resonance imaging	↑	↓	↓	Riyvlin *et al.* 1995
Transcranial sonograph	↑	General ↑	In basal ganglia	Becker *et al.* 1995; Berg *et al.* 2002b
Quantitative				
Flame spectrophotometry	↑ 1.77(S)	0.81(NSC)	1.20(NSC)	Riederer *et al.* 1985
Inductively coupled plasma spectroscopy	↑ 1.35(S)(t)	1.04(NSC)(l)	↓ 0.71 (SK)(l)	Dexter *et al.* 1989b
	↑ 1.31(S)(zc)	0.83(NSC)(m)	↓ 0.71(S)(m)	Dexter *et al.* 1991
Laser microprobe mass analysis	↑ 1.45(S)			Good *et al.* 1992
Extended X-ray absorption fine structure	↑ 2.01(S)	1.23(NSC)	↑ 1.43(S)(l)	Griffiths *et al.* 1999
Atomic absorption and atomic emission spectroscopy	NSC	caudate N. NSC	NE	Uitti *et al.* 1989
FerrochemII serum iron/TIBC analyser	NSC	NSC	↑	Loeffler *et al.* 1995
Mössbauer spectroscopy	NSC			Galazka-Friedman *et al.* 1996

Table modified from Goetz *et al.* (2004).

Values are expressed as a change in iron mean values in PD compared with mean values in normal subjects.

S, significant change; NSC, no significant change; t, total; zc, zona compacta; l, lateral; m, medial; NE, not estimated.

et al. 1996). These discrepancies are most likely to be related to variability in method sensitivity. For instance, Mössbauer spectroscopy only detects ^{57}Fe, which is a low occurrence isotope in the brain (Gerlach *et al.* 1995), this may account for the undetected iron elevation. Another factor which may also contribute to the absence of iron changes could be the brain tissue obtained from patients with different duration of the disease. Indeed, no nigral iron changes (Dexter *et al.* 1992) have been found in pre-symptomatic PD or incidental Lewy body disease (although this has been disputed) or mild PD (Riederer *et al.* 1989). In contrast, the elevation of iron in the SN in severe PD is well documented (Table 3).

Similarly, MRI studies support the positive correlation between degree of nigral iron elevation and severity of the motor symptoms (Bartzokis *et al.* 1999) and not duration of the disease (Wallis *et al.* 2008). Instead, excessive iron deposits in the subthalamic nucleus were correlated to the duration of the disease (Kosta *et al.* 2006). An increase of MRI field-dependent relaxation rate (R_2) (FDRI) in the SN has been found in early onset PD, whereas in late onset PD there was a decrease in FDRI (Bartzokis *et al.* 1999). An increased MRI (FDRI) corresponds to an elevation in specific iron pool (ferritin iron is known to increase R_2) (Vymazal *et al.* 1999; Behnke *et al.* 2009). These findings are consistent with reduction of nigral ferritin levels in older PD patients using biochemical analysis (Dexter *et al.* 1993) and conform to the hypothesis of some malfunction in brain iron metabolism/regulation. Also, PRIME magnetic resonance sequence has shown a positive correlation to the reduction in iron levels in the putamen and disease duration (Graham *et al.* 2000). Although, this finding contradicts the significant reduction in iron content found only in the globus pallidus region (medial and lateral) in PD (Dexter *et al.* 1993; Table 3) using biochemical analysis. The sensitivity and specificity of MRI in the detection of iron deposition needs to be carefully considered before commenting on such anomalies (Haacke *et al.* 2005). Nevertheless, most of these semi-quantitative methods for brain analysis have complemented and reflected the iron changes obtained using biochemical analysis of postmortem tissue (Table 3). More importantly, *in vivo* MRI evaluation, TCS and histochemical methods have proved to be useful tools for *in vivo* characterisation and localisation of brain iron neurodegenerative disorders.

Transcranial sonography allows the two-dimensional visualisation of the brain. TCS studies have depicted an iron-elicited increase in echogenicity in the basal ganglia and SN in PD (Becker *et al.* 1995). Similarly, augmented brain iron levels have been reported in a group of asymptomatic subjects of which 60% exhibited a reduction of striatal radiolabelled fluoro-dopa thus reflecting savaged dopaminergic nigrostriatal pathway (Berg *et al.* 1999; Iron-induced neurodegeneration; oxidative stress section). However, critical remarks are related to the role of hyperechogenicity of SN in PD because nearly 10% of healthy individuals (Berg *et al.* 1999, 2002a,b), patients with depression (Walter *et al.* 2007; Hoeppner *et al.* 2009) and patients with Attention Deficiency Hyperactivity Disorder (Romanos *et al.* 2010) all show significant hyperechogenicity of SN while not demon-

strating PD. Nevertheless, hyperechogenicity has been related to increased iron in the SN (Berg *et al.* 2002a,b; Zecca *et al.* 2005).

The significant elevation of total iron in PD in the SN, an area marked with pathological changes suggests an integral role of the iron-related oxidative neuronal destruction. Extensive studies using postmortem brain tissue from PD patients have furnished burgeoning evidence for the involvement of oxidative stress in the pathogenesis of the disease process. These findings suggest that oxidative cellular damages represent a series of molecular event eventually leading to the destruction of nigral neurons. Oxidative stress and its relevance to PD will be discussed in greater detail in ''consequences of increased iron in Parkinson's disease'' section.

The sources of augmented iron could be (i) dysregulation of iron homeostasis caused by pathological variation in iron tissue distribution or (ii) a malfunction in molecules involved in sequestrating excess iron such as caused by pathological variation in iron tissue distribution ferritin and NM or (iii) ageing is associated with accumulation of bound iron in the brain. Nevertheless, these putative pathways are likely to be interlinked.

Potential mechanism(s)/sources of the elevated iron levels in PD

Iron homeostasis

In view of the fact that either iron deficiency or iron overload can elicit cellular deleterious events, therefore, its distribution and storage is tightly regulated in the human body. Maintenance is also important as it has no regulated means of excretion and it readily reacts with peroxides to produce cellular toxic-free radicals.

Cellular iron homeostasis is largely maintained by the expression of proteins associated with its uptake, utilisation and storage. The regulation of iron is heavily dependent on its cellular access mediated via transferrin receptors and its storage protein ferritin. Iron (Fe^{2+}) absorbed from the duodenum binds to transferrin and circulates in the blood. Subsequently, the iron is taken up into the cells via transferrin receptors, where it is stored in the centre of metalloproteins. Excess iron in mucosal cells is stored as ferritin and is lost when these cells are shed in the gut. Iron-mediated cellular toxicity ensues when excess iron levels overwhelm the binding capacity of transferrin. Transferrin regulates the expression of the hormone, hepcidin. This hormone controls the expression of the iron transporter ferroportin and thereby regulating intestinal iron absorption and iron release (Nemeth 2008).

The iron regulation is dependent on iron-responsive element-binding proteins present in the cytosol. Iron-responsive element-binding protein is capable of undergoing reversible change depending on iron availability. In the brain, there are many pathways in which iron can be transferred across the blood–brain barrier (BBB) including the transferrin receptor-mediated uptake, divalent cation metal transporter (DCT1) and lactoferrin receptor (Ponka 2004).

Studies employing radiolabelled iron-transferrin (Moos and Morgan 2004) suggest that iron transport across the BBB occurs mainly via receptor-mediated endocytosis of iron-containing transferrin. Then, the brain capillary endothelial cells absorb iron-bound transferrin from the blood via receptor-mediated endocytosis. This is followed by the release of iron from the endosome into the brain interstitial fluid by the iron exporter ferroportin (Wu *et al.* 2004). Once in the brain interstitium, the iron may bind to large molecules such as transferrin or lactoferrin. Also, transferrin secreted from the oligodendrocytes plays a vital role in binding and transporting iron in the brain interstitium. The presence of transferrin receptors on neurons and not glial cells lead to the suggestion that glia may uptake iron via a mechanism not involving transferrin receptor. Indeed, the marked presence of ferritin in glia indicates that these cells do acquire iron.

It is vital to consider the biochemical and anatomical aspects of normal physiological iron regulation to postulate its role in neurodegeneration. Dysfunction of brain iron homeostasis may ascribe for the changes of brain iron in PD.

This abnormality in iron regulation may in turn be associated to some genetic component, for instance, the disorders highlighted in Table 2. More importantly, a mutation of genes related to iron transport and binding was demonstrated by the high frequency of G2578S transferrin polymorphisms in late onset PD only (Borie *et al.* 2002). Indeed, MRI-FDRI evaluations of brain iron in PD (Fig. 1a) have illustrated a dysfunction in iron homeostasis that may be different in the early onset PD in contrast to late onset PD (Bartzokis *et al.* 1999).

A recent *in vitro* study reported L-dopa induced increased expression of DCT1 and thus an increase of cellular ferrous uptake, suggesting that active iron may also be associated to levodopa related neurotoxicity in cortical neurons (Fang *et al.* 2009). More importantly, an elevated expression of an isoform of DCT1 has been found in SN in PD (Salazar *et al.* 2008). This may ascribe for the mechanism associated with iron accumulation observed in the disorder. Similarly in MPTP-treated mice, there was an increased expression of DCT1 in the ventral mesencephalon and a corresponding iron deposition and dopaminergic cell loss. Thus, DCT1 plays an instrumental role in iron accumulation and consequent oxidative-mediated cell damage.

Alternatively, there may be some non-genetic causes involved in the misregulation of iron at various stages, for instance, uptake and release, storage, intracellular metabolism (Ke and Qian 2007).

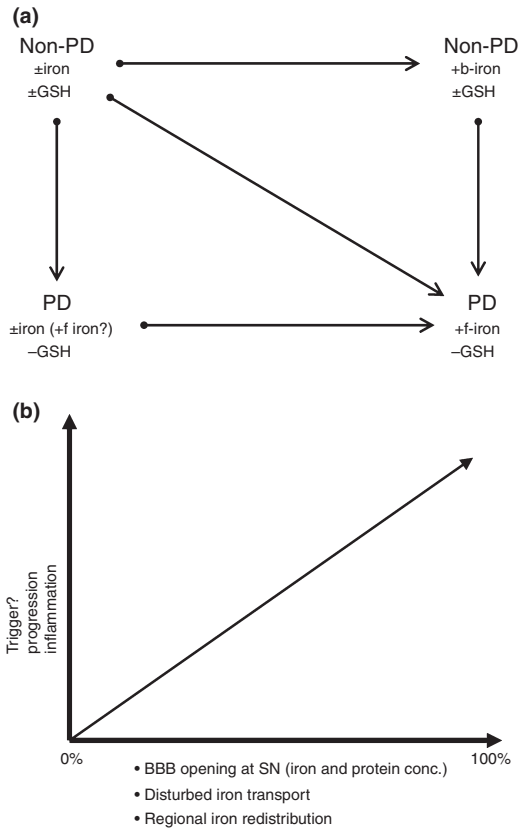

Fig. 1 (a) A hypothetical model based on conflicting publications that demonstrate no or significant involvement of iron pathology in PD; (±) physiological concentration of iron or GSH, (+) increase, (–) decrease, (b-iron) bound iron (ferritin, neuromelanin), (f-iron) free iron, (GSH) reduced glutathione. (b) A hypothetical model characterising variation of blood–brain barrier (BBB) disturbances causing variation in SN iron and protein uptake from the periphery. However, other possibilites of increased SN iron may be a disturbed iron transport mechanisms or regional iron redistribution. Variations in triggering and in the progression of PD pathology and inflammatory processes would be respective consequences.

A damaged or 'leaky' BBB in the SN (Fig. 1b) may serve as a potential peripheral source for the elevated iron content or even allow entry to neurotoxins into the brain and the resultant accumulation of proteins (Oesterreicher *et al.* 1994; Lewy body formation section). The p-glycoprotein plays a regulatory role in the maintenance of BBB function (Tan *et al.* 2002). It serves as an efflux pump that does not normally allow verapamil hydrochloride (a substrate for p-glycoprotein) to cross the BBB. However, PET studies have shown a high uptake of radiolabelled verapamil into the frontal white matter areas in advanced PD and progressive supranuclear palsy patients (Bartels *et al.* 2008). This probably mirrors frontal dysfunction of affected frontostriatal pathways. In early PD patients, in contrast, there is a lower verapamil uptake in the mid brain and frontal regions thereby demonstrating some malfunction of p-glycoprotein system in the BBB in the later stages of the disease. This dysfunction in the BBB is probably exacerbated with increasing age of the patients in view of the reduced function of p-glycoprotein in the BBB with respect to ageing (Bauer *et al.* 2008).

An enhanced expression of DCT1 in the dopaminergic neurons in the SN in PD (Qian and Wang 1998), may contribute to an increase of iron transport to this region. Also, the elevation of nigral lactotransferrin receptors in PD may represent a pathway for the iron deposition into this area (Faucheux *et al.* 1995; Leveugle *et al.* 1996). However, the reduction of transferrin receptor binding in the SN in PD (Faucheux *et al.* 1995) may represent its down-regulation in response to the raised nigral iron content.

It has been suggested that most of the iron transported via transferrin receptor is concentrated in ferritin and NM. Therefore, analysis of the ferritin and NM system provide vital clues in the elucidating the mechanism(s) attributing to the increased iron content observed in the illness.

Ferritin

Ferritin is the most common iron storage protein in the brain. It comprises two types of sub-units: heavy (H) and light (L), which work in complementary ways to store intracellular iron (Connor *et al.* 1994). H-ferritin is found mainly in the neurons, where it sequesters iron and is found in organs where there is little iron storage and high iron utilisation. In contrast, L-ferritin is associated with iron storage and is expressed on microglia and also detected in NM granules. Oligodendrocytes express both H- and L-ferritin (Zecca *et al.* 2004).

Iron bound to ferritin is considered non-toxic as it does not allow iron to react with molecules in a manner that can evoke cellular damage. However, a breakdown of protective ferritin complex can lead to an overload in free iron, which in turn can be highly neurotoxic via the production of free radicals and mediation of lipid peroxidation of cell membranes. After the brain uses the iron it has stored, it may leave the cells with the aid of copper-associated protein ceruloplasmin.

Ferritin may be associated with the pathogenesis and/or the clinical features of the disease. This is based on the mutation of genes encoding for L-ferritin and parkinsonian features observed in autosomal dominant adult-onset basal ganglia disease (Curtis *et al.* 2001). Nonetheless, there have been contradictory findings reported in the changes in nigral ferritin levels in PD. An up-regulation of ferritin expression in PD (Jellinger *et al.* 1990) would be the normal physiological response corresponding to the increase of iron content observed in PD. However, a significant decrease of SN ferritin levels in PD was found by others (Dexter *et al.* 1991; Faucheux *et al.* 2002). Furthermore, the ferritin in SN in PD has been reported to be more heavily laden with iron compared with normal subjects (Griffiths *et al.* 1999). This would suggest that inadequate ferritin is present, which is unable to handle the iron overload. The ferritin deficiency has been contributed to the sustained activity of iron

regulatory protein-binding activity that represses ferritin synthesis (Faucheux *et al.* 2002). This clearly supports the notion of a dysfunction in iron homeostasis. A reduction of serum ferritin and iron was reported in PD (Connor *et al.* 1995). This clearly supports a misregulation of iron handling in the brain and periphery in PD. More importantly, it demonstrates that normal up-regulation of ferritin associated to ageing is absent in PD (Connor *et al.* 1995). Similarly, a disruption of iron homeostasis is also implicated in Alzheimer's disease, although a decrease in the iron transport protein transferrin is involved and not the iron storage protein (Connor *et al.* 1992).

Another pathway that may contribute to the increased iron in PD may involve the release of iron from ferritin mediated by endogenous or exogenous reactive chemical species through reductive mechanisms (Lapenna *et al.* 1995). Indeed, glial cells generate significant amounts of toxic superoxide- and nitric oxide-derived free radicals from L-arginine. Subsequently, these free radicals can release iron from ferritin and nitric oxide can inhibit activity of mitochondrial complexes I and II, thereby exacerbating oxidative damage (Yoshida *et al.* 1995). Furthermore, superoxide and nitric oxide can combine to yield highly cellular toxic peroxynitryl radical. Exogenous dopaminergic toxins such as 6-hydroxydopamine (6-OHDA) can also release iron from ferritin, leading to the iron-generated free radical-induced lipid peroxidation of cells (Double *et al.* 1998). The involvement of iron is confirmed by the abolishment of lipid peroxidation in the presence of an iron chelator. Oxidation of endogenous dopamine can also produce 6-OHDA, which in turn releases the iron from the storage protein ferritin. Indeed, i*n vitro* studies have shown 6-OHDA can be produced from dopamine under oxidative stress conditions (Napolitano *et al.* 1995). Therefore, perhaps this occurs in the disease state and the release of 6-OHDA exacerbates the free radical-operated oxidative stress in the SN. Interestingly, the detection of a '6-OHDA' -like compound in the urine of PD patients supports this contention (Andrew *et al.* 1993).

However, ferritin is localised primarily in glial cells (mainly in the oligodendrocytes) (Connor *et al.* 1994); it therefore appears unlikely that free radicals produced in the glia would diffuse across the intracellular space and destroy about 70% or more of the dopaminergic nigral neurons. In contrast, the presence of L-ferritin in NM granules (Tribl *et al.* 2009) would support the involvement of cytotoxic mechanism operating within the SNc neurons. Nevertheless, the role of ferritin and its pathology in glia for PD and its progression waits for being elucidated in detail.

Neuromelanin

Neuromelanin (NM) is a brownish-black pigment. NM is another iron sequestrate found in high concentrations in the dopaminergic and noradrenergic neurons in SN and locus coeruleus (LC) respectively. It is produced by the auto-oxidation of dopamine or noradrenaline. However, NM does not only represent a product of dopamine autoxidation (Sulzer and Zecca 2000; Sulzer *et al.* 2000, Tribl *et al.* 2005), because in PD patients treated with high concentrations of the dopamine precursor levodopa (L-dopa), an increase in NM content in the remaining surviving neurons has not been reported.

Neuromelanin may serve a physiological role in the iron storage. Indeed, about 50% of the total NM is saturated with iron under normal physiological conditions, suggesting a neuroprotective role by virtue of sequestering redox active iron. The association of NM to iron is highlighted by findings using particle-induced X-ray emission (Morawski *et al.* 2005). Evidence suggests that NM acts to reduce potentially toxic iron by chelating it in the cytosol of neurons (Zucca *et al.* 2006). The dihydroxyindole units present in the melanic groups of NM is attributed to its preference to chelate iron in comparison with other metals (Zecca *et al.* 2004). Iron has been found to be directly bound to the NM in the SN (Ben-Shachar and Youdim 1993; Zecca *et al.* 2006) and this contributes for the increased signal emitted in PD (Kienzl *et al.* 1995). Interestingly, Tribl *et al.* (2009) have demonstrated the presence of L-ferritin as a component of NM granules in human SN. This finding galvanises the notion that NM does not only represent a breakdown product of dopamine and noradrenaline, but also serves a physiological role in iron homeostasis and thus is neuroprotective against free iron-mediated oxidative stress. Additionally, this reflects the operation of two iron storage systems within the nigral NM containing neurons, one that is NM mediated and the other is an L-ferritin based.

In PD, there is significantly less iron bound to NM resulting in an increase in the availability of redox-active (free) iron in the SN. NM may therefore be involved in maintenance of intra neuronal iron homeostasis. Furthermore, this elevation has been shown to correlate with neuronal cell loss in this area. This supports the involvement of NM mediated modulation of iron reactivity in PD (Faucheux *et al.* 2003).

Interestingly, the degenerative process appears to exhibit a pre-dilection to the NM-containing nigral neurons with high iron content and no calbindin (calcium-binding protein) D28K. These cells are more susceptible to iron-mediated oxidative stress. Calbindin D28K preserves neuronal cells as a result of blocking calcium-mediated apoptosis-induced cell death. Thus, the remaining surviving neurons in PD do not contain NM, show low iron levels and are calbindin D28K positive (Hirsch 1994; Saper 1999). Thus, this preferential neuronal loss illustrates a pattern of selective degeneration and the role of calcium ions in executing cell death. It also implicates NM-derived iron in the mediation of oxidative damage and thereby highlighting the involvement of iron in the pathogenesis of PD. Therefore, perhaps the iron remaining from these degenerated neurons contributes to its

increase observed in SN homogenates from postmortem brain tissue. However, the neuropathological findings indicate that the ventral tier of SNc (an area poor in NM) bears the onslaught of the disease in contrast to the pigmented-rich dorsal tier that is spared (Gibb 1992). This may suggest a protective role of NM, where it may directly be involved in the inactivation of free radicals or indirectly by chelating iron. This role is demonstrated by *in vitro* studies, where NM significantly reduced basal and iron-stimulated lipid peroxidation in rat cortex (Ben-Shachar *et al.* 1991). NM has indeed been reported to be formed also in pre-motor cortex (Ward *et al.* 2009).

However, other studies have demonstrated the cytotoxic role of NM; indeed addition of iron saturated NM produced significant cell damage (Double *et al.* 1999). Also, NM added to primary cultured neurons produced reactive oxygen species via (i) free radicals produced from iron liberated from NM and (ii) oxidation of semi-quinone radicals (Depboylu *et al.* 2007). *In vitro* studies using SH-SY5Y cells have shown NM to cause oxidation of reduced GSH and apoptotic cell death (Naoi *et al.* 2008). Indeed, nuclear magnetic resonance spectroscopy analysis shows that the NM protective role may be compromised in PD as illustrated by the reduction in its ability to bind to iron (Aime *et al.* 2000). This reduction in NM ability to bind to excess iron may be due to an alteration in its binding ability as a consequence of the disease process or alternatively perhaps its binding capacity is overwhelmed by elevated iron content. Consequently, this would result in an increase in the availability of free iron, which would in turn initiate oxidative damage to the neurons. This is in accordance with the increase in redox activity of iron in NM aggregates from PD patients, where about 70% of the melanised neurons are lost (Faucheux *et al.* 2003).

Ageing

Ageing is associated with cell damage, cell repair and progressive inflammation. The normal accumulation of iron in the brain is associated to ageing. In particular, iron accumulates in the SN with respect to age until the fourth decade after which it stabilises (Zecca *et al.* 2001). Similarly, NM accumulation is also associated with ageing in normal subjects; however, in PD, a significant decrease of NM was demonstrated (Zecca *et al.* 2002). This deposition has also been associated to the cognitive decline in the elderly. Interestingly, iron deficiency anaemia has been shown to adversely affect cognition and motor performance in human and animals (Khedr *et al.* 2008). This illustrates the importance of maintaining the regulation of iron homeostasis and the fine balance of iron that exists in the brain and periphery.

An up-regulation of ferritin expression occurs with ageing; however, this system is not operational in PD (Dexter *et al.* 1994), perhaps because of the malfunctioning of iron homeostasis in the disorder.

In PD, neuronal degeneration is observed in both the SN and LC, although the LC is less markedly affected. A comparative analysis of iron-related neuronal susceptibility in the SN and LC during ageing (Zecca *et al.* 2004) illustrated: (i) a higher iron content in the SN compared with LC; (ii) a higher ferritin : iron ratio in the LC in contrast to the SN; (iii) similar NM levels; however, the nigral NM contained higher iron levels compared with LC. The higher iron content in SN could be due to its steady increase with respect to age, whereas in LC the iron content remains constant throughout the life. Consequently, the higher iron content present in the SN in comparison with the LC may ascribe for its vulnerability to iron-mediated oxidative damage in disease state in PD.

Reactive oxygen species are considered to be contributors to the ageing process. Endogenously produced free radicals normally generated by metabolic processes evoke oxidative modifications to lipids, proteins and genetic components. Ageing may pose as an important risk factor in the expression of idiopathic PD. It has been suggested that PD is a disease of 'accelerated ageing' (Fahn and Sulzer 2004). However, the pattern of neuronal cell loss within SN is different. Neuronal loss caused by ageing demonstrates regional variations within SNc, where the greatest cell loss is observed in the dorsal region (6.9% per decade) and the least in the lateral ventral region (2.1% per decade; Fearnely and Lees 1991). Whereas in PD, the neuronal loss appears to be mainly concentrated in the lateral ventral tier (90%) of SNc, in contrast in ageing there is a preferential cell loss in the dorsal tier (56%) of the SNc. Thus, other factors must be involved in the aetiology of the disease that confers to the susceptibility chiefly of the ventral area of the SNc.

Consequences of increased iron in Parkinson's disease

Iron-induced neurodegeneration; oxidative stress

The increased iron-associated MRI contrasts in SN in living PD patients exhibited a direct correlation to the motor performance (Gorell *et al.* 1995). Interestingly, MRI assessment of age-related iron accumulation in the striatum has been shown to be inversely correlated to decline in motor performance in rhesus monkeys (Cass *et al.* 2007). Also, intranigral administration of iron produces selective dopaminergic neuronal loss and consequently eliciting parkinsonian features both biochemically and behaviourally (Riederer and Wesemann 1995; Youdim 2003). Iron deposition in the subthalamic nucleus has been associated with the duration of the disease. This may mirror an association between the deposition of iron and clinical manifestation of PD. Thus, augmentation of the nigral iron in PD may directly correlate with the severity of clinical symptoms.

Indeed, in pre-symptomatic PD or incidental Lewy body disease (although this remains debatable) there are not any changes in iron or ferritin content in SN or manifestation of clinical symptoms of PD (Dexter *et al.* 1994). Dexter *et al.* (1994) found no significant changes in striatal dopamine levels in incidental Lewy body disease suggesting that nigral iron accumulation may be secondary to neuronal loss. Thus, iron changes observed in the SN in PD are more likely to represent a secondary phenomenon (Hirsch and Faucheux 1998). Interestingly, some have found no changes in nigral iron content in PD (Uitti *et al.* 1989; Loeffler *et al.* 1995; Galazka-Friedman *et al.* 1996; Friedman *et al.* 2009). However, it may well be that total iron measurement does not reflect the pathological status. At unchanged total iron concentration, an increased free iron concentration could well be relevant to induce changes leading to oxidative stress and pathological dopaminergic transmission.

However, Becker *et al.* (1995) and Zecca *et al.* (2004) reported an elevation of nigral iron in a few subjects with incidental Lewy body disease. Subsequently, they also found an increased nigral echogenicity in some healthy subjects with decreased striatal uptake of radiolabelled fluoro-dopa (Zecca *et al.* 2005). Clearly, these diverse findings warrant for further clarification using a greater number of subjects with asymptomatic PD. Methodological issues may also play a critical role in this arena and may attribute to these conflicting findings to the different methods employed (Gerlach *et al.* 2006).

Alternatively, the differences or even overt PD may suggest sub-groups as proposed by one of us (Peter Riederer Fig. 1a,b) of asymptomatic PD, incidental Lewy body disease, one sub-group with no significant changes in nigral iron and ferritin content or striatal dopamine, a marked decrease in GSH in SN, plus Lewy body pathology (Riederer *et al.* 1989; Sian *et al.* 1991; Dexter *et al.* 1994) and a second sub-group exhibiting increase in nigral-free iron content, reduction in striatal dopamine, plus Lewy body pathology (Zecca *et al.* 2005). This concept of sub-groups concords with (i) the heterogeneity proposed in early symptomatic PD (Lewis *et al.* 2005) including closed or open BBB for iron transport (Oesterreicher *et al.* 1994) (Fig 1b) and (ii) the findings from a recent study in which SN hyperechogenicity has been detected in depressed patients with mild motor dysfunction (Walter *et al.* 2007; Hoeppner *et al.* 2009). It was suggested that these depressed patients may subsequently pose an elevated risk of developing clinically defined PD. Thus, the stage at which iron executes an involvement in disease process may be different and thus characterising sub-groups of the illness. Then, the elevation of nigral-free iron in PD may initiate and/or contribute to the ongoing degenerative processes resulting in cell death. However, as long as iron taken up into the SN is bound to NM or ferritin it is not toxic even at high concentrations as shown in nearly 10% of healthy individuals with SN hyperechogenicity (Berg *et al.* 2002a,b) and in patients with attention-deficit hyperactivity disorder (Romanos *et al.* 2010). If subgroups based on variation of SN free iron content can be substantiated it remains interesting to study their phenotypes, disease progress and possibly therapeutic strategies. Also treatment with iron-chelators may be envisaged as early as iron toxicity is established. There are two strategies under discussion as follows: (i) usage of a peripherally acting iron chelator, like desferoxamine which according to our hypothesis would chelate free iron in SN by crossing an open BBB at the site of SN only and (ii) usage of a BBB crossing iron chelator, like M30, M30S or HLA 20 (as developed by Varinel, Inc., West Chester, PA, USA; Weinreb *et al.* 2010).

There are a number of mechanisms proposed in the pathogenesis of dopaminergic neuronal destruction in the SN in PD. These include mitochondrial dysfunction, oxidative and nitrative stress, inflammation, excitotoxicity and a failure in ubiquitin-proteasome system (UPS). There does not appear to be one cause of PD and indeed there seems to be a complex interplay between these various mechanisms. For instance, oxidative stress could evoke a defect in the activity of complex 1 in mitochondria and vice versa. It is almost like a cyclic set of events that are closely interlinked and finally share a common pathway leading to cell death via apoptosis (Jenner and Olanow 2006). Additionally, Zhu *et al.* (2004) have suggested an association between oxidative stress and non-apoptotic cell death that involves autophagic cellular processes.

Oxidative stress in Parkinson's disease

By definition, free-radical species contain one or more unpaired electron(s). Free radicals such as reactive oxygen species or reactive nitrogen species are products of normal metabolism. Under physiological conditions, the cellular defence system, such as antioxidant GSH, superoxide dismutase and catalase could inactivate these cytotoxic species.

However, in the disease state there may be an over-production of free radicals which may overwhelm the cellular protective mechanisms. Alternatively, the defence processes may be compromised and thus not functioning at optimal level. Under such circumstances, oxidative stress would ensue and trigger the cascade of events leading to cellular destruction. Free radical-mediated oxidative damage occurs in PD at various sites within the cell, such as lipid peroxidation of cellular membranes, damage of DNA, and produces toxic products such as 4-hydroxynonenal (HNE), which can react with protein and interfere with cellular function.

At present, there is evidence for the involvement of both reactive oxygen and nitrogen species in mediating oxidative stress. Free radicals are highly reactive and unstable; therefore, they cannot be directly employed as a measure of oxidative stress. Thus, other indices are used to demonstrate its occurrence. Changes in the cellular antioxidant

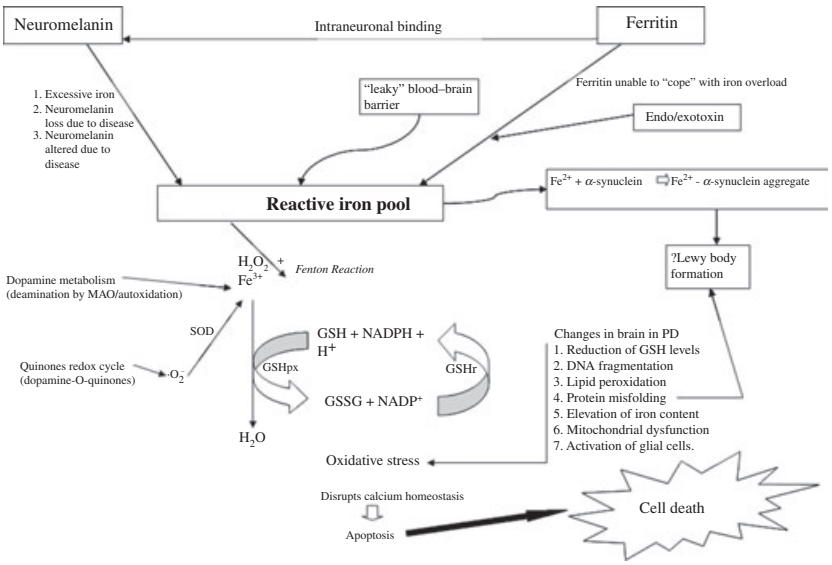

Fig. 2 Iron-mediated neuronal cell death in Parkinson's disease. Putative pathway sources for iron release, such as ferritin, neuromelanin (NM) or neurotoxins. Neurotoxins, such as MPTP, kainate, 6-hydroxydopamine (6-OHDA) can mediate release of iron. Alternatively, some malfunction in the blood–brain barrier, 'leaky', may also increase the access of iron into the brain. Subsequently, this reactive-free iron may react via the Fenton reaction with the hydrogen peroxide produced from example monoamine oxidase metabolised dopamine, to yield toxic and hydroxyl (OH·) free radical species. In addition, the reactive iron may cause α-synuclein to aggregate, which may also lead to the generation of OH· and/or Lewy body formations. These highly reactive OH· radicals may orchestrate a cascade of cellular deleterious events such as oxidative stress. Antioxidants such as GSH that comprises a major cellular defence system attempts to protect the cells from the onslaught of events elicited by oxidative stress, such as DNA damage, protein misfolding/damage, lipid peroxidation of the cell membrane, 'consumption' of cellular antioxidants. Finally, oxidative stress may disturb the cellular calcium homeostasis, thereby resulting in excitotoxicity and finally apoptotic-induced cell death. The elevated iron levels in the substantia nigra may thus play an instrumental role in the vulnerability and degeneration of this area, which is characteristic to Parkinson's disease. GSHpx, glutathione peroxidase; SOD, superoxide dismutase; GSHr, glutathione reductase.

system are also employed as a marker for oxidative damage (Jenner 1993).

It has been suggested that SNc normally exhibits high levels of basal oxidative stress and this is augmented in PD. Studies using postmortem brain tissue have provided compelling evidence for the involvement of oxidative stress in the pathogenesis of idiopathic PD (Fig. 2). The following changes in the SN in PD have been documented.

Lipid peroxidation of cell membranes can be catastrophic for cell viabilty. It can also result in the HNE production. Indeed, immunocytochemistry of the SN in PD shows elevated HNE concentrations (Yoritaka *et al.* 1996). Furthermore, increased levels of lipid peroxidation products (e.g. malondialdehyde) and a corresponding reduction of the substrate polyunsaturated fatty acids are found in the SN in Parkinsonian patients (Dexter *et al.* 1989a; Montine *et al.* 2004). The presence of free reactive iron can continue the propagation of lipid peroxidation. Iron can react with lipid hydroperoxides to produce alkoxyl radical, which in turn may react with polyunsaturated fatty acids, the substrate for lipid peroxidation. Oxidative stress can produce denaturation or aggregation of proteins and marked DNA damage. Reactive oxygen or nitrogen species may also oxidise proteins leading to some change in their structure and consequent dysfunction or inactivation of enzymes. These species may produce direct damage and consequent proteasomal dysfunction (Reinheckel *et al.* 1998).

Gene defects in familial PD associated with abnormal proteins such as three-point mutations of α-synuclein causes inheritable early onset PD (Olivares *et al.* 2009) or mutations of enzymes related to ubiquitination (such as Parkin) has lead to the notion of an altered UPS. This change in the UPS system may play an integral role in the pathogenesis of the disease. Oxidative stress may impair the UPS; this may in turn accelerate the degenerative process. Indeed, processes involved in oxidative damage such as lipid peroxidation may affect the proteasome system. This concept is supported by the modification of α-synuclein by acrolein (a product of lipid peroxidation) in the dopaminergic neurons in SN in PD (Shamoto-Nagai *et al.* 2007). This modification may result in protein aggregation, impair the proteasome system and evoke other cellular deleterious effects.

The ubiquitin system consists of the proteolytic enzyme, 26S proteasome. The 26S proteasome exerts ubiquitin-dependent proteolysis of oxidised or nitrated proteins. *In vitro* studies have demonstrated that iron released from NM may induce oxidative stress, which in turn decreases the activity of mitochondrial complex 1 activity. This would reduce ATP

generated and the activity of ATP-dependent 26S proteasome leading to the resultant protein aggregation in the neurons (Shamoto-Nagai *et al.* 2004). These protein aggregates may represent precursors for LB formation (McNaught *et al.* 2003). Thus, the failure to clear away proteins such as excess α-synuclein may result in the formation of α-synuclein-immunopositive LB and neuronal destruction.

Alternatively, protein aggregation may induce mitochondrial dysfunction and release of proapoptotic molecules that orchestrate cell death (Shults 2006). The availability of free iron (released from NM) may mediate oxidative damage, resulting in the formation of the oxidative product, HNE. Subsequently, HNE can also damage the 26S proteasome, which in turn may lead to its dysfunction and production of free radicals and oxidative and nitrative stress.

Interestingly, cells treated with HNE produce a series of events that implicate that the gathering of ubiquinated proteins and dysfunction of proteasomal activity may induce mitochondrial-dependent apoptotic dopaminergic cell destruction. These events include a decrease in proteasomal activity, a depletion of GSH and oxidative damage to lipids, DNA and proteins and eventually apoptotic cell death (Hyun *et al.* 2002). The HNE-mediated events bear a marked similarity to the changes reported in the SN in PD and thus support their involvement in the disease.

The hydroxyl radicals are associated with the formation of 8-hydroxy-2-deoxy-guanine from DNA and mark the occurrence of oxidative damage to DNA. Alternatively, this may represent a change in the cellular redox state to a more oxidising environment. Similarly, peroxynitrite can also induce DNA damage and result in the production of 8-hydroxy-2-deoxy-guanine (Byun *et al.* 1999).

The antioxidant GSH shows a marked depletion (40%) but GSSG remained unaltered (Sian *et al.* 1991, 1994; Sofic *et al.* 1992). The maintenance of GSH : GSSG ratio is vital for the cell survival. This depletion of GSH mirrors a compromised cellular protective capacity conferring a vulnerability of the dopaminergic nigral neurons to the cytotoxic species produced from dopamine metabolism through either autoxidation or deamination via monoamine oxidase. Furthermore, the antioxidant capacity of SN in PD was found to be lower compared with normal subjects (Sofic *et al.* 2006) thus highlighting a compromised antioxidant cellular defence system and thus accounting for a pre-dilection to oxidative damage. The importance of GSH in the pathogenesis of PD is illustrated as follows: (i) it is selectively found in the SN, that bears the brunt of the disease, (ii) this change is disease-specific and is not found in other basal ganglia disorders (Sian *et al.* 1992) and finally (iii) a similar depletion in nigral GSH content was found in asymptomatic PD (Sian *et al.* 1991), incidental Lewy body disease. Incidental Lewy body disease occurs in 10–15% of the population above 60 years that show no clinical features of PD. There were no changes in iron and ferritin levels or mitochondrial dysfunction. This reflects that the GSH deficit in SN represents an early event in the progression of the disease. This antioxidant depletion may represent a compromised state of a major cellular defence system in the primary asymptomatic stages of the disease, thereby increasing the vulnerability of dopaminergic neurons to oxidative stress and propagating cell death (Fig 1a).

The changes in other potential antioxidant enzymes such as GSH peroxidase, superoxide dismutase and catalase in the SN in PD remain discordant.

Interestingly, in a recent study depletion of GSH levels (*ca.* 50%) in rat mid brain dopaminergic cells by buthionine sulphoxamine (an inhibitor for GSH synthesis) treatment resulted in an increase of cellular iron-labile pool, reactive oxygen and nitrogen species (Kaur *et al.* 2009). Furthermore, the elevation of iron was found to be dependent on hydrogen peroxide and protein synthesis (via transferrin receptor translation). Indeed, it has been suggested that nitric oxide may inhibit GSH reductase resulting in changes in GSH levels (Barker *et al.* 1996).

This may suggest that the early GSH reduction in SN in asymptomatic PD (Sian *et al.* 1991) may ascribe (via production of hydrogen peroxide) for the subsequent iron elevation found in symptomatic PD (Dexter *et al.* 1994). However, although these findings are striking, caution needs to be exercised when translating *in vitro* findings, which may provide a simplistic explanation of the sequence of processes that occur in the isolated system. This is in gross contrast with complex biochemical events occurring *in vivo* and more importantly in the disease state. Indeed, in PD a significant iron reduction was found in the globus pallidus (Dexter *et al.* 1994), whereas there were no changes in GSH in this area (Sian *et al.* 1991).

The mismanagement of cellular iron resulting in the enhanced availability of free iron may represent a key mediator of oxidative stress in later symptomatic stage of PD.

Iron mediates oxidative stress primarily via reactive oxygen species. Animal studies have shown furnished support for the iron released from NM and its cellular toxic role in the brain. Intranigral administration of iron in adult rats leads to neuronal dopaminergic loss in the SN and depletion of striatal dopamine content (Wesemann *et al.* 1995). Consequently, this confirms the role of free iron as a neurotoxin via oxidative stress in the disease process. Thus, any mechanism(s) that reduce(s) or impair(s) a major antioxidant system like GSH-GSSG cycle and augments free reactive iron precipitates iron-mediated processes and will amplify oxidative stress.

Further evidence for the involvement of free radicals in the cascade of dopaminergic cell death is furnished by the two neurotoxins 6-OHDA and MPTP (MPP+, the toxic metabolite). They execute dopaminergic neuronal death primarily via free radical-mediated mitochondrial dysfunction. More importantly, they mimic many pathobiochemical processes

and behaviour observed in the PD and have therefore proved to serve as a valuable model for the disease.

Mitochondrial dysfunction in PD marked by the significant reduction in complex I activity in SN (Reichmann and Riederer 1989), may also contribute to oxidative stress. The involvement of nitric oxide is highlighted, because it produces reversible/irreversible inhibition of complex 1 of the mitochondrial respiratory chain (Bolanos *et al.* 1997). A reduction of complex 1 would result in the decrease of ATP production. In turn, this ATP depletion may consequently disturb other ATP -dependent mechanisms, for instance, the high levels of plasma membrane ATP-dependent sodium/potassium transporter present in the mid brain dopaminergic neurons, particularly the SN (Seutin *et al.* 1996). Other potential ATP dependent sites may be within the mitochondria include aconitase.

Aconitase is involved in iron and citric acid handling. Thus, the mitochondrial dysfunction may itself contribute to the overall disturbance in iron homeostasis in the mid brain. Alternatively, iron-generated reactive species may inhibit mitochondrial complex 1 activity (Harley *et al.* 1993; Shamoto-Nagai *et al.* 2004). In view of the absence of any nigral changes of iron in incidental Lewy body disease (Dexter *et al.* 1994), the elevated iron levels are unlikely to initiate oxidative stress and mitochondrial dysfunction, but may nevertheless magnify them.

The destruction of the dopaminergic neurons may elicit some inflammatory response that assigns for the active microgliosis detected in the SN in PD (McGeer and McGeer 2004; Consequences of increased iron in Parkinson's disease section). Indeed, cyclo-oxygenase, Cox-2 appears to be expressed by SN neurons, therefore, suggesting that dying dopaminergic neurons may trigger gliosis. Cox-2 produces prostagladins that may evoke an inflammatory response and thus gliosis. Subsequently, microglia activation may in turn mediate oxidative-induced neurotoxicity through the production of reactive-free radicals and indirectly via the release of iron from ferritin (Fig 1b). Indeed, glial activation produces an increase of immunoreactivity for nitric oxide synthase in the SN in PD (Hunot *et al.* 1996). Therefore, although microgliosis may present a secondary event in the degenerative process, it may nevertheless exacerbate the cellular-destructive cascade. Strikingly, patients with MPTP-induced Parkinsonism show continual degeneration long after the dopaminergic toxin has been cleared away (this may be due to the MPP+ binding to NM). Therefore, microglial activation could contribute to this pathway of ongoing cell death. More importantly, this activated glia can be detected by imaging techniques, which would serve as a vital tool for development of selective anti-inflammatory therapies in PD (Block *et al.* 2007).

The microgliosis occurring mainly in the SNc may decrease the binding capacity of NM or ferritin for iron, resulting in the release of free iron which in turn elicits oxidative damage. Indeed, nitric oxide (released by inflammation) has been shown to displace iron from ferritin (Reif and Simmons 1990). Pro-inflammatory cytokines, hydrogen peroxide (products of dopamine metabolism) may stimulate the expression of glial heme oxygenase-1 (Schipper 2004). This enzyme degrades heme to free iron. This is in accordance with the increased staining of heme oxygenase-1 in LB of nigral astrocytes in PD (Schipper 2000). Consequently, an over expression of this enzyme would contribute to the marked iron deposition found in PD, which may mediate cellular damage. Additionally, the free iron itself can activate microglia and release cytokines and nitric oxide (Zecca *et al.* 2004).

Lipocalin-2 is a neutrophil gelatinase-related lipocalin (ligand-binding protein). Its main role appears to be cellular protection by virtue of sequestrating iron through human siderophore and thereby preventing it from producing toxic oxygen radicals. Strikingly, oxidative stress and inflammation can induce its expression and serve as a protective response. Thus, the nigral gliosis in PD may also serve as a compensatory protective response (Weizer-Stern *et al.* 2006; Hu *et al.* 2009).

A recent study has suggested that the extracellular NM remaining after the loss of melanised dopaminergic neurons in PD may also activate microglia, thereby contributing to the inflammatory reaction resulting in further cell destruction (Wilms *et al.* 2003). This may be of particular significance in the early phase of the disease, when there is a rapid loss of pigmented neurons. Consequently, the release of NM from the dying cells may evoke some cellular destructing inflammatory process(es) (Orr *et al.* 2002). Thus, the humoral immune response to NM in PD (Orr *et al.* 2005; Double *et al.* 2009), may reflect a primary role of inflammation in the cascade of nigral degeneration. Consequently, this may confer to the characteristic susceptibility of pigmented neurons to destruction observed in the disease. However, in the later stages of the disease, it seems unlikely that NM remaining in the 'sick neurons' plays a major contributor role in the inflammatory cascade. Indeed, the nigral NM content was found to be decreased in PD and this is exhibited by the pale appearance of the SN in PD.

Interestingly, there are biological associations of PD with inflammation, diabetes and cancer (Moran and Graeber 2008). The development of cancer may contain an element of inflammation. This notion is endorsed by the reduction of the incidence (40%) of colon cancer in the prophylactic use of aspirin. Similarly, regular intake of aspirin or other non-steroidal inflammatory drugs has shown to significantly reduce the risk for PD (Chen *et al.* 2003).This may provide vital insights in the aetiology and pathogenesis underlying the progressive neuronal destruction in PD. In turn, this would provide valuable direction in potential treatment aimed at halting or hindering the progression of neuronal destruction. The fundamental pathological characteristics of PD are a marked dopaminergic cell loss (> 70%) coupled

with the presence of LB in the remaining surviving neurons in the SN.

Lewy body formation

Lewy bodies are eosinophilic inclusions that are observed in all areas exhibiting neuronal loss, such as dopamine neuron synapses and axons of SN, LC, basal nucleus of Meynert and dorsal motor nucleus of the vagus. Although they seem to be protective in early phases of PD they are considered as pathological hallmark for PD. Their presence in the SN is imperative for the diagnosis PD at postmortem. Nevertheless, they are not exclusive to PD and are observed in other disease, such as diffuse Lewy body disease and neurodegeneration with brain iron accumulation.

These large intracytoplasmic inclusions lack an outer membrane and thus appear to have a 'halo' outside its inner core. LB is highly ubiquinated and primarily consists of α-synuclein (particularly in the halo). The lipoprotein α-synuclein is involved in the intracellular transport of many lipids and proteins (Perez and Hastings 2004). It is localised in close vicinity to synaptic vesicles (Spillantini and Goedert 2000) and may therefore be associated to neurotransmitter release. The α-synuclein gene (PARK1) has been identified in a small numbers of families presenting with familial PD (Polymeropoulos *et al.* 1997).

Protein aggregation is a common characteristic of many neurodegenerative disorders and therefore this process may play a key role in their pathogenesis (Irvine *et al.* 2008). Although the precise mechanisms involved in LB formation is unclear, nevertheless it appears to be associated to protein aggregation. Interestingly, Goetz *et al.* (2004) have found that lipid peroxidation can produce protein aggregation. In fact, this might be protective for neurons until a threshold is reached at which LBs become toxic. Iron has shown to promote α-synuclein aggregation that can be blocked by the addition of an iron chelator (Hashimoto *et al.* 1999). It has been postulated that free iron released from NM affects the mitochondrial UPS resulting in a failure to clear away proteins such as α-synuclein. Consequently, this leads to its aggregation into α-synuclein immunopositive LB and cell death (McNaught *et al.* 2003).

The highly toxic peroxynitrite radical derived from nitric oxide may be involved in the formation of LB. Indeed, the marker for peroxynitrite, 3-nitrotyrosine, has been detected in LB (Giasson *et al.* 2000). The association of α-synuclein with iron in PD is based on their presence in LB. Iron has been found to accumulate within LB found in PD (Castellani *et al.* 2000). Furthermore, iron has been implicated in the polymerisation of α-synuclein. Therefore, aggregation of α-synuclein (associated with LB formation) may lead to accumulation of redox active iron in the cytosol; consequently, iron-generated free radicals may be released via Fenton reaction. This concords with the view that iron-mediated oxidative damage may be mediated via α-synuclein oligomerisation which occurs during development of PD pathology (Olivares *et al.* 2009). Also, the depleted ferritin content may represent a 'deficiency' of iron sequestrant processes, leading to the accumulation of free iron. Indeed, the free iron present in LB of SN in PD has been shown to exist in a state suitable to elicit oxidative stress (Castellani *et al.* 2000). However, at the present time the mechanism as to how mutant α-synuclein may cause dopaminergic cell death remains to be resolved. What is the role of wild-type α-synuclein in sporadic PD? Furthermore, α-synuclein is a common protein not exclusive to LB or PD only. Indeed, it is found in other neurodegenerative disorders (such as MSA, dementia with LB) for which there must be different pathogenic mechanisms involved (Spillantini and Goedert 2000). The increase in free iron in the SN in PD is associated with the generation of toxic-free radicals derived from hydrogen peroxide (Fenton reaction) which in turn may mediate lipid peroxidation and secondary dopaminergic cell destruction (Stankiewicz *et al.* 2007). Furthermore, BE-M17 neuroblastoma cells that over-express either the wild-type or mutated form of α-synuclein, exhibit a marked susceptibility to iron-mediated cell damage (Ostrerova-Golts 2000). It has therefore been postulated that interaction of iron with α-synuclein induces aggregate formation and production of advanced glycosylation end products, which are associated with the cross-linking of LB (Muench *et al.* 2000). It has been hypothesised from *in vitro* studies (Volles *et al.* 2001) that before formation of large aggregates, α-synuclein produces small oligomers called protofibrils that can be stabilised by dopamine-quinone. Dopamine quinones and semi-quinones are present in NM. This would implicate an involvement of some NM-derived reactive dopamine with α-synuclein. The association of NM in the pathological events occurring in PD is further demonstrated by preservation of the non-NM containing dopaminergic neurons of the ventral tegmental area in contrast to the marked loss of NM containing dopaminergic in the SN in PD. Interestingly, two dopaminergic neurotoxins, MPTP and paraquat that mimic many of the changes observed in PD, also produce α-synuclein aggregation. Thus, protein aggregation may be involved in the pathogenesis of PD. The role of LB in PD remains unclear, whether it contributes to the pathogenesis of the illness or if it simply represents a consequence of the neurodegenerative process. It is unlikely that it represents a cause of the disease as it is absent in PARK2 autosomal recessive inherited juvenile PD. However, it is present in the asymptomatic phase (in incidental Lewy body disease), and thus represents an early change in the course of the disease. Interestingly, α-synuclein has been detected in CSF and plasma in PD patients. This has lead to the suggestion that α-synuclein may be released from degenerating dopaminergic neurons and therefore may be employed as marker for the illness (Olivares *et al.* 2009).

Iron changes in PD; a cause or consequence of oxidative stress

Monkeys treated with MPTP (neurotoxic metabolite MPP+), have shown an elevation of iron content to be a secondary event (He *et al.* 2003). Similarly, there is an absence of significant increase in iron content in the SN in the early asymptomatic stage of PD (incidental Lewy body disease; Dexter *et al.* 1994) in contrast to the marked elevation of nigral iron in symptomatic PD. This suggests that the iron changes observed in SN in PD are most likely to represent a secondary event in the neuronal degeneration cascade, nevertheless it may (via oxidative stress-mediated pathways) exacerbate and contribute to the overall cellular destructive process prevalent in the disorder.

The final pathway leading to cell death may be apoptotic 'programmed' cell death. This mechanism has been strongly implicated in experimental PD models. Above all, the up-regulation of anti-apoptotic proteins has been shown to block MPTP-mediated cell death (Przedborksi and Vila 2001). The involvement of calcium-mediated cell death is illustrated by the sparing of the highly calbindin (calcium-binding protein) expressed dopaminergic neurons in the ventral tegmental area (Iacopino *et al.* 1992). However, recent publications also point iron as an inducer of apoptotic mechanisms (Miwa *et al.* 2010; Yu *et al.* 2010).

Overall, the activity of free or poorly liganded iron in combination with hydrogen peroxide or superoxide underpins its cytotoxic role in a spectrum of diseases, such as degenerative disorders (e.g. PD), chronic inflammatory and vascular disease (Kell 2009). Alterations in levels of iron and GSH in the SN endorse the involvement of oxygen reactive species in the mediation of oxidative stress. Interestingly, expression of a PARK7 gene related to early onset familial PD appeared to be protective against oxidative stress and regulate stroke-induced damage (Aleyasin *et al.* 2007). This would suggest to non-oxidative stress-mediated degeneration in this form of PD.

Although at the present time it remains unresolved as to whether iron represents a cause or consequence of dopaminergic neuronal cell death, nevertheless iron executes its cytotoxic effects via oxidative damage. In view of the radical role of oxidative stress in the pathogenesis of PD, the overload of iron may serve as a mediator that can evoke many distressing sequelae via generating free radicals. Indeed, lesions of the rat median forebrain resulted in the consequent deposition of iron in the SNc similar to that observed in PD (Oesterreicher *et al.* 1994). The marked decrease of nigral GSH in incidental Lewy body disease (Sian *et al.* 1991) suggests the occurrence of oxidative stress in the asymptomatic phase of the disease. However, it is unclear whether oxidative stress is an initiator of neuronal degeneration or a consequence of it. In fact, it has been questioned whether pre-clinical or incidental Lewy body disease, such as Braak stages I and II reflect early stages of PD at all. Nevertheless, in the later stages of the disease it is most likely to exert cumulative and irreversible cytotoxic effects in the cascade of events leading to the dopaminergic degeneration characteristic of PD.

Although dopaminergic neurons present the main area of focus in PD, nevertheless gliosis or activation of glia may serve an integral role in the progression of dopamine cellular destruction. Indeed, most of the biochemical changes (GSH, complex 1 and 26S proteasomal activity) found in PD is most likely to occur in glial cells. This concept is supported by histochemical studies that demonstrate cellular localisation of GSH (Sian *et al.* 1994a,b), and iron (Morris and Edwardson 1994) in the glia. Similarly, the reduction of mitochondrial complex 1 activity (Mizuno *et al.* 1989; Reichmann and Riederer 1989; Schapira *et al.* 1989) is most likely to occur in glial cells as opposed to 'sick neurons' (Benzi *et al.* 1991). Also the magnitude of these alterations is 30–40% in homogenates of SN, which is far too great to represent changes occurring in the few remaining neurons (1–2%). They are most likely to occur in glial cells. The occurrence of oxidative stress in these glia cells would result in their activation and release of cytokines, reactive oxygen and nitrogen species and propagate oxidative damage to the dopaminergic neurons.

Recent findings strongly support the involvement of the UPS in the pathogenesis of PD. Elevated iron (released from NM) in SN may affect the UPS in mitochondria resulting in the accumulation of proteins such as α-synuclein. Altered proteolysis is associated with dopaminergic cell death and Lewy body formation in both idiopathic and familial PD (Fornai *et al.* 2005). Furthermore, a reduction in activity of three proteasomal enzymes in the SNc was found in sporadic PD (McNaught *et al.* 2003).

Conclusions

The pathogenesis of PD appears to be labyrinth, which most probably involves a myriad of pathogenic factors operating in concert. Additionally, the complexity is further illustrated by the extensive distribution of pathology in other non-dopaminergic areas, such as noradrenaline neurons of the LC, 5-hydroxtryptamine neurons of the dorsal raphe, cholinergic neurons of the nucleus basalis of Meynert and the peripheral autonomic nervous system. However, the loss of dopaminergic neurons in the SNc leading to the degeneration of the nigrostriatal pathway and resulting in the marked reduction in striatal dopamine appears to be pivotal in the presentation of the clinical features of the disease. This is demonstrated by the remarkable response of most of the motoric symptoms to dopamine replacement therapy. Furthermore, the importance of SNc in PD has been challenged (Braak *et al.* 2004) by findings that suggest that PD pathology begins in the brain stem and olfactory areas

and eventually observed in other brain regions. Nevertheless, this concept of the operation of different pathogenic mechanisms is reflected by difference in symptoms and pathology of patients within the same family and with the same mutation (Zimprich *et al.* 2004). Thus, PD may represent a spectrum of related diseases with a constellation of a number of potential pathogenic pathways. Indeed, this concept of PD as a spectrum disease is illustrated by the absence of changes in SN iron levels in some asymptomatic PD subjects (Dexter *et al.* 1994), in contrast to the elevated nigral iron content reported by others in such subjects (Berg *et al.* 1999). Thus, the occurrence of the elevated nigral iron content in the course of the disease progression (Fig 1a and b) may be dependent on the PD sub-group involved with possible variation in phenotypes, disease progression and therapeutic strategies. Furthermore, the alterations of brain iron levels are most likely to be attributed to a dysfunction in its homeostasis or metabolism. The regulation of iron storage in NM-pigmented SN neurons involves a dual system, NM and L-ferritin operated (Tribl *et al.* 2009). In PD, the loss of NM cells in SN coupled with the alteration of NM structure itself may compromise its ability to sequester the redox active metal, leading to the augmented iron levels in this area. Additionally, the reduced ferritin levels reported by some groups (Dexter *et al.* 1991; Faucheux *et al.* 2002) may further compromise the iron storage mechanisms, consequently resulting in more free iron. Subsequently, this excess free iron may elicit oxidative or nitrative stress via production of reactive-free radicals and trigger the cascade of cell destruction. These series of events could evoke and propagate further cellular degeneration. Indeed, superoxide and nitric oxide radicals may induce the release of iron from ferritin (Gerlach *et al.* 2006), thereby contributing to the raised iron levels. Consequently, the excess iron could initiate the pro-oxidant role of NM, resulting in the formation of more reactive oxygen species and precipitating oxidative/nitrative stress (Lopiano *et al.* 2000). These cyclic sequences of events are so closely inter-linked that it is difficult to ascertain the initiator/inducer of neuronal degeneration.

Nevertheless, the role of iron either in the mediation or/and in contribution to oxidative stress induced neuronal degeneration in the pathogenesis of PD is well supported. Furthermore, the nigral changes in iron probably work in concert with other factors in executing neuronal cell death.

References

Aime S., Bergamasco B., Casu M., Digilio G., Fasano M. and Giraudu S. (2000) Isolation of ^{3}C NMR characterisation of insoluble proteinaceous fraction from substantia nigra of patients with Parkinson's disease. *Mov. Disorders* **15**, 977–981.

Aleyasin H., Rousseaux W. C., Philips M., Kim R. H., Bland J. R., Callaghan S., Slack R. S., During M. J., Mak T. W. and Park D. S. (2007) The Parkinson's disease gene DJ-1 is also a key regulator of stroke induced damage. *Proc. Natl Acad. Sci. USA* **107**, 18748–18753.

Andrew R., Watson D. G., Bet S. A., Midgley J. M., Wenlong H. and Perry R. K. (1993) The determination of 6-hydroxydopamines and other trace amines in the urine of parkinsonian patients and normal controls. *Neurochem. Res.* **18**, 1175–1177.

Barker J. E., Heales S. J. and Cassidy A. (1996) Depletion of brain glutathione reductase activity: an enzyme susceptible to oxidative stress. *Brain Res.* **716**, 118–122.

Bartels A., Willemsen A. T., Kotekaas R., Jong B. M., De Vries R., Klerk O., Portman A. and Leenders K. L. (2008) Decreased blood brain barrier P-glycoprotein function in the progression of Parkinson's disease, PSP and MSA. *J. Neural Transm.* **115**, 1001–1009.

Bartzokis G., Cumming J. L., Markham C. H., Marmarelis P. Z., Treciokas L. J., Tishler T. A., Marder S. R. and Mintz J. (1999) MRI evaluation of brain iron earlier and later onset Parkinson's disease and normal subjects. *Magn. Reson. Imaging* **17**, 213–222.

Bauer M., Karch R., Abrahim A., Wagner C. C., Kletter K., Mueller M. and Langer O. (2008) Decreased blood–brain barrier P-glycoprotein function with aging. *BMC Pharmacol.* **8**(Suppl. 1), A48.

Beard J. L. and Connor J. R. (2003) Iron status and neural functioning. *Annu. Rev. Nutr.* **23**, 41–58.

Becker G., Seufert J., Reichmann H. and Reiners K. (1995) Degeneration of substantia nigra in chronic Parkinson's disease visualized by transcranial color-coded real time sonography. *Neurology* **45**, 443–454.

Behnke S., Schroeder U., Dillmann U., Bucholz G. G., Schreckenberger M., Fuss G., Reith W., Berg D. and Krick C. M. (2009) Hyperechogenicity of the substantia nigra in healthy controls is related to MRI changes and to neuronal loss as determined by F-Dopa PET. *NeuroImage* **47**, 1237–1243.

Ben-Shachar D. and Youdim M. B. (1993) Iron, melanin and dopamine interaction: relevance to Parkinson's disease. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **17**, 139–150.

Ben-Shachar D., Riederer P. and Youdim M. B. H. (1991) Iron-melanin interaction and lipid peroxidation: implications for Parkinson's disease. *J. Neurochem.* **57**, 1609–1614.

Benzi G., Curti D. and Pastoris O. (1991) Sequential damage in mitochondrial complexes by peroxidative stress. *Neurochem. Res.* **16**, 1295–1302.

Berg D., Becker G., Zeiler B., Tucha O. and Hofmann E. (1999) Vulnerabilty of the nigrostriatal system as detected by transcranial ultrasound. *Neurol* **53**, 1026–1031.

Berg D., Becker G., Riederer P. and Riess O. (2002a) Iron in neurodegenerative disorders. *Neurotoxic Res.* **8**, 637–653.

Berg D., Roggendorf W., Schröder U. *et al.* (2002b) Echogenicity of the Substantia Nigra: association with increased iron content and marker for susceptibility to nigrostriatal injury. *Arch. Neurol.* **59**, 999–1005.

Block M., Zecca L. and Hong J.-S. (2007) Microglia-mediated neurotoxicity; uncovering the molecular mechanisms. *Nat. Rev. Neurosci.* **8**, 57–69.

Boissier de Sauvages F. (1768) Nosologie methodice sistens morbeorum clesses juxte. Sydenhemi mentem and botericorum rodinem. Amsterdam. Fratelli de Tournes.

Bolaños J. P., Almeida A., Fernández E., Medina J. M., Land J. M., Clark J. B. and Heales S. J. (1997) Potential mechanisms for nitric oxide-mediated impairment of brain mitochondrial energy metabolism. *Biochem. Soc. Trans.* **25**, 944–949.

Borie C., Gasparini F., Verpillat P., Bonnet A. M., Agid Y., Hetet G., Brice A., Dürr A. and Grandchamp B. (2002) French Parkinson's disease genetic study group. Association study between iron related genes polymorphism and Parkinson's disease. *J. Neurol.* **249**, 801–804.

Braak H., Ghebremedhin E., Rub U., Bratzke H. and Del Tredici K. (2004) Stages in development of Parkinson's disease related pathology. *Cell Tissue Res.* **318**, 121–134.

Byun J., Henderson J. P., Mueller D. M. and Heinecke J. W. (1999) 8-Nitro-2-deoxyguanosine, a specific marker for reactive oxygen species, is generated by the myeloperoxidase-hydrogen peroxide-nitrite system of activated human phagocytes. *Biochemistry* **38**, 2590–2600.

Cass W. A., Grondin R., Anderson A. H., Zhang Z., Hardy P. A., Hussey-Anderson L. K., Rayens W. S., Gerhardt G. A. and Gash D. M. (2007) Iron accumulation in the striatum predicts ageing-related decline in motor function in rhesus monkeys. *Neurobiol. Aging* **28**, 258–271.

Castellani R. J., Siedlak S. L., Perry G. and Smith M. A. (2000) Sequestration of iron by Lewy bodies in Parkinson's disease. *Acta Neuropathol.* **100**, 111–114.

Chen H., Zhang S. M. and Hernan M. A. (2003) Nonsteroidal anti-inflammmatory drugs and the risk of Parkinson's disease. *Arch. Neurol.* **60**, 1059–1064.

Connor J. R., Synder B. S., Beard J. L., Fine R. and Mufson E. (1992) Regional distribution of iron and iron-regulatory proteins in the brain aging and Alzheimer's disease. *J. Neurosci. Res.* **31**, 327–335.

Connor J. R., Boeshore K. L., Bekovic S. A. and Menzies S. L. (1994) Isoforms of ferritin have a specific cellular distribution in the brain. *J. Neurosci. Res.* **37**, 461–465.

Connor J. R., Synder B. S., Arosio P., Loeffler D. A. and Lewitt P. (1995) A quantitative analysis of isoferritins in selected regions of aged, Parkinsonian and Alzheimer's diseased brains. *J. Neurochem.* **65**, 717–724.

Connor J. R., Arosio P., Loeffler D. A. and Lewitt P. (2004) Decreased transferrin receptor expression by neuromelanin cells in restless leg syndrome. *Neurology* **62**, 1563–1567.

Curtis Ar., Fey C., Morris C. M., Bindoff L. A., Ince P. G., Chinery P. F., Coulthard A., Jackson M. *et al.* (2001) Mutation in the gene encoding ferritin light polypeptide causes dominant adult-onset basal ganglia disease. *Nat. Genet.* **28**, 350–354.

Depboylu C., Matusch A., Tribl F., Zory M., Michel P. P., Riederer P., Gerlach M., Becker S., Oertal W. H. and Hoglinger G. U. (2007) Glia protects neurons against extracellular human neuromelanin. *Neurodeg. Dis.* **4**, 218–226.

Dexter D. T., Wells F. R., Agid F., Agid Y., Lees A. J., Jenner P. and Marsden C. D. (1987) Increased nigral iron content in postmortem parkinsonian brain. *Lancet* **341**, 1219–1220.

Dexter D. T., Carter C. J., Wells F. R., Javoy-Agid F. and Agid Y. (1989a) Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease. *J. Neurochem.* **52**, 381–389.

Dexter D. T., Wells F. R., Lees A. J., Agid F., Agid Y., Jenner P. and Marsden C. D. (1989b) Increased nigral iron content and alterations in other metal ions occurring in brain in Parkinson's disease. *J. Neurochem.* **52**, 1830–1836.

Dexter D. T., Carter C. J., Wells F. R., Javoy-Agid F., Agid Y., Lees A., Jenner P. and Marsden C. D. (1991) Alterations in the levels of iron, ferritin and other trace metals in Parkinson's disease and other neurodegenerative diseases affecting the basal ganglia. *Brain* **114**, 1953–1975.

Dexter D. T., Jenner P., Schapira A. H. and Marsden C. D. (1992) Alterations in levels of iron, ferritin, and other trace metals in neurodegenerative diseases affecting the basal ganglia.Ann. *Neurol* **32**, S94–S100.

Dexter D. T., Sian J., Jenner P. and Marsden C. D. (1993) Implications of alterations in trace element levels in brain in Parkinson's disease and other neurological disorders affecting the basal ganglia. *Adv. Neurol.* **60**, 273–281.

Dexter D. T., Sian J., Rose S. *et al.* (1994) Indices of oxidative stress and mitochondrial function in individuals with incidental Lewy body disease. *Ann. Neurol.* **35**, 38–44.

Double K. L., Maywald M., Schmittel M., Riederer P. and Gerlach M. (1998) In vitro studies of ferritin iron release and neurotoxicity. *J. Neurochem.* **70**, 2492–2499.

Double K. L., Riederer P. and Gerlach M. (1999) The significance of neuromelanin for neurodegeneration in Parkinson'sdisease. *Drug News Perspect.* **12**, 333–340.

Double K. L., Rowe D. B., Carew-Jones F. M. *et al.* (2009) Anti-melanin antibodies are increased in sera in Parkinson's disease. *Exp. Neurol.* **217**, 297–301.

Earle K. M. (1968) Studies on Parkinson's disease including X-ray fluorescent spectroscopy of formalin fixed brain tissue. *J. Neuropathol. Exp. Neurol.* **27**, 1–14.

Fahn S. and Sulzer D. (2004) Neurodegeneration and neuroprotection in Parkinson's disease. *NeuroRx* **1**, 139–154.

Fang D., Zhong-ming Q., Zhu L., Wu Xiao M. W., Yung W. H., Ting T. Y. and Ya K. (2009) L-Dopa neurotoxicity is mediated by up-regulation of DMT1-IRE expression. *PLoS ONE* **4**, e4593.

Faucheux B. A., Nillesse N., Damier P., Spik G., Mouatt-Prigent A. *et al.* (1995) Expression of lactoferritin receptors is increased in the mesencephalon of patients with Parkinson's disease. *Proc. Natl Acad. Sci. USA* **92**, 9603–9607.

Faucheux B. A., Damier P. and Spik G. (2002) Lack of upregulation of ferritin is associated with sustained iron regulatory protein-1 binding activity in the substantia nigra of patients of Parkinson's disease. *J. Neurochem.* **83**, 320–330.

Faucheux B. A., Martin M. E., Beaumont C., Hauw J. J., Agid Y. and Hirsch E. C. (2003) Neuromelanin associated redox active iron is increased in the substantia nigra of patients with Parkinson's disease. *J. Neurochem.* **86**, 1142–1148.

Fearnely J. M. and Lees A. J. (1991) Ageing and Parkinson's disease: substantia nigra regional selectivity. *Brain* **114**, 2283–2301.

Fornai F., Schluter O. M., Lenzi P., Gesi M., Ruffoli R. and Ferruci M. (2005) Parkinson-like syndrome induced by continuous MPTP infusion: convergent roles of the ubiquitin proteasome system and alpha-synuclein. *Proc. Natl Acad. Sci. USA* **102**, 3413–3418.

Friedman A., Galazka-Friedman J. and Koziorowski D. (2009) Iron as a cause of Parkinson disease – a myth or a well established hypothesis? *Parkinsonism Relat. Disord.* **15**(Suppl. 3), S212–S214.

Galazka-Friedman J., Bauminger E. R. and Friedman A. (1996) Iron in parkinsonian and control substantia nigra: a Moessbauer spectroscopy study. *Mov. Disord.* **11**, 8–16.

Gerlach M., Trautwein A., Zecca L., Youdim M. B. H. and Riederer P. (1995) Moessbauer spectroscopic studies of purified neuromelanin isolated from substantia nigra. *J. Neurochem.* **65**, 923–926.

Gerlach M., Double K. L., Youdim M. B. H. and Riederer P. (2006) Potential sources of increased iron in the substantia nigra of parkinsonian patients. *J. Neural Transm.* **70**, 133–142.

Giasson B. I., Duda Jem Murray I. V., Chen Q. and Souza J. M. (2000) Oxidative damage linked to neurodegeneration by selective alpha-synuclein nitration in synucleinopathy lesions. *Science* **3**, 985–989.

Gibb W. R. (1992) Melanin, tyrosine hydroxylase, calbindin and substance P in the human mid brain and substantia nigra in relation to nigrostriatal projections and differential neuronal susceptibility in Parkinson's disease. *Brain Res.* **581**, 283–291.

Goetz M. E., Double K., Gerlach M., Youdim M. B. H. and Riederer P. (2004) The relevance of iron in the pathogenesis of Parkinson's disease. *Ann. N Y Acad. Sci.* **1012**, 193–208.

Good P. F., Olanow C. W. and Perl D. P. (1992) Neuromelanin-containing neurons of the substantia nigra accumulate iron and aluminum in Parkinson's disease: a LAMMA study. *Brain Res.* **593**, 343–346.

Gorell J. M., Ordidge R. J., Brown G. G., Deniau J. C., Buderer N. M. and Helpern J. A. (1995) Increased iron-related MRI contrast in the substantia nigra in Parkinson's disease. *Neurology* **45**, 1138–1143.

Graham J. M., Paley M. N., Grunewald R. A., Hoggard N. and Griffiths P. D. (2000) Brain iron deposition in Parkinson's disease imaged

using the PRIME magnetic resonance sequence. *Brain* **123**, 2423–2431.

Griffiths P. D., Dobson B. R., Jones G. R. and Clarke D. T. (1999) Iron in the basal ganglia in Parkinson's disease: an *in vitro* study using X-ray absorption fine structure and cryo-electron microscopy. *Brain* **122**, 667–673.

Gutteridge J. M. (1994) Hydroxyl radicals, iron, oxidative stress and neurodegeneration. *Ann. N Y Acad. Sci.* **738**, 201–213.

Haacke E. M., Cheng N. Y., House M. J., Liu Q., Neelavalli J., Ogg R. J., Khan A., Ayaz M., Kirsch W. and Obenaus A. (2005) Imaging iron stores in the brain using magnetic resonance imaging. *Magn. Reson. Imaging* **23**, 1–25.

Harley A., Cooper J. M. and Schapira A. H. (1993) Iron induced oxidative stress and mitochondrial dysfunction: relevance to Parkinson's disease. *Brain Res.* **627**, 349–353.

Hashimoto M., Takeda A., Hsu L. J., Takenouchi T. and Masliah E. (1999) Role of cytochrome c as a stimulator of alpha-synuclein aggregation in Lewy body disease. *J. Biol. Chem.* **274**, 28849–28852.

He Y., Thong P. S., Lee T., Leong S. K., Mao B. Y., Dong F. and Watt F. (2003) Dopaminergic cell death precedes iron elevation in MPTP-injected monkeys. *Free Rad. Biol. Med.* **35**, 540–547.

Hirsch E. C. (1994) Biochemistry of Parkinson's disease with special reference to the dopaminergic systems. *Mol. Neurobiol.* **9**, 135–142.

Hirsch E. C. and Faucheux B. A. (1998) Iron metabolism and Parkinson's disease. *Mov. Disord.* **13**, 39–45.

Hoeppner J., Prudente-Morrissey L., Herppertz S. C., Benecke R. and Walter U. (2009) Substantia nigra hyperechogenicity in depressive subjects relates to motor asymmetry and impaired word fluency. *Eur. Arch. Psychiatry Clin. Neurosci.* **259**, 92–97.

Hu L., Hittelman W., Lu T., Ji P., Arlinghaus R. *et al.* (2009) NGAL decreases E-cadherin-mediated cell–cell adhesion and increases cell motility and invasion through Rac1 in colon carcinoma cells. *Lab. Invest.* **89**, 531–548.

Hunot S., Boissiere F. and Faucheux B. (1996) Nitric oxide synthase and neuronal vulnerability in Parkinson's disease. *Neuroscience* **72**, 355–363.

Hyun D., Lee M., Halliwell B. and Jenner P. (2002) Proteasomal dysfunction induced by 4-hydroxy-2,3-transnonenal, an end product of lipid peroxidation: a mechanism contributing to neurodegeneration. *J. Neurochem.* **83**, 360–370.

Iacopino A., Christakos S., German D., Sonsalla P. K. and Altar C. A. (1992) Calindin-D28K containing neurons in animal models of neurodegeneration: possible protection from excitotoxicity. *Brain Res. Mol. Brain Res.* **13**, 251–261.

Irvine G. B., El-Agnaf O. M., Shankar G. M. and Walsh D. M. (2008) Protein aggregation in the brain: the molecular basis for Alzheimer's and Parkinson's disease. *Mol. Med.* **14**, 451–464.

Jellinger K., Paulus W., Grundke-Iqbal I., Riederer P. and Youdim M. B. H. (1990) Brain iron and ferritin in Parkinson's disease and Alzheimer's disease. *J. Neural Transm.* **2**, 327–340.

Jellinger K., Kienzl E., Rumpelmair G., Riederer P., Stachelberger H., Ben-Shachar D. and Youdim M. B. (1992) Iron-melanin complex in substantia nigra of parkinsonian brains: an x-ray microanalysis. *J. Neurochem.* **59**, 1168–1171.

Jenner P. (1993) Altered mitochondrial function, iron metabolism and glutathione levels in Parkinson's disease. *Acta Neurol. Scand. Suppl.* **146**, 6–13.

Jenner P. and Olanow C. W. (2006) The pathogenesis of cell death in Parkinson's disease. *Neurology* **66**, S24–S36.

Kaur D., Lee D., Ragapolan S. and Andersen J. K. (2009) Glutathione depletion in immortalized midbrain-derived dopaminergic neurons results in increases in the labile iron pool: Implications for Parkinson's disease. *Free Radic. Biol. Med.* **46**, 593–598.

Ke Y. and Qian Z. M. (2007) Brain iron metabolism: neurobiology and neurochemistry. *Prog. Neurobiol.* **83**, 149–173.

Kell D. B. (2009) Iron behaving badly: inappropriate iron chelation as a major contributor to the aetiology of vascular and other progressive inflammatory and degenerative diseases. *BMC Med. Genomics* **2**, 2.

Khedr E., Hamed S. A., Elbeih E., El-Shereef H., Ahamad Y. and Ahmad S. (2008) Iron states and cognitive abilities in young adults: neuropsychological and neurophysiological assessment. *Eur. Arch. Psychiatry Clin. Neurosci.* **258**, 489–496.

Kienzl E., Puchinger L., Jellinger K., Linert W., Stachelberger H. and Jameson R. (1995) The role of transition metals in the pathogenesis of Parkinson's disease. *J. Neurol. Sci.* **134**, 69–78.

Kosta P., Argyrpoulou M. I. and Markoula S. (2006) MRI evaluation of the basal ganglia size and iron content in patients with Parkinson's disease. *J. Neurol.* **253**, 26–32.

Lapenna D., Degioia S., Ciofani G. and Cuccurullo F. (1995) Captopril induces iron release from ferritin and oxidative stress. *J. Pharm. Pharmacol.* **47**, 59–61.

Leveugle B., Faucheux B., Bouras C., Nillesse N., Spik G., Hirsch E., Agid Y. and Hof P. (1996) Cellular distribution of iron-binding protein lactotransferrin in the mesencephalon of Parkinson's disease. *Acta Neuropathol.* **91**, 566–572.

Lewis S., Fotynie T., Blackwell A., Robbins T., Owen A. and Barker R. (2005) Heterogeneity of Parkinson's disease in the early clinical stages using a data driven approach. *J. Neurol. Neurosurg. Psychiatry* **76**, 343–348.

Lhermitte J., Kraus W. M. and McAlpine A. D. (1924) On the occurrence of abnormal deposits of iron in brain in parkinsonism with special reference to its localisation. *J. Neurol. Psychopathology* **5**, 195–208.

Loeffler D. A., Connor J. R., Juneau P. I., Snyder B. S., Kanaley L., DeMaggio A. J. *et al.* (1995) Transferrin and iron in normal, Alzheimer's disease, and Parkinson's disease brain regions. *J. Neurochem.* **65**, 710–716.

Lopiano L., Chiesa M., Digilio G., Giraudo S., Bergamasco B., Torre E. and Fasano M. (2000) Q-band EPR investigations of neuromelanin control and Parkinson's disease patients. *Biochem. Biophys. Acta* **1500**, 306–312.

McGeer P. L. and McGeer E. (2004) Inflammation and neurodegeneration in Parkinson's disease. *Parkinsonism Relat. Disord.* **10**, S3–S7.

McNaught K. S., Belizaire R., Isacson O., Jenner P. and Olanow C. W. (2003) Altered proteasomal function in sporadic Parkinson's disease. *Exp. Neurol.* **179**, 38–46.

Miwa C. P., de Lima M. N., Scalco F., Vedana G., Mattos R., Fernandez L. L., Hilbig A., Schröder K. and Vianna M. R. (2010). Neonatal iron treatment increases apoptotic markers in hippocampal and cortical areas of adult rats. *Neurotoxicity Res. [Epub ahead of print] PMID: 20369315.*

Mizuno Y., Ohta S., Tanaka M., Takamiya S., Suzuki K. *et al.* (1989) Deficiencies in complex I subunits of the respiratory chain in Parkinson's disease. *Biochem. Biophys. Res. Commun.* **163**, 1450–1455.

Montine K. S., Quinn J. F., Zhang J., Fessel J. P. and Roberts L. J. 2nd, Morrow J. D. and Montine T. J. (2004) Isoprostanes and related products of lipid peroxidation in neurodegenerative diseases. *Chem. Phys. Lipids* **128**, 117–124.

Moos T. and Morgan E. H. (2004) The metabolism of neuronal iron and its pathogenic role in neurological disease. *Ann. N Y Acad. Sci.* **1012**, 14–26.

Moran L. B. and Graeber M. B. (2008) Towards a pathway definition of Parkinson's disease: a complex disorder with links to cancer, diabetes and inflammation. *Neurogenetics* **9**, 1–13.

Morawski M., Meinecke Ch., Reinert T., Dorffel A. C., Riederer P., Arendt T. and Butz T. (2005) Determination of trace elements in the human substantia nigra. *Nucl. Instrum. Methods Phys. Res. B* **231**, 224–228.

Morris C. M. and Edwardson J. A. (1994) Iron histochemistry of the substantia nigra in Parkinson's disease. *Neurodegeneration* **3**, 277–282.

Muench G., Lüth H. J., Wong A., Arendt T., Hirsch E. *et al.* (2000) Crosslinking of alpha-synuclein by advanced glycation end products – an early pathophysiological step in Lewy body formation? *J. Chem. Neuroanat.* **20**, 253–257.

Naoi M., Maruyama W., Yi H., Yamaaoka Y., Shagamoto-Nagai M., Akao Y., Gerlach M. and Tanaka Riederer P. (2008) Neuromelanin selectively induces apoptosis in dopaminergic SH-SY5Y cells by deglutathionylation in mitochondria: involvement of protein and melanin component. *J. Neurochem.* **105**, 2489–2500.

Napolitano A., Crescenzi O., Pezzella A. and Prota G. (1995) Generation of neurotoxin 6-hydroxydopamine by peroxidase/hydrogen peroxide oxidation of dopamine. *J. Med. Chem.* **38**, 917–922.

Nemeth E. (2008) Iron regulation and erythropoesis. *Curr. Opin. Hematol.* **15**, 169–175.

Oesterreicher E., Sengstock G. J., Riederer P., Olanow C. W., Dunn A. J. and Arendash G. (1994) Degeneration of nigrostriatal dopaminergic neurons increases iron within the substantia nigra: a histochemical and neurochemical study. *Brain Res.* **660**, 8–18.

Olivares D., Huang X., Branden L., Greig N. H. and Rogers J. T. (2009) Physiological and pathological role of alpha synuclein in Parkinson's disease through iron mediated oxidative stress. The role of putative iron responsive element. *Int. J. Mol. Sci.* **10**, 1226–1260.

Orr C. F., Rowe D. B. and Halliday G. M. (2002) An inflammatory review of Parkinson's disease. *Prog. Neurobiol.* **68**, 325–340.

Orr C. F., Rowe D. B., Mizuno Y., Mori H. and Halliday G. (2005) A possible role for humoral immunity in the pathogenesis of Parkinson's disease. *Brain* **128**, 2665–2674.

Ostrerova-Golts N. (2000) The A53T alpha-synuclein mutation increases iron dependent aggregation and toxicity. *J. Neurochem.* **20**, 6048–6054.

Parkinson J. (1817). An essay on the shaking palsy. *J. Neuropsychiatry Clin. Neurosci.. 2002 Spring;* **14**:223–236; discussion 222.

Perez R. G. and Hastings T. G. (2004) Could a loss of alpha-synuclein function put dopaminergic neurons at risk? *J. Neurochem.* **89**, 1318–1324.

Polymeropoulos M. H., Lavedan C., Leroy E., Ide S. E., Dehejia A., Dutra A. *et al.* (1997) Mutation in the alpha synuclein gene identified in families with Parkinson's disease. *Science* **276**, 2045–2047.

Ponka P. (2004) Hereditary causes of distribution iron homeostasis in the central nervous system. *Ann. N Y Acad. Sci.* **1012**, 267–281.

Przedborksi S. and Vila M. (2001) MPTP: a review of its mechanisms of neurotoxicity. *Clin. Neurosci. Res.* **1**, 407–418.

Qian Z. M. and Wang Q. (1998) Expression of iron transport proteins and excessive iron accumulation in the brain in neurodegenerative disorders. *Brain Res. Brain Res. Rev.* **27**, 257–267.

Rausch W. D., Hirata Y., Nagatsu T., Riederer P. and Jellinger K. (1988) Tyrosine hydroxylase activity in caudate nucleus from Parkinson's disease: effects of iron and phosphorylating agents. *J. Neurochem.* **50**, 202–208.

Reichmann H. and Riederer P. (1989) Biochemische Analyse der Atmungskettenkomplexe verschiedener Hirnregionen von Patienten mit M. Parkinson und anderen Basalganglienerkrankungen. *Symposium zu einem Foerderschwerpunkt des BMFT* **44**, 23–25.

Reinheckel T., Sitte N. and Ulrich O. (1998) Comparitive resistance of the 20S and 26S proteosames to oxidative stress. *Biochem. J. Biol. Chem.* **274**, 23787–23793.

Riederer P. and Wesemann W., eds (1995) Parkinson's disease: experimental models and therapy. *J. Neural Transm. Suppl.* **46**. Springer Verlag Wien/New York, Wien.

Riederer P., Sofic E., Rausch W. D., Kruzik P. and Youdim M. B. H. (1985) Dopaminforschung heute und morgen – L-dopa in der Zukunft, in L-Dopa Substitution der Parkinson-Krankheit (Riederer P. and Umek H., eds), pp. 127–144. Springer Verlag Wien, New York.

Riederer P., Rausch W. D., Schmidt B., Kruzik P. and Konradi C. (1988) Biochemical fundamentals of Parkinsons's disease. *Mt Sinai J. Med.* **55**, 21–28.

Riederer P., Sofic E., Rausch W. D., Schmidt B., Reynolds G. P., Jellinger K. and Youdim M. B. H. (1989) Transition metals, ferritin, glutathione and ascorbic acid in parkinsonian brains. *J. Neurochem.* **52**, 515–520.

Reif D. and Simmons R. (1990) Nitric oxide mediates iron release from ferritin. *Arch. Biochem. Biophys.* **283**, 537–541.

Romanos M., Weise D., Schliesser M., Schecklmann M., Löffler J., Warnke A., Gerlach M., Classen J. and Mehler-Wex C. (2010) Structural abnormality of the substantia nigra in children with attention-deficit hyperactivity disorder. *J. Psychiatry Neurosci.* **35**, 55–58.

Ryvlin P., Broussolle E., Piollet H., Viallet F., Khalfallah Y. and Chazot G. (1995) Magnetic resonance imaging evidence of decreased putamenal iron content in idiopathic Parkinson's disease. *Arch. Neurol.* **52**, 583–588.

Salazar J., Mena N., Hunot S., Prigent A., Alvarez-Fischer D. *et al.* (2008) Divalent metal transporter (DMTI) contributes to the neurodegeneration in animal models of Parkinson's disease. *Proc. Natl Acad. Sci. USA* **105**, 18578–18583.

Saper C. B. (1999) 'Life a thief in the night'': the selectivity of degeneration in Parkinson's disease. *Brain* **122**, 1401–1402.

Schapira A. H., Cooper J. M., Dexter D. T., Jenner P., Clark J. B. and Marsden C. D. (1989) Mitochondrial complex 1 deficiency in Parkinson's disease. *Lancet* **1**, 1269.

Schipper H. M. (2000) Heme oxygenase-1, role in brain ageing and neurodegeneration. *Exp. Gerontol.* **35**, 821–830.

Schipper H. M. (2004) Heme oxygenase expression in human central nervous system disorders. *Free Radic. Biol. Med.* **37**, 1995–2011.

Schmidt M. B. (1940) Störungen des Eisenstoffwechsels und ihre Folgen. *Ergebnisse der Pathologie* **XXXV**, 105–208.

Seutin V., Shen K. Z., North R. A. and Johnson S. W. (1996) Sulfonylurea-sensitive potassium current evoked by sodium-loading in rat midbrain dopamine neurons. *Neuroscience* **71**, 709–719.

Shamoto-Nagai M., Maruyama W., Akao Y., Osawa T., Tribl F., Gerlach M., Zecca L., Riederer P. and Naoi M. (2004) Neuromelanin inhibits enzymatic activity of 26S proteosome in human dopaminergic SH-SY5Y cells. *J. Neural Transm.* **111**, 1253–1265.

Shamoto-Nagai M., Maruyama W., Hashizume Y., Yoshida M., Osawa T., Riederer P. and Naoi M. (2007) In parkinsonian substantia nigra, alpha-synuclein is modified by acrolein, a lipid peroxidation product and accumulates in the dopamine neurons with inhibition of proteasome activity. *J. Neural Transm.* **114**, 1559–1567.

Shults C. W. (2006) Lewy bodies. *Proc Natl. Acad Sci USA* **103**, 1661–1668.

Sian J., Dexter D. T. and Jenner P. (1991) Decrease in nigral glutathione in Parkinson's disease. *Br. J. Pharmacol.* **104**, 281.

Sian J., Dexter D. T., Lees A. J., Daniel S., Jenner P. and Marsden C. D. (1994a) Glutathione-related enzymes in brain in Parkinson's disease. *Ann. Neurol.* **36**, 356–361.

Sian J., Dexter D. T., Jenner P., Lees A. J., Daniel S., Agid Y., Javoy-Agid F., Jenner P. and Marsden C. D. (1994b) Alterations in glutathione levels in neurodegenerative disorders affecting the basal ganglia. *Ann. Neurol.* **36**, 348–355.

Sofic E., Riederer P., Heinsen G. and Youdim M. B. H. (1988) Increased iron (III) and total iron content in post mortem substantia nigra of parkinsonian brain J. *Neural Transm.* **74**, 199–205.

Sofic E., Lange K. W., Jellinger K. and Riederer P. (1992) Reduced and oxidized glutathione in the substantia nigra of patients with Parkinson's disease. *Neurosci. Lett.* **142**, 128–130.

Sofic E., Sapcanin A., Tahirovic I., Gavrankapetanovic I., Jellinger K., Reynolds G. P., Tatschner T. and Riederer P. (2006) Antioxidant capacity in postmortem brain tissues of Parkinson's and Alzheimer's diseases. *J. Neural Transm. Suppl.* **71**, 39–43.

Spillantini M. G. and Goedert M. (2000) The alpha-synucleinopathies: Parkinson's disease, dementia with Lewy bodies and multiple system atrophy. *Ann. N Y Acad. Sci.* **920**, 16–27.

Stankiewicz J., Scott S. P., Neema M., Arora A., Batt C. and Bakshi R. (2007) Iron in chronic brain disorders: imaging and neurotherapeutic implications. *Neurotherapeutics* **4**, 371–386.

Sulzer D. and Zecca L. (2000) Untraneuronal dopamine-quinone synthesis: a review. *Neurotox. Res.,* **1**, 181–195.

Sulzer D., Bogulavsky J., Larsen K., Behr G. *et al.* (2000) Neuromelanin biosynthesis driven by excess cytosolic catecholamines not accumulated by synaptic vesicles. *Proc. Natl Acad. Sci. USA,* **87**, 11869–11874.

Tan K. H., Purcell W. M., Heales S. J., McLeod J. D. and Hurst R. D. (2002) Evaluation of the role of P-glycoprotein in inflammation induced blood-brain barrier damage. *Neuroreport* **13**, 2593–2597.

Tanner C. M., Ottman R., Goldman S. M., Ellenberg J., Chan P., Mayeux R. *et al.* (1999) Parkinson's disease in twins: an etiologic study. *JAMA* **281**, 341–366.

Tribl F., Gerlach M., Marcus K., Asan E., Tatschner A., Meyer H. E., Bringmann G. and Riederer P. (2005) Subcellular proteomics of neuromelanin granules isolated from the human brain. *Mol. Cell Proteomics* **4**, 945–957.

Tribl F., Asan E., Arzberger T., Tatschner T., Lagenfeld E., Meyer H. E., Bringmann G., Riederer P. *et al.* (2009) Identification of L-ferritin in neuromelanin granules of the substantia nigra – a targeted proteomics approach. *Mol. Cell Proteomics* **8**, 1832–1838.

Uitti R. J., Rajput A. H., Rozdilsky B., Bickis M., Wollin T. and Yuen W. K. (1989) Regional metal concentrations in Parkinson's disease, other chronic neurological disease, and control brains. *Can. J. Neurol. Sci.* **16**, 310–314.

Volles M. J., Lees S. J., Rochert J. C., Shtilerman M. D., Ding T. T., Kessler J. C. *et al.* (2001) Vesicle permeabilisation by protofibrillar alpha-synuclein: implications for the pathogenesis and treatment of Parkinson's disease. *Biochemistry* **40**, 7812–7819.

Vymazal J., Righini A., Brooks R. A., Canesi M., Mariani C., Leonardi M. and Pezzoli G. (1999) T1 and T2 in the brain of healthy subjects, patients with Parkinson disease, and patients with multiple system atrophy: relation to iron content. *Radiology* **211**, 489–495.

Wallis L. I., Paley M. N., Graham J. M., Grunewald R. A., Wignall E. L., Joy H. M. and Griffiths P. D. (2008) MRI assessment of basal ganglia iron deposition in Parkinson's disease. *J. Magn. Reson. Imaging* **28**, 1061–1067.

Walter U., Hoeppner J., Prudente-Morrissey L., Horowski S., Herpertz S. C. and Benecke R. (2007) Parkinson's disease-like midbrain sonography abnormalities are frequent in depressive disorders. *Brain* **130**(Pt 7), 1799–1807.

Ward W. C., Zucca F. A., Bellei C., Zecca L. and Simon J. D. (2009) Neuromelanins in various regions of human brain are associated with native and oxidized isoprenoid lipids. *Arch. Biochem. Biophys.* **484**, 94–99.

Weinreb O., Amit T., Bar-Am O. and Youdim M. B. (2010) Rasagiline: a novel anti-parkinsonian monoamine oxidase-b inhibitor with neuroprotective activity. *Prog. Neurobiol.* **92**, 330–344.

Weizer-Stern O., Adamsky K., Amariglio N., Levin C. *et al.* (2006) Downregulation of hepcidin and haemojuvelin expression in the hepatocyte cell-line HepG2 induced by thalassaemic sera. *Br. J. Haematol.* **135**, 129–138.

Wesemann W., Solbach M., Nafe R., Grote C., Sontag K. H., Riederer P., Jellinger K., Mennel H. D. and Clement H. W. (1995) Effect of lazaroid U-74389 on iron-induced reduction of striatal dopamine metabolism. *J. Neural Transm.* **46**, 175–182.

Wills A. J., Sawle G. V., Guilbert P. R. and Curtis A. R. (2002) Palatal tremor and cognitive decline in neuroferritinopathy. *J. Neurol. Neurosurg. Psychiatry,* **73**, 91–93.

Wilms H., Rosenstiel P., Sievers J., Deuschi G., Zecca L. and Lucius R. (2003) Activation of microglia by human neuromelanin is NF-kappaB dependent and involves p38 mitogen-activated protein kinase; implications for Parkinson's disease. *FASEB J.* **17**, 500–502.

Wichmann T. and DeLong M. R. (1993) Pathophysiology of parkinsonian motor abnormalities. *Adv. Neurol.* **60**, 53–61.

Wu L. J., Leenders A. G., Cooperman S., Meyron-Holtz E., Smith S., Land W. *et al.* (2004) Expression of iron transporter ferroportin in synaptic vesicles and the blood brain barrier. *Brain Res.* **1001**, 108–117.

Yoritaka A., Hattori N. and Uchida K. (1996) Immunohistochemical detection of 4-hydroxynonenal protein adducts in Parkinson's disease. *Proc. Natl Acad. Sci. USA* **93**, 2696–2701.

Yoshida T., Tanaka M., Sotomatsu A. and Hirai S. (1995) Activated microglia cause superoxide-mediated release of iron from ferritin. *Neurosci. Lett.* **190**, 21–24.

Youdim M. B. H. (2003) Rasagiline: an anti-Parkinson drug with neuroprotectivity. *Exp. Rev. Neurother.* **3**, 737–749.

Youdim M. B. H. and Riederer P. (1993) The role of iron in senescence of dopaminergic neurons in Parkinson's disease. *J. Neural Transm. Suppl.* **40**, 57–67.

Youdim M. B. H. and Riederer P. (2004) Iron in the brain, normal and pathological, in Encyclopedia of Neuroscience (Adelman G. and Smith B., eds), pp. 984–987. Elsevier, Amsterdam.

Yu H. C., Feng S. F., Chao P. L. and Lin A. M. (2010). Anti-inflammatory effect of pioglitazone on iron-induced oxidative injury in the nigrostriatal dopaminergic system. *Neuropathol. Appl. Neurobiol.* **36**, 612–622.

Zecca L., Gallorini M., Schunermann V., Trautwein A. X. and Gerlach M. (2001) Iron, neuromelanin and ferritin of normal subjects at different ages, consequences for iron storage and neurodegenerative processes. *J. Neurochem.* **76**, 1766–1773.

Zecca L., Tampellini D., Gatti A., Crippa R., Eisner M., Sulzer D. *et al.* (2002) The neuromelanin of human substantia nigra and its interaction with metals. *J. Neural Transm.* **109**, 663–672.

Zecca L., Youdim M. B. H., Riederer P., Connor J. R. and Crichton R. R. (2004) Iron, brain ageing and neurodegenerative disorders. *Nat. Rev. Neurosci.* **5**, 863–873.

Zecca L., Berg D., Arzberger T., Ruprecht P., Rausch W. D., Musicco M., Tampellini D., Riederer P., Gerlach M. and Becker G. (2005) In vivo detection of iron and neuromelanin by transcranial sonography: a new approach for early detection of substantia nigra damage. *Mov. Disord.* **20**, 1278–1285.

Zhu M., Rajamani S., Kaylor J., Hans Zhou F. *et al.* (2004) The flavonoids baicalein inhibits fibrilliation of alpha synuclein and disaggregates existing fibrils. *J. Biol. Chem.* **279**, 26846–26857.

Zimprich A., Biksup S., Leitner P. *et al*. (2004) Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron* **44**, 601–607.

Zucca F. A.., Bellei C., Giannelli S., Terreni M. R.., Gallorini M., Rizzio E., Pezzoli G., Albertini A. and Zecca L. (2006) Neuromelanin and iron in human locus coeruleus and substantia nigra during aging: consequences for neuronal vulnerability. *J. Neural Transm.* **113**, 757–767.