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Mitochondrial
dysfunction

Depleted
glutathione

Iron
accumulation

Diminished
copper

**Nexus between mitochondrial function, iron, copper and glutathione in
Parkinson's disease**

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Abstract

Parkinson's disease is neuropathologically characterised by loss of catecholamine neurons in vulnerable brain regions including substantia nigra pars compacta and locus coeruleus. This review discusses how the susceptibility of these regions is defined by their shared biochemical characteristics that differentiate them from other neurons. Parkinson's disease is biochemically characterised by mitochondrial dysfunction, accumulation of iron, diminished copper content and depleted glutathione levels in these regions. This review also discusses this neuropathology, and provides evidence for how these pathological features are mechanistically linked to each other. This leads to the conclusion that disruption of mitochondrial function, or iron, copper or glutathione metabolism in isolation provokes the pathological impairment of them all. This creates a vicious cycle that drives pathology leading to mitochondrial failure and neuronal cell death in vulnerable brain regions.

Progression of Parkinson's disease pathology

Parkinson's disease (PD) is neuropathologically characterised by the death of specific neuronal populations and the appearance of α -synuclein-containing Lewy bodies. These two pathological hallmarks exhibit regional differences in their rate of progression. Lewy body pathology occurs before overt neuronal loss, and appears first in olfactory nucleus and dorsal motor nucleus of the vagus, progressing to locus coeruleus and then substantia nigra and many other brain stem nuclei and eventually cortex (Braak and Del Tredici, 2017; Braak et al., 2003). Cell loss is observed most profoundly from the dopaminergic substantia nigra pars compacta, and also neuromelanin-containing locus coeruleus, with cell death also seen in the dorsal motor nucleus of the vagus, and peripheral nervous system noradrenergic neurons innervating the heart and skin, with more moderate neuronal death observed in the ventral tegmental area and other brain stem nuclei at later stages (Alberico et al., 2015; Halliday et al., 2005; Surmeier et al., 2017; Surmeier and Sulzer, 2013). Hence there is a disconnect between α -synuclein pathology and neuronal loss. These neuropathological features give rise to motor symptoms including bradykinesia, muscular rigidity and resting tremor, and non-motor features including olfactory symptoms, cognitive impairment, psychiatric symptoms and autonomic dysfunction.

Loss of dopaminergic neurons in the substantia nigra pars compacta is the most universal feature of PD, and is responsible for generating the overt motor symptoms associated with parkinsonism. However, substantia nigra pars compacta dopaminergic neuron loss is also evident in other conditions including multiple system atrophy and progressive supranuclear palsy (Dickson, 2012). Neuronal loss from the locus coeruleus generally occurs later and is not quite as profound as degeneration in the substantia nigra pars compacta, and is also observed in Alzheimer's disease and Down syndrome (German et al., 1992; Kelly et al., 2017).

Mitochondrial impairment in Parkinson's disease

Mitochondrial impairment occurs early in PD pathogenesis (Lin and Beal, 2006). There is abundant evidence for impaired activity of mitochondrial complex I in PD post-mortem tissue, profoundly from SN, but also other brain regions including frontal cortex and the periphery (Bindoff et al., 1989; Mann et al., 1994; Mizuno et al., 1989; Parker et al., 1989; Parker et al., 2008; Schapira et al., 1990; Schapira et al., 1989). Furthermore, mutations in genes associated with familial PD are involved in mitochondrial dysfunction, including α -synuclein, PINK1, Parkin, DJ-1 and LRRK2 (Ariga et al., 2013; Esteves et al., 2014; Nakamura, 2013; Truban et al., 2017).

Further evidence for mitochondrial impairment in the pathogenesis of PD comes from the selective sensitivity of the substantia nigra and locus coeruleus to mitochondrial toxins, including 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-hydroxydopamine, rotenone and paraquat (Giannoccaro et al., 2017; Masilamoni et al., 2017). All are complex I inhibitors and administration causes neuronal death and gives rise to parkinsonism in animal models. In humans, MPTP administration causes parkinsonism (Langston et al., 1983), while environmental exposure to mitochondrial toxins increases the likelihood of developing PD (Tanner et al., 2011).

The sensitivity of the substantia nigra to MPTP may be due in part to the unique metabolism of the toxin in this region. MPTP is metabolised to 1-methyl-4-phenylpyridinium (MPP^+) within astrocytes by monoamine oxidase B, the latter required for dopamine metabolism. MPP^+ is the toxic metabolite that potently inhibits complex I, and traffics from astrocytes into neurons via dopamine transporters.

Inhibition of astrocytic dopamine transporters blocks MPP⁺ export from astrocytes and prevents MPTP toxicity in mice (Cui et al., 2009). Thus neurons are selectively vulnerable to MPP⁺ over glia, and substantia nigra dopaminergic neurons are particularly sensitive to MPTP due to the presence of dopamine transporters, facilitating the uptake of the toxic metabolite MPP⁺.

The presence of dopamine concurrent with elevated iron is often postulated to contribute to the sensitivity of substantia nigra neurons. Dopamine is metabolised by monoamine oxidases in a reaction generating hydrogen peroxide. Dopamine can also be oxidised (eventually) to neuromelanin, giving the characteristic black colour to the tissue (substantia nigra is Latin for black substance). In the presence of iron, multiple toxic species can be generated from dopamine and its metabolites, evidence for which has variously been shown in vitro and in vivo (Hare and Double, 2016; Zucca et al., 2015). However, this is unlikely to be the sole reason for neuronal degeneration in PD as other vulnerable regions such as the locus coeruleus do not contain high iron levels, and the frontline treatment for PD, L-DOPA, which is metabolised to dopamine, does not greatly accelerate disease progression, which would be expected if dopamine was the central toxic species.

One salient factor that differentiates degenerating neurons from relatively insensitive neurons relates to basal mitochondrial stress. Many neuronal populations exhibit autonomous pacemaking axonal firing, that is axonal signalling in the absence of synaptic input, including vulnerable regions in PD such as the substantia nigra pars compacta, locus coeruleus and dorsal motor nucleus of the vagus, as well as relatively insensitive ventral tegmental area and Purkinje neurons. However, the vulnerable neurons have an unusual reliance on Cav1 calcium channels (Chan et al., 2007) and have relatively poor calcium buffering (Foehring et al., 2009; Goldberg et al., 2012) as compared to ventral tegmental area or Purkinje neurons (Liang et al., 1996; Schmidt et al., 2007). This pacemaking calcium signalling requires elevated mitochondrial activity, leading to oxidative stress. Indeed neurons in the substantia nigra and dorsal motor nucleus of the vagus, but not ventral tegmental area, exhibit elevated mitochondrial oxidative stress under basal conditions (Goldberg et al., 2012; Guzman et al., 2010; Horowitz et al., 2011). This appears to be a characteristic that differentiates vulnerable neuronal populations in regions including substantia nigra,

locus coeruleus and dorsal motor nucleus of the vagus from relatively insensitive regions such as ventral tegmental area neurons (Surmeier et al., 2016; Surmeier and Sulzer, 2013). Further evidence for a mechanistic involvement of Cav1 channels in pathological mitochondrial stress includes an amplification of this basal oxidative stress in the absence of antioxidant DJ-1, which can be prevented by inhibition of Cav1 channels by isradipine (Goldberg et al., 2012; Guzman et al., 2010). Accordingly, isradipine is currently being investigated in a phase III clinical trial (NCT02168842) following a successful phase II trial which indicated a trend towards efficacy (ParkinsonStudyGroup, 2013).

Hence neurons in vulnerable regions including the substantia nigra, locus coeruleus and dorsal motor nucleus of the vagus are under elevated basal oxidative stress and are highly susceptible to mitochondrial impairment. Other biochemical features of vulnerable regions in PD include accumulation of iron, and depletion of copper and glutathione (Table 1). These will now be discussed in relation to their effect on mitochondrial function.

Iron accumulates in vulnerable regions in Parkinson's disease

It is well established from examination of post-mortem tissue that iron is elevated in normal substantia nigra as compared to other brain regions, and is specifically further elevated in PD (Davies et al., 2013; Dexter et al., 1991; Dexter et al., 1990; Dexter et al., 1987; Dexter et al., 1989; Griffiths et al., 1999; Riederer et al., 1989; Sofic et al., 1991; Sofic et al., 1988). In addition, substantia nigra iron is selectively increased in monogenetic forms of PD as measured by transcranial sonography (Schweitzer et al., 2007) and in autosomal recessive juvenile parkinsonism (Takanashi et al., 2001). Iron is also relatively high in other regions, such as caudate, putamen and globus pallidus (Davies et al., 2013; Zucca et al., 2015), but has been reported to decrease in these regions in PD (Popescu et al., 2009). Although the locus coeruleus degenerates almost as profoundly as substantia nigra pars compacta, it contains very little iron (Zecca et al., 2004). Nevertheless, iron still accumulates in neuromelanin-containing neurons in the locus coeruleus in PD (Davies et al., 2014).

In mice, the relatively insensitive substantia nigra pars reticulata contains approximately 45% more iron than the substantia nigra pars compacta, although pars

compacta iron is still elevated over the adjacent ventral tegmental area (Hare et al., 2014). Both the ventral tegmental area and substantia nigra pars compacta contain tyrosine hydroxylase-positive dopaminergic neurons, while the substantia nigra pars reticulata does not. Hence the sensitivity of pars compacta neurons to degeneration may be due to their unique characteristic of both elevated iron and presence of dopamine (Hare et al., 2014), although this would not explain the sensitivity of neurons in the locus coeruleus, where iron levels are approximately 7-fold lower than substantia nigra (Zecca et al., 2004). Toxicity of iron is also determined by the relative abundance of suitable ligands such as ferritin. For example, the locus coeruleus has approximately 10-fold less L-ferritin than the substantia nigra (Zecca et al., 2004). An early histochemical study corroborates the differential distribution of iron between the substantia nigra pars compacta and pars reticulata (Morris and Edwardson, 1994), as does recent magnetic resonance imaging of PD patients: a region of the substantia nigra pars compacta referred to as the dorsolateral nigral hyperintensity (corresponding to lower iron than the adjacent medial pars compacta and pars reticulata regions) is consistently lost in PD (Mahlknecht et al., 2017), corresponding to accumulation of iron in this region.

Histological stains for iron in the substantia nigra find most iron associated with glia and non-pigmented (neuromelanin-free) neurons (Morris and Edwardson, 1994; Zecca et al., 2004). Most increased iron is associated with glial ferritin (Griffiths et al., 1999; Hare et al., 2014; Morris and Edwardson, 1994; Zecca et al., 2001), and ferritin levels have been found to be both elevated and decreased in post-mortem PD substantia nigra (Dexter et al., 1991; Dexter et al., 1990; Riederer et al., 1989). Neuronal neuromelanin also robustly binds iron (Double et al., 2003). Although apparently not detectable by histological stains, elevated iron has been detected by X-ray fluorescence in neuromelanin in substantia nigra dopaminergic neurons and locus coeruleus noradrenergic neurons in PD (Davies et al., 2014; Oakley et al., 2007) and may be the major form of iron storage in neurons (Zecca et al., 2001).

In models of PD, substantia nigra iron is elevated in response to MPTP, rotenone and 6-hydroxydopamine in monkeys, mice and rats (Devos et al., 2014; Hare et al., 2014; Lee et al., 2009b; Lv et al., 2011; Mastroberardino et al., 2009; Mena et al., 2011; Mena et al., 2015; Mochizuki et al., 1994; Oestreicher et al., 1994; Temlett et al.,

1994). This elevation in iron is restricted to the substantia nigra, with no change in iron in the neighbouring dopaminergic ventral tegmental area (Lv et al., 2011; Mastroberardino et al., 2009), and the elevated iron has been localised to both neurons and glia (Oestreicher et al., 1994).

The pathological importance of iron is ably demonstrated by experiments in which sequestering iron is neuroprotective. Decreasing labile iron by treatment of mice with chelators including deferoxamine, deferiprone and clioquinol, among others, or overexpressing ferritin invariably prevents the loss of substantia nigra dopaminergic neurons and rescues motor deficits in mouse models of PD (Devos et al., 2014; Kaur et al., 2003; Lee et al., 2009b; Lei et al., 2015; Lei et al., 2012; Mastroberardino et al., 2009; Mena et al., 2011; Mena et al., 2015). Importantly, deferiprone was assessed in a recent phase II clinical trial in PD patients and exhibited efficacy on the unified Parkinson's disease rating scale (Devos et al., 2014). This constitutes the first compelling evidence for efficacy of iron chelation therapy for the treatment of PD.

The iron-mitochondria nexus

Mitochondria play a key role in regulating iron metabolism. They are the site of Fe-S cluster assembly and heme synthesis. Fe-S clusters are integral for the function of many proteins including complex I of the electron transport chain and other iron metabolism proteins (Liddell, 2015). Of these, the bifunctional cytosolic protein aconitase/iron regulatory protein 1 is central to the regulation of iron metabolism. In iron-replete cells when Fe-S cluster assembly is sufficient, aconitase/iron regulatory protein 1 binds an Fe-S cluster, promoting aconitase activity. However, under low iron conditions, Fe-S cluster assembly is impaired, resulting in insufficient Fe-S cluster binding. Under these conditions, iron regulatory protein 1 lacks aconitase activity and exhibits an iron regulatory role, binding to iron-responsive elements on the mRNA of iron trafficking genes, increasing translation of iron import proteins such as transferrin receptor and divalent metal transporter 1, and downregulating iron storage (ferritin) and export (ferroportin). Together, this coordinates iron trafficking proteins to facilitate cellular iron accumulation (Muhlenhoff et al., 2015).

Pathological disruption of Fe-S cluster assembly leads to aberrant iron regulatory protein signalling and a false iron starvation phenotype due to lack of Fe-S cluster binding by iron regulatory protein 1. In diseases such as Friedreich's ataxia (Rotig et

al., 1997) and several other conditions involving disrupted Fe-S assembly (Stehling et al., 2014), this leads to mitochondrial iron accumulation in a futile attempt to fuel Fe-S cluster assembly.

Analysis of iron regulatory proteins in post-mortem tissue implies that iron regulatory protein signalling is disrupted in PD. Firstly, there is no clear change in ferritin levels in PD substantia nigra, with levels variously reported as increased (Riederer et al., 1989), decreased (Dexter et al., 1991; Dexter et al., 1990) or unchanged (Mann et al., 1994). This discrepancy may be due to methodological differences (Dexter et al., 1991; Mann et al., 1994). The elevated iron in PD substantia nigra without a clear concomitant increase in ferritin is consistent with a dysregulation of iron regulatory protein signalling and likely increase in free or labile iron (Hirsch, 2006). The iron import protein divalent metal transporter 1 (containing an iron-responsive element) is also increased in PD substantia nigra, indicative of dysregulated iron regulatory protein signalling (Ayton et al., 2015). In mice treated with MPTP, the increased iron content is accompanied by decreased ferroportin, also indicative of dysregulated iron regulatory protein signalling (Lee et al., 2009b). On the other hand, transferrin receptor expression is reportedly low in normal SN (Faucheux et al., 1993), and although there is no loss of transferrin receptor from the SN in PD (Ayton et al., 2015; Faucheux et al., 1993), a specific loss of transferrin receptor from neuromelanin-containing neurons in SN has been reported (Faucheux et al., 1997), and there is a loss of transferrin receptor in 6-hydroxydopamine and MPTP-treated rats and mice (Gal et al., 2010; He et al., 1999; You et al., 2016). This decreased transferrin receptor expression concomitant with elevated cellular iron levels suggest normal iron regulatory protein signalling in PD, in contrast to other iron trafficking proteins. However, it is important to note that iron regulatory protein-independent modulation of transferrin receptor levels has been observed (Kaur et al., 2009).

Mitochondrial inhibition impairs Fe-S cluster synthesis, which leads to mitochondrial iron accumulation (Stehling et al., 2014). For example, the PD-related mitochondrial toxin rotenone impairs Fe-S cluster assembly and causes iron regulatory protein-mediated mitochondrial iron accumulation in vitro (Mena et al., 2011) and in rodents (Mastroberardino et al., 2009; Mena et al., 2015). Hence it is likely that the iron accumulation evident in response to complex I inhibition by other mitochondrial

toxins is also driven by impaired Fe-S cluster assembly and aberrant iron regulatory protein signalling.

Elevated mitochondrial iron causes oxidative stress, impairs mitochondrial function and leads to neuronal death (Munoz et al., 2016). Sequestering mitochondrial iron by overexpression of mitochondrial ferritin protects neuronal cells from multiple mitochondrial toxins in vitro (Shi et al., 2010; Wang et al., 2016; You et al., 2016). Furthermore, iron chelators that can access mitochondrial iron, including deferiprone, show efficacy in the MPTP mouse model (Devos et al., 2014; Mena et al., 2015). As discussed above, deferiprone recently exhibited efficacy in a phase II clinical trial in PD patients (Devos et al., 2014). Hence a pathologically relevant pool of elevated iron appears to be localised to mitochondria in PD. This elevation may be driven by aberrant iron regulatory protein signalling as a result of mitochondrial dysfunction.

In addition to disrupting Fe-S cluster assembly, mitochondrial dysfunction increases oxidative stress. Iron binding by aconitase/iron regulatory protein 1 is exquisitely sensitive to radicals such as superoxide (Gardner and Fridovich, 1991) and nitric oxide (Jaffrey et al., 1994). Hence iron regulatory protein signalling may be additionally dysregulated by the elevated mitochondrial stress abundantly evident in PD, disrupting the binding of Fe-S clusters to iron regulatory protein 1.

Together these studies show that normal iron metabolism requires adequate mitochondrial activity. The impaired mitochondrial activity evident in PD can disrupt iron regulation, causing accumulation of mitochondrial iron. Conversely, iron accumulation causes oxidative stress, disrupting mitochondrial function. Hence both aberrant iron metabolism and mitochondrial dysfunction impair each other, and both are evident in PD.

Copper is depleted from vulnerable regions in Parkinson's disease

In normal brain, the substantia nigra and locus coeruleus are highly enriched for copper as compared to other brain regions, with approximately 2-3-fold more copper in these regions compared to cortex (Davies et al., 2014; Davies et al., 2013; Dexter et al., 1991; Rios et al., 1995). Accordingly, a major cuproprotein superoxide dismutase 1 is highly expressed in the substantia nigra and striatum of mice (Pardo et al., 1995),

and seems to be specific to the substantia nigra pars compacta and not pars reticulata (Hare et al., 2014). Furthermore, the copper importer Ctr1 is abundant in the pars compacta but not pars reticulata (Davies et al., 2013). Hence it is likely that the elevated copper is restricted to the substantia nigra pars compacta, and indicates that copper content is another factor that distinguishes the vulnerable pars compacta from the adjacent insensitive pars reticulata in normal brain (Table 1).

In PD, the copper contents of the substantia nigra and locus coeruleus are selectively decreased by approximately 50% compared to control, with no significant change in other regions (Ayton et al., 2013; Davies et al., 2014; Dexter et al., 1991; Dexter et al., 1987; Dexter et al., 1989; Popescu et al., 2009). This decrease has been detected specifically within neuromelanin-containing neurons in both the substantia nigra pars compacta and locus coeruleus (Davies et al., 2014). Furthermore, Ctr1 is decreased in these neurons, but not from neuromelanin-containing neurons in the ventral tegmental area (Davies et al., 2014). That copper and Ctr1 are lost from both substantia nigra pars compacta and locus coeruleus, but not ventral tegmental area neurons in PD indicates that copper loss in PD is specific to the vulnerable areas (Davies et al., 2014), consistent with impaired capacity to uptake copper in PD.

Copper deficiency impairs mitochondrial function via insufficient supply of copper to complex IV. Indeed, complex IV activity is impaired in LRRK2 mutant PD patients (Mortiboys et al., 2015) and in mutated PINK1 and DJ-1 patient fibroblasts and animal models (Lopez-Fabuel et al., 2017). Hence disrupted copper entry into the substantia nigra in PD can directly impair mitochondrial activity.

Copper deficiency also impacts the activity of other cuproproteins. There is a profound loss of the copper-dependent activity of ceruloplasmin, despite no change in ceruloplasmin protein level in PD substantia nigra (Ayton et al., 2013) and cerebrospinal fluid (Olivieri et al., 2011). Ceruloplasmin is a major cuproenzyme considered the major systemic copper carrier, and is found glycosylphosphatidylinositol-anchored to the extracellular surface of astrocytes in the brain (Jeong and David, 2003). Enzymatic activity of ceruloplasmin requires occupancy of all six copper sites, formation of which is dependent on copper supply to the secretory pathway (Hellman et al., 2002).

In mice, MPTP alters copper levels in an apparently dose-dependent manner. MPTP at a total dose of 150mg/kg causes approximately 50% decrease in striatum and midbrain copper levels, whereas 90mg/kg causes only small decrease (Rios et al., 1995), and 40mg/kg actually increases substantia nigra copper by 19% (Ayton et al., 2013). Regardless, ceruloplasmin activity is severely decreased in MPTP-treated mice (Ayton et al., 2013), indicating substantial disruption of copper metabolism in response to this toxin.

Copper-dependent iron trafficking

Ceruloplasmin activity coordinates cellular copper and iron levels via its key role in iron export. Ceruloplasmin is a ferroxidase responsible for oxidising Fe^{2+} exported by ferroportin to Fe^{3+} , to promote iron binding to transferrin and prevent Fe^{2+} -mediated oxidation reactions. Deletion of ceruloplasmin in mice causes glial iron accumulation (Jeong and David, 2003, 2006), elevated substantia nigra iron and the development of parkinsonism (Ayton et al., 2013; Patel et al., 2002). This can be rescued by iron chelation via deferiprone (Ayton et al., 2013). Lack of ceruloplasmin also exacerbates MPTP toxicity in mice, presumably due to elevated iron, a hypothesis supported by similar exacerbation of MPTP toxicity in conjunction with iron loading (You et al., 2015). Conversely, peripheral administration of ceruloplasmin protects against MPTP toxicity, indicating improved iron trafficking provides protection against MPTP (Ayton et al., 2013). In PD patients, the effectiveness of deferiprone iron chelation therapy correlates with the extent of ceruloplasmin loss (Grolez et al., 2015), suggesting loss of ceruloplasmin correlates with pathological iron accumulation.

Aceruloplasminemia patients harbouring loss-of-function mutations in ceruloplasmin also have disrupted iron metabolism, with glial iron accumulation (Gonzalez-Cuyar et al., 2008; Oide et al., 2006) and certain mutations are associated with elevated nigral iron and parkinsonism (Hochstrasser et al., 2004; Hochstrasser et al., 2005; McNeill et al., 2008). If iron accumulation is central to PD pathogenesis, it could be expected that loss of ceruloplasmin should be more strongly associated with parkinsonism. That the relationship is relatively mild can be explained by two aspects. Firstly, ceruloplasmin is mainly expressed in astrocytes and lack of ceruloplasmin function causes glial iron accumulation (Jeong and David, 2003), possibly eliciting only minimal disruption to

neuronal iron metabolism. Secondly, amyloid precursor protein provides complementary iron trafficking activity. Amyloid precursor protein is also a cuproprotein, and has been shown to be involved in iron export in association with ferroportin (Duce et al., 2010; Wong et al., 2014). Deletion of amyloid precursor protein from mice causes nigral iron accumulation, neuron loss and parkinsonism (Ayton et al., 2015). Iron chelation partially prevents nigral neuron loss in amyloid precursor protein knockout mice and overexpression of amyloid precursor protein protects against MPTP toxicity in wild type mice (Ayton et al., 2015). Correlating with iron accumulation, amyloid precursor protein expression is diminished in the substantia nigra of PD patients and by MPP⁺ treatment in cultured neuronal cells (Ayton et al., 2015). While amyloid precursor protein appears to colocalise with tyrosine hydroxylase-positive neurons in human substantia nigra (Ayton et al., 2015), astrocytes also express amyloid precursor protein in vivo (Banati et al., 1995; Clarner et al., 2011; Gehrmann et al., 1995), although its role in glial iron trafficking is yet to be specifically determined.

Both ceruloplasmin (Mazumder et al., 2006) and amyloid precursor protein expression in astrocytes are strongly upregulated by inflammation (Banati et al., 1995; Clarner et al., 2011; Gehrmann et al., 1995). That the clear inflammation evident in PD (Rocha et al., 2015) is not accompanied by increased ceruloplasmin or amyloid precursor protein activity further indicates that there is an insufficient supply of copper to meet the requirements of these cuproproteins in PD.

Disrupted copper uptake in PD substantia nigra not only prevents adequate supply of copper for the enzymatic activity of amyloid precursor protein, but also disturbs its subcellular localisation. Copper regulates amyloid precursor protein trafficking from the perinuclear region to the cell surface (Acevedo et al., 2011) in a phosphorylation-dependent manner, and copper depletion prevents this trafficking (Acevedo et al., 2014). Disrupted amyloid precursor protein trafficking to cell surface is evident in tau knockout mice, which exhibit iron accumulation and parkinsonism (Lei et al., 2012). Hence impaired amyloid precursor protein trafficking due to copper depletion contributes to iron accumulation.

In addition to diminished copper availability via decreased Ctr1 expression, ceruloplasmin and amyloid precursor protein activity can be further decreased by oxidative stress. In PD patients, ceruloplasmin is oxidised in cerebrospinal fluid, causing release of copper and loss of ferroxidase activity, and the extent of ceruloplasmin oxidation correlates with PD clinical progression (Olivieri et al., 2011). In mice, MPTP treatment causes loss of both ceruloplasmin activity (Ayton et al., 2013) and amyloid precursor protein expression (Ayton et al., 2015).

In addition to inflammation, amyloid precursor protein expression is also regulated by iron regulatory protein signalling. Amyloid precursor protein contains an iron-responsive element promoting its translation under iron-replete conditions (Rogers et al., 2002). Amyloid precursor protein expression is inhibited in a manner dependent on aberrant iron regulatory protein signalling, possibly involving nitric oxide (Ayton et al., 2015). Hence disrupted iron regulatory protein signalling evident in PD as a result of mitochondrial and oxidative stress (as discussed above) contributes to iron accumulation via impaired amyloid precursor protein expression. In this way, amyloid precursor protein regulation differs from ceruloplasmin, and explains why amyloid precursor protein expression is decreased (Ayton et al., 2015) while ceruloplasmin expression is maintained in PD, the latter losing copper-dependent activity (Ayton et al., 2013) due to diminished copper availability.

The therapeutic efficacy of modulating copper levels in the substantia nigra has been assessed. The copper drug diacetylbis(N(4)-methylthiosemicarbazonato)copper^{II} (Cu^{II}(atsm)) exerts potent neuroprotective effects in multiple animal models of PD, preventing dopaminergic neuron loss and rescuing motor impairments (Hung et al., 2012). A positron emission tomography imaging study demonstrates that Cu^{II}(atsm) selectively localises to the striatum in PD patients (Ikawa et al., 2011). However, the mechanism/s by which Cu^{II}(atsm) elicits its neuroprotective effects are unclear. Cu^{II}(atsm) has been demonstrated to deliver bioavailable copper to endogenous proteins (Roberts et al., 2014), hence the protection afforded by Cu^{II}(atsm) in PD models may involve copper delivery, enhancing the activity of cuproproteins including complex IV, ceruloplasmin and amyloid precursor protein, correcting mitochondrial impairment and iron trafficking, respectively. In addition, Cu^{II}(atsm) effectively detoxifies nitric oxide (Hung et al., 2012). As discussed above, amyloid

precursor protein expression is hypothesised to be suppressed by nitric oxide-induced disruption of iron regulatory protein signalling (Ayton et al., 2015). Therefore, Cu^{II} (atm) could correct aberrant iron regulatory protein signalling by degrading nitric oxide, correcting expression of amyloid precursor protein and other iron metabolism proteins, and thereby limit toxic iron accumulation.

These studies demonstrate that the substantia nigra in PD is characterised by disruption of copper trafficking and insufficiency of ceruloplasmin and amyloid precursor protein, two key cuproproteins directly involved in iron trafficking. Further, these studies demonstrate that aberrant iron trafficking is sufficient to instigate neuronal death in the substantia nigra, implicating iron accumulation per se in the pathogenesis of PD.

Glutathione is depleted from substantia nigra in Parkinson's disease

A robust feature of PD is the selective loss of glutathione from the substantia nigra. Many studies have reported substantial glutathione depletion (30-50%), coupled with elevated proportion of oxidised glutathione in post-mortem PD substantia nigra tissue (Fitzmaurice et al., 2003; Pearce et al., 1997; Perry and Yong, 1986; Sian et al., 1994a; Sian et al., 1994b; Sofic et al., 1992). Although this has yet to be verified in PD patients in the clinic, recent advances in magnetic resonance spectroscopy technology have shown decreased substantia nigra glutathione in MPTP-treated marmosets (Heo et al., 2017). A clinical trial (NCT01470027) investigating magnetic resonance spectroscopy detection of glutathione in PD patients has recently been completed but yet to report. A histochemical study showed a profound loss of glutathione from surviving dopaminergic neurons in PD substantia nigra, while glutathione was maintained in glia (Pearce et al., 1997), hence it may be an early feature in PD. Depletion of glutathione from rat brain substantia nigra is sufficient to induce dopaminergic neuron death and motor impairment (Garrido et al., 2011). Three small phase I/II clinical trials assessing glutathione delivery to PD patients have produced mild yet positive results (Hauser et al., 2009; Mischley et al., 2015; Sechi et al., 1996). Hence loss of glutathione per se is sufficient to kill substantia nigra neurons, and therapeutic rescue of glutathione levels may provide clinical benefit.

Relationship between glutathione and mitochondria

Glutathione plays several important roles in mitochondria, and lack of glutathione causes mitochondrial dysfunction (Jha et al., 2000; Lee et al., 2009a). Conversely, mitochondrial and oxidative stress are well known to deplete cellular glutathione levels, e.g. MPTP causes loss of glutathione in mice (Kaur et al., 2003; Yong et al., 1986). In addition to its well-known functions as an antioxidant and substrate for glutathione peroxidases, glutathione is also critical for maintaining the redox status of proteins via glutaredoxins. Glutaredoxins catalyse the reduction of glutathione-protein mixed thiols. Glutaredoxin 2 is a mitochondrial isoform in mammalian cells (Gladyshev et al., 2001). Deletion of glutaredoxin 2 causes mitochondrial defects including glutathionylation of complex I and IV and uncoupling protein-3, disrupting their activity (Mailloux et al., 2013; Wu et al., 2011; Wu et al., 2014). Furthermore, glutaredoxin 2 activity is dependent on glutathione levels (Lee et al., 2009a). Hence loss of glutathione can disrupt mitochondrial function, and aberrant mitochondrial function can deplete glutathione levels.

Glutathione is required for copper uptake

Glutathione plays a key role in copper metabolism. Early studies showed more than 60% of cellular copper is associated with glutathione in hepatoma cell lines (Freedman et al., 1989). Furthermore, glutathione is required for copper accumulation by HEK293 cells (Maryon et al., 2013). Depletion of cellular glutathione by buthionine sulfoximine treatment robustly decreases copper uptake, an effect which is not altered by knockdown or overexpression of copper chaperone for superoxide dismutase or Atox1, suggesting glutathione binds copper imported by the plasma membrane transporter Ctr1 as an intermediate before being bound by higher affinity chaperones (Maryon et al., 2013). Indeed, Banci et al (Banci et al., 2010) proposed copper is trafficked along affinity gradients, likely trafficked from glutathione to higher affinity chaperones such as Atox1, copper chaperone for superoxide dismutase and Cox17, then to their very high affinity final cuproproteins. In addition to copper uptake, a high level of reduced glutathione is also required to maintain Atox1 in a reduced state, facilitating copper trafficking to the secretory pathway (Hatori et al., 2012; Hatori et al., 2016). In vivo, depletion of glutathione in drosophila gives rise to a neuronal copper deficiency phenotype that can be partially rescued by copper (Mercer et al., 2016). Hence glutathione is required for copper uptake and must be maintained in reduced form for appropriate copper trafficking. In PD, depleted

glutathione in the SN may impair copper trafficking, leading to the observed decreased copper levels.

Glutathione modulates iron metabolism via interaction with Fe-S clusters

Glutathione is involved in the regulation of iron. In yeast, severe depletion of glutathione impairs mitochondrial Fe-S cluster assembly and evokes a profound iron starvation phenotype (Kumar et al., 2011). In various neuronal cell lines and drosophila, while glutathione depletion invariably causes iron accumulation, the reported involvement of iron regulatory protein signalling is unclear, variously reported as increased, decreased or unchanged (Kaur et al., 2009; Lee et al., 2009a; Wang et al., 2016). Of note, when glutathione is depleted, mitochondria are reported as the predominant site of iron accumulation (Lee et al., 2009a), and overexpression of mitochondrial ferritin normalises iron content and expression of iron-related proteins (Wang et al., 2016), suggesting mitochondria are the key organelle affected by glutathione depletion. Conversely, elevated iron decreases glutathione, in brains of amyloid precursor protein knockout mice fed a high iron diet (Duce et al., 2010), and frataxin-null yeast (Auchere et al., 2008). Hence depleted glutathione can drive iron accumulation, and elevated iron can deplete glutathione levels.

Glutathione can regulate iron metabolism by interacting with Fe-S clusters and modulating their trafficking and assembly in mitochondria. As described above, Fe-S cluster assembly and trafficking are essential for coordination of iron metabolism via iron regulatory proteins (Muhlenhoff et al., 2015). Glutathione has recently been shown to directly coordinate with Fe-S clusters, potentially playing a role in trafficking Fe-S clusters (Fidai et al., 2016). Furthermore, glutathione interacts with Fe-S clusters via glutaredoxins. In addition to its role in thiol redox control, glutaredoxin 2 interacts with Fe-S clusters in mitochondria (Hoff et al., 2009). In-solution studies show that this coordination is dependent on the presence of reduced glutathione: when reduced glutathione becomes limiting or under oxidising conditions, the Fe-S cluster dissociates from glutaredoxin 2 (Berndt et al., 2007; Mitra and Elliott, 2009). Knockdown of glutaredoxin 2 in neuronal cells produces the same mitochondrial iron accumulation phenotype as glutathione depletion (Lee et al., 2009a). These results suggest that the depletion of glutathione described above induces the iron accumulation phenotype via impaired glutaredoxin 2 activity.

In addition to glutaredoxin 2, a second mitochondrial glutaredoxin, glutaredoxin 5 is required for mitochondrial Fe-S cluster assembly, a role conserved from yeast to humans (Rodriguez-Manzanque et al., 2002; Ye et al., 2010). Glutaredoxin 5-mediated Fe-S cluster assembly is also required for mitochondrial heme synthesis (Wingert et al., 2005; Ye et al., 2010). Knockdown of glutaredoxin 5 in erythroblasts results in an iron starvation phenotype and mitochondrial iron accumulation (Ye et al., 2010). Mutation in glutaredoxin 5 gives rise to sideroblastic anemia, with erythrocytes exhibiting impaired heme synthesis and mitochondrial iron accumulation (Ye et al., 2010). This effect appears to be restricted to erythrocytes, most likely due to their high requirement for heme synthesis and expression of erythrocyte-specific isoform of the heme synthesis protein 5'aminolevulinate synthase 2, which is repressed by iron regulatory protein signalling, unlike ubiquitous 5'aminolevulinate synthase 1 (Ye et al., 2010). Nevertheless, while fibroblasts from these patients do not exhibit iron accumulation, they do have altered expression of iron trafficking proteins (Ye et al., 2010). Given the apparent higher demand for iron in the substantia nigra, altered supply of glutathione to glutaredoxin 5 may be sufficient to impair Fe-S cluster and heme biogenesis in the substantia nigra, contributing to iron dysregulation.

As mitochondrial dysfunction can deplete glutathione levels, and given the elevated mitochondrial stress evident in normal substantia nigra, glutathione loss may be an early event instigating the observed deficits in iron and copper. Depleted glutathione will decrease copper trafficking and copper levels, leading to decreased activity of cuproproteins such as complex IV, ceruloplasmin and amyloid precursor protein, causing mitochondrial impairment and leading to aberrant iron metabolism, respectively, hence glutathione is required for mitochondrial function, copper uptake and trafficking, and coordinating appropriate iron metabolism. Conversely, iron and/or mitochondrial dysfunction both deplete glutathione levels.

Glutathione is regulated by the transcription factor Nrf2

Nrf2 is a transcription factor that regulates a battery of phase II antioxidant genes. These include NAD(P)H quinone dehydrogenase 1, heme oxygenase 1, ferritin and many enzymes of glutathione metabolism, including glutamate-cysteine ligase, the rate-limiting enzyme of glutathione synthesis. Under normal conditions, Nrf2 is

constitutively targeted for proteasomal degradation in the cytosol by its negative regulator, Keap1. Hence under normal conditions, Nrf2 signalling is very low. However, under conditions of oxidative stress, Nrf2 dissociates from Keap1 and translocates and accumulates in the nucleus where it binds to antioxidant response elements, promoting the transcription of hundreds of genes (Liddell, 2017). Expression of Nrf2 is not required for the maintenance of basal glutathione content (Liddell et al., 2016), but is required for upregulation of glutathione in response to stimulation (McMahon et al., 2001).

Nrf2 signalling in the substantia nigra is complex. In normal human post-mortem substantia nigra, Nrf2 is mainly cytosolic, whereas nuclear Nrf2 is strongly elevated in PD patients (Ramsey et al., 2007). However, this does not appear to translate into a functional increase in antioxidant proteins, as there is no difference in glutamate-cysteine ligase activity in the substantia nigra of post-mortem PD brain compared to age-matched controls (Sian et al., 1994b), and glutathione content is decreased (see above). Interestingly, γ -glutamyl transferase is selectively increased in the substantia nigra of PD brain (Sian et al., 1994b). That γ -glutamyl transferase is the only one of several Nrf2-regulated enzymes of glutathione metabolism to be changed in PD (Sian et al., 1994b) suggests that it may also be regulated by factors other than Nrf2. γ -Glutamyl transferase is involved in trafficking glutathione from astrocytes to neurons (Hirrlinger and Dringen, 2009), and increased activity could indicate increased export of glutathione from astrocytes as a response to oxidative stress.

In mice, Nrf2 signalling is elevated in normal substantia nigra compared to other brain regions (Chen et al., 2009; Jakel et al., 2005; Lv et al., 2015), providing further evidence that the substantia nigra is experiencing elevated oxidative stress under normal conditions (as discussed above). However, as in human PD substantia nigra, translation of Nrf2 signalling to functional antioxidant proteins in mouse substantia nigra appears to be impaired, with lower specific NAD(P)H quinone dehydrogenase 1 activity in substantia nigra than striatum (Chen et al., 2009).

In mouse models of PD, Nrf2 signalling in substantia nigra is also further elevated in response to MPTP (Chen et al., 2009; Ozkan et al., 2016; Swanson et al., 2013), 6-hydroxydopamine (Jakel et al., 2005), and in the A53T α -synuclein model (Gan et al.,

2012). However, all these models exhibit severe pathology. Together these studies indicate that endogenous Nrf2 signalling is insufficient to counter localised elevated oxidative stress in both normal and PD substantia nigra.

Although endogenous Nrf2 appears to be insufficient to counter oxidative stress in PD, it remains a critical determinant of dopaminergic neuron survival and PD pathology. Ablation of Nrf2 in mouse models of PD worsens survival, while overexpression of Nrf2 is protective (Chen et al., 2009; Gan et al., 2012; Jakel et al., 2007). Furthermore, a genetic association has been determined between NFE2L2 (Nrf2 gene) variants and PD risk (Todorovic et al., 2015; von Otter et al., 2014). Hence increasing Nrf2 activity enhances resistance to PD symptoms.

Many Nrf2-inducing agents are being investigated for the treatment of PD, including dimethyl fumarate (Ahuja et al., 2016; Campolo et al., 2017; Lastres-Becker et al., 2016), triterpenoids (Kaidery et al., 2013), curcumin (Cui et al., 2016; Khatri and Juvekar, 2016), and resveratrol (Gaballah et al., 2016). Meanwhile, PD drugs deprenyl and apomorphine have been shown to activate Nrf2 in vitro (Hara et al., 2006; Nakaso et al., 2006; Xiao et al., 2011).

Copper regulates Nrf2 activation

Nrf2 activation is influenced by copper. Experimental exposure to harmful doses of copper induces Nrf2 signalling (Jiang et al., 2014; Song et al., 2014), which is blocked in Nrf2-deficient cells (Song et al., 2014). Nrf2 is also induced by subtoxic levels of copper. The neuroprotective compound pyrrolidine dithiocarbamate increases brain copper levels, induces Nrf2 signalling (Liddell et al., 2016) and improves cognitive deficits in Alzheimer's disease model mice (Cheng et al., 2006; Malm et al., 2007). As discussed above, Cu^{II}(atm) exhibits robust efficacy in multiple animal models of PD (Hung et al., 2012), and has been demonstrated to deliver bioavailable copper to endogenous proteins (Roberts et al., 2014), hence the protection afforded by Cu^{II}(atm) in PD models may involve copper delivery. Cu^{II}(atm) has recently been shown to activate Nrf2 in heart (Srivastava et al., 2016), therefore Cu^{II}(atm) may be protective in PD models by activating Nrf2 in a copper-dependent mechanism. These studies demonstrate that therapeutically increasing copper activates protective Nrf2 signalling. This suggests that endogenous copper

modulates Nrf2. The copper depletion evident in PD substantia nigra may contribute to the impaired Nrf2 signalling evident in PD. As Nrf2 is a master regulator of glutathione and cellular antioxidant mechanisms, copper depletion may disrupt glutathione levels, which can drive aberrant iron metabolism, and mitochondrial and oxidative stress via impaired Nrf2 signalling.

While stimulating Nrf2 signalling prevents neuronal death in models of PD, it remains to be determined whether restoring glutathione levels is central to this protective effect, and whether this occurs via correcting iron and/or copper metabolism, preventing mitochondrial impairment or generalised restoration of redox balance, all of which are functions implicated for glutathione.

Ferroptosis, a glutathione-dependent cell death pathway, in Parkinson's disease

Ferroptosis is a recently described distinct form of non-apoptotic programmed cell death involving disruption of glutathione resulting in iron-mediated lipid peroxidation and cell death (Dixon et al., 2012; Yang et al., 2014). An essential regulator of ferroptosis is glutathione peroxidase 4 (Yang et al., 2014), which unlike other glutathione peroxidases, can detoxify membranous lipid hydroperoxides (Brigelius-Flohe and Maiorino, 2013). Ferroptosis can be experimentally induced by depletion of glutathione by buthionine sulfoximine or erastin, thus limiting glutathione availability as a substrate for glutathione peroxidase 4, or by direct inhibition or deletion of glutathione peroxidase 4 (Friedmann Angeli et al., 2014; Yang et al., 2014).

Ferroptosis causes mitochondrial abnormalities including presence of shrunken and ruptured mitochondria (Dixon et al., 2012; Friedmann Angeli et al., 2014).

Mitochondrial iron is also implicated, with sequestration of mitochondrial iron by overexpression of mitochondrial ferritin preventing erastin toxicity in a neuronal cell line and drosophila (Wang et al., 2016). Autophagic degradation of ferritin increases labile iron, decreases glutathione and increases sensitivity to erastin in fibroblasts and cancer cells, indicating elevated iron exacerbates ferroptosis (Hou et al., 2016). On the other hand, chelation of iron, for example by deferoxamine or deferiprone, blocks ferroptosis (Dixon et al., 2012; Do Van et al., 2016; Friedmann Angeli et al., 2014). Hence ferroptosis is modulated by iron availability. The exact mechanisms of ferroptosis are still being elucidated, but the presence of acyl-CoA synthetase long-chain family member 4 is required for ferroptosis to occur (Doll et al., 2017). This

protein esterifies two fatty acyls (arachidonoyl and adrenoyl) into the phospholipids phosphatidylethanolamines (Kagan et al., 2017) and its inhibition is sufficient to block ferroptosis (Doll et al., 2017; Kagan et al., 2017). These long unsaturated fatty acids are more prone to lipid peroxidation, which can be inhibited by lipid radical scavengers ferrostatin-1, liproxstatin-1 and vitamin E analogues, preventing cell death (Dixon et al., 2012; Friedmann Angeli et al., 2014).

Originally described for cancer cells (Dixon et al., 2012; Yang et al., 2014), ferroptosis has been studied in the context of neurodegeneration. Induction of ferroptosis via conditional ablation of glutathione peroxidase 4 from neurons in adult mice induces a rapid paralysis phenotype coupled with muscle atrophy and death (Chen et al., 2015). These mice display features of ferroptosis, and phenotype progression is slightly delayed by vitamin E administration (Chen et al., 2015). Conditional ablation of glutathione peroxidase 4 from forebrain neurons in adult mice induces cognitive deficits accompanied by hippocampal degeneration and markers of ferroptosis including elevated lipid peroxidation (Hambright et al., 2017). When fed a vitamin E deficient diet, hippocampal degeneration in these mice was greatly accelerated, along with the appearance of a prominent locomotor impairment (Hambright et al., 2017). Neurodegeneration was abrogated by treatment with liproxstatin-1 (Hambright et al., 2017).

Given that ferroptosis involves mitochondrial impairment, elevated iron and diminished glutathione, all cardinal features of PD, ferroptosis has also been studied in relation to PD (Guiney et al., 2017). In vitro, the ferroptosis inhibitor ferrostatin-1 prevents rotenone and MPP⁺ toxicity in a neuronal cell line (Ito et al., 2017; Kabiraj et al., 2015). In a melanoma cell line, inhibition of complex I causes ferroptosis, which can be prevented by ferrostatin-1 or overexpression of glutathione peroxidase 4, and exacerbated by knockdown of glutathione peroxidase 4 (Basit et al., 2017). Perhaps the best evidence for the involvement of ferroptosis in PD comes from a study which found LUHMES cells, a human dopaminergic neuron precursor cell line, were highly sensitive to erastin as compared to other neuronal cell lines (Do Van et al., 2016). In this study, ferrostatin-1 prevented the toxicity of glutathione depletion by erastin, buthionine sulfoximine and N-ethylmaleimide, the PD-related toxins MPP⁺, dopamine, rotenone and paraquat, and glutamate in LUHMES cells. Iron chelation by

deferiprone also protected against many of these toxins (Do Van et al., 2016). Examination of post-mortem PD brain found a substantial loss of mRNA for proteins inhibited by erastin and a 6-fold increase in glutathione peroxidase 4 mRNA in substantia nigra but not other regions including striatum and cortex (Do Van et al., 2016). Whether the latter translates to increased glutathione peroxidase 4 activity is unclear. Glutathione peroxidase 4 protein is decreased in the substantia nigra of post-mortem PD brains compared to controls, but increased relative to surviving cell density (Bellinger et al., 2011). Erastin inhibits components of system X_c^- (Dixon et al., 2012) but their mRNA are elevated in erastin-treated LUHMES cells in a presumably compensatory mechanism (Do Van et al., 2016). Glutathione peroxidase 4 mRNA in PD substantia nigra could be similarly upregulated to compensate for loss of activity, or could be upregulated in surviving neurons. Regardless, it appears that key components regulating ferroptosis are altered in PD. Finally, MPTP toxicity in mice can be prevented by pre-treatment with ferrostatin-1 (Do Van et al., 2016). This suggests that the vulnerable neurons in PD may be dying via ferroptosis, and that inhibition of ferroptosis may be neuroprotective in PD.

Conclusions

Parkinson's disease is biochemically characterised by the disruption of mitochondrial function and the metabolism of iron, copper and glutathione. These changes are specific to the degenerating brain regions in PD and together determine regional vulnerability. Emerging evidence indicates that the recently described cell death pathway ferroptosis may be critical in PD (Guiney et al., 2017). While ferroptosis is initiated primarily by disruption of glutathione and exacerbated by elevated iron, this review discusses how disruption of mitochondrial function, iron, copper or glutathione metabolism alone is sufficient to impair all other factors (Figure 1). Therefore disruption of any one of these features can instigate a self-perpetuating cycle of increasing pathology, leading to neuronal cell death and PD. Therapeutically targeting each of these factors provides protection in animal models of PD, and are being pursued in the clinic. This includes directly abrogating iron accumulation via iron chelators such as deferiprone, mitochondrial stress via calcium channel blockers such as isradipine, glutathione depletion via glutathione administration or Nrf2 activation, and copper depletion via copper drugs such as Cu^{II} (atsm). Given the interdependent nature of these pathological features, although treatments target a

specific pathology, they are likely to abrogate pathology and restore function of all these factors. Therefore, in regards to treatment, perhaps it is not relevant which impairment/s occur first. However, in order to elucidate the natural course of the disease at a biochemical level and provide biomarkers for early intervention, longitudinal studies are required to determine the sequence of events, utilising for example advances in clinical imaging techniques for mitochondrial function, iron and glutathione content.

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References

- Acevedo, K.M., Hung, Y.H., Dalziel, A.H., Li, Q.X., Laughton, K., Wikhe, K., Rembach, A., Roberts, B., Masters, C.L., Bush, A.I., Camakaris, J., 2011. Copper promotes the trafficking of the amyloid precursor protein. *J Biol Chem* 286, 8252-8262.
- Acevedo, K.M., Opazo, C.M., Norrish, D., Challis, L.M., Li, Q.X., White, A.R., Bush, A.I., Camakaris, J., 2014. Phosphorylation of amyloid precursor protein at threonine 668 is essential for its copper-responsive trafficking in SH-SY5Y neuroblastoma cells. *J Biol Chem* 289, 11007-11019.
- Ahuja, M., Ammal Kaidery, N., Yang, L., Calingasan, N., Smirnova, N., Gaisin, A., Gaisina, I.N., Gazaryan, I., Hushpulan, D.M., Kaddour-Djebbar, I., Bollag, W.B., Morgan, J.C., Ratan, R.R., Starkov, A.A., Beal, M.F., Thomas, B., 2016. Distinct Nrf2 Signaling Mechanisms of Fumaric Acid Esters and Their Role in Neuroprotection against 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine-Induced Experimental Parkinson's-Like Disease. *J Neurosci* 36, 6332-6351.
- Alberico, S.L., Cassell, M.D., Narayanan, N.S., 2015. The Vulnerable Ventral Tegmental Area in Parkinson's Disease. *Basal Ganglia* 5, 51-55.
- Ariga, H., Takahashi-Niki, K., Kato, I., Maita, H., Niki, T., Iguchi-Ariga, S.M., 2013. Neuroprotective function of DJ-1 in Parkinson's disease. *Oxid Med Cell Longev* 2013, 683920.
- Auchere, F., Santos, R., Planamente, S., Lesuisse, E., Camadro, J.M., 2008. Glutathione-dependent redox status of frataxin-deficient cells in a yeast model of Friedreich's ataxia. *Hum Mol Genet* 17, 2790-2802.
- Ayton, S., Lei, P., Duce, J.A., Wong, B.X., Sedjahtera, A., Adlard, P.A., Bush, A.I., Finkelstein, D.I., 2013. Ceruloplasmin dysfunction and therapeutic potential for Parkinson disease. *Ann Neurol* 73, 554-559.
- Ayton, S., Lei, P., Hare, D.J., Duce, J.A., George, J.L., Adlard, P.A., McLean, C., Rogers, J.T., Cherny, R.A., Finkelstein, D.I., Bush, A.I., 2015. Parkinson's disease iron deposition caused by nitric oxide-induced loss of beta-amyloid precursor protein. *J Neurosci* 35, 3591-3597.
- Banati, R.B., Gehrmann, J., Wiessner, C., Hossmann, K.A., Kreutzberg, G.W., 1995. Glial expression of the beta-amyloid precursor protein (APP) in global ischemia. *J Cereb Blood Flow Metab* 15, 647-654.
- Banci, L., Bertini, I., Ciofi-Baffoni, S., Kozyreva, T., Zovo, K., Palumaa, P., 2010. Affinity gradients drive copper to cellular destinations. *Nature* 465, 645-648.
- Basit, F., van Oppen, L.M., Schockel, L., Bossenbroek, H.M., van Emst-de Vries, S.E., Hermeling, J.C., Grefte, S., Kopitz, C., Heroult, M., Hgm Willems, P., Koopman, W.J., 2017. Mitochondrial complex I inhibition triggers a mitophagy-dependent ROS increase leading to necroptosis and ferroptosis in melanoma cells. *Cell Death Dis* 8, e2716.
- Bellinger, F.P., Bellinger, M.T., Seale, L.A., Takemoto, A.S., Raman, A.V., Miki, T., Manning-Bog, A.B., Berry, M.J., White, L.R., Ross, G.W., 2011. Glutathione Peroxidase 4 is associated with Neuromelanin in Substantia Nigra and Dystrophic Axons in Putamen of Parkinson's brain. *Mol Neurodegener* 6, 8.
- Berndt, C., Hudemann, C., Hanschmann, E.M., Axelsson, R., Holmgren, A., Lillig, C.H., 2007. How does iron-sulfur cluster coordination regulate the activity of human glutaredoxin 2? *Antioxid Redox Signal* 9, 151-157.
- Bindoff, L.A., Birch-Machin, M., Cartlidge, N.E., Parker, W.D., Jr., Turnbull, D.M., 1989. Mitochondrial function in Parkinson's disease. *Lancet* 2, 49.

- Braak, H., Del Tredici, K., 2017. Neuropathological Staging of Brain Pathology in Sporadic Parkinson's disease: Separating the Wheat from the Chaff. *J Parkinsons Dis* 7, S73-s87.
- Braak, H., Del Tredici, K., Rub, U., de Vos, R.A., Jansen Steur, E.N., Braak, E., 2003. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging* 24, 197-211.
- Brigelius-Flohe, R., Maiorino, M., 2013. Glutathione peroxidases. *Biochim Biophys Acta* 1830, 3289-3303.
- Campolo, M., Casili, G., Biundo, F., Crupi, R., Cordaro, M., Cuzzocrea, S., Esposito, E., 2017. The Neuroprotective Effect of Dimethyl Fumarate in an MPTP-Mouse Model of Parkinson's Disease: Involvement of Reactive Oxygen Species/Nuclear Factor-kappaB/Nuclear Transcription Factor Related to NF-E2. *Antioxid Redox Signal*.
- Chan, C.S., Guzman, J.N., Ilijic, E., Mercer, J.N., Rick, C., Tkatch, T., Meredith, G.E., Surmeier, D.J., 2007. 'Rejuvenation' protects neurons in mouse models of Parkinson's disease. *Nature* 447, 1081-1086.
- Chen, L., Hambright, W.S., Na, R., Ran, Q., 2015. Ablation of the Ferroptosis Inhibitor Glutathione Peroxidase 4 in Neurons Results in Rapid Motor Neuron Degeneration and Paralysis. *J Biol Chem* 290, 28097-28106.
- Chen, P.C., Vargas, M.R., Pani, A.K., Smeyne, R.J., Johnson, D.A., Kan, Y.W., Johnson, J.A., 2009. Nrf2-mediated neuroprotection in the MPTP mouse model of Parkinson's disease: Critical role for the astrocyte. *Proc Natl Acad Sci U S A* 106, 2933-2938.
- Cheng, G., Whitehead, S.N., Hachinski, V., Cechetto, D.F., 2006. Effects of pyrrolidine dithiocarbamate on beta-amyloid (25-35)-induced inflammatory responses and memory deficits in the rat. *Neurobiol Dis* 23, 140-151.
- Clarner, T., Buschmann, J.P., Beyer, C., Kipp, M., 2011. Glial amyloid precursor protein expression is restricted to astrocytes in an experimental toxic model of multiple sclerosis. *J Mol Neurosci* 43, 268-274.
- Cui, M., Aras, R., Christian, W.V., Rappold, P.M., Hatwar, M., Panza, J., Jackson-Lewis, V., Javitch, J.A., Ballatori, N., Przedborski, S., Tieu, K., 2009. The organic cation transporter-3 is a pivotal modulator of neurodegeneration in the nigrostriatal dopaminergic pathway. *Proc Natl Acad Sci U S A* 106, 8043-8048.
- Cui, Q., Li, X., Zhu, H., 2016. Curcumin ameliorates dopaminergic neuronal oxidative damage via activation of the Akt/Nrf2 pathway. *Mol Med Rep* 13, 1381-1388.
- Davies, K.M., Bohic, S., Carmona, A., Ortega, R., Cottam, V., Hare, D.J., Finberg, J.P., Reyes, S., Halliday, G.M., Mercer, J.F., Double, K.L., 2014. Copper pathology in vulnerable brain regions in Parkinson's disease. *Neurobiol Aging* 35, 858-866.
- Davies, K.M., Hare, D.J., Cottam, V., Chen, N., Hilgers, L., Halliday, G., Mercer, J.F., Double, K.L., 2013. Localization of copper and copper transporters in the human brain. *Metallomics* 5, 43-51.
- Devos, D., Moreau, C., Devedjian, J.C., Kluza, J., Petrault, M., Laloux, C., Jonneaux, A., Ryckewaert, G., Garcon, G., Rouaix, N., Duhamel, A., Jissendi, P., Dujardin, K., Auger, F., Ravasi, L., Hopes, L., Grolez, G., Firdaus, W., Sablonniere, B., Strubi-Vuillaume, I., Zahr, N., Destee, A., Corvol, J.C., Poltl, D., Leist, M., Rose, C., Defebvre, L., Marchetti, P., Cabantchik, Z.I., Bordet, R., 2014. Targeting Chelatable Iron as a Therapeutic Modality in Parkinson's Disease. *Antioxid Redox Signal* 21, 195-210.

- Dexter, D.T., Carayon, A., Javoy-Agid, F., Agid, Y., Wells, F.R., Daniel, S.E., Lees, A.J., Jenner, P., 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 (Pt 4), 1953-1975.
- Dexter, D.T., Carayon, A., Vidailhet, M., Ruberg, M., Agid, F., Agid, Y., Lees, A.J., Wells, F.R., Jenner, P., Marsden, C.D., 1990. Decreased ferritin levels in brain in Parkinson's disease. *J Neurochem* 55, 16-20.
- Dexter, D.T., Wells, F.R., Agid, F., Agid, Y., Lees, A.J., Jenner, P., Marsden, C.D., 1987. Increased nigral iron content in postmortem parkinsonian brain. *Lancet* 2, 1219-1220.
- Dexter, D.T., Wells, F.R., Lees, A.J., Agid, F., Agid, Y., Jenner, P., Marsden, C.D., 1989. Increased nigral iron content and alterations in other metal ions occurring in brain in Parkinson's disease. *J Neurochem* 52, 1830-1836.
- Dickson, D.W., 2012. Parkinson's disease and parkinsonism: neuropathology. *Cold Spring Harb Perspect Med* 2.
- Dixon, S.J., Lemberg, K.M., Lamprecht, M.R., Skouta, R., Zaitsev, E.M., Gleason, C.E., Patel, D.N., Bauer, A.J., Cantley, A.M., Yang, W.S., Morrison, B., 3rd, Stockwell, B.R., 2012. Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* 149, 1060-1072.
- Do Van, B., Gouel, F., Jonneaux, A., Timmerman, K., Gele, P., Petraut, M., Bastide, M., Laloux, C., Moreau, C., Bordet, R., Devos, D., Devedjian, J.C., 2016. Ferroptosis, a newly characterized form of cell death in Parkinson's disease that is regulated by PKC. *Neurobiol Dis* 94, 169-178.
- Doll, S., Proneth, B., Tyurina, Y.Y., Panzilius, E., Kobayashi, S., Ingold, I., Irmeler, M., Beckers, J., Aichler, M., Walch, A., Prokisch, H., Trumbach, D., Mao, G., Qu, F., Bayir, H., Fullekrug, J., Scheel, C.H., Wurst, W., Schick, J.A., Kagan, V.E., Angeli, J.P., Conrad, M., 2017. ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. *Nat Chem Biol* 13, 91-98.
- Double, K.L., Gerlach, M., Schunemann, V., Trautwein, A.X., Zecca, L., Gallorini, M., Youdim, M.B., Riederer, P., Ben-Shachar, D., 2003. Iron-binding characteristics of neuromelanin of the human substantia nigra. *Biochem Pharmacol* 66, 489-494.
- Duce, J.A., Tsatsanis, A., Cater, M.A., James, S.A., Robb, E., Wikke, K., Leong, S.L., Perez, K., Johanssen, T., Greenough, M.A., Cho, H.H., Galatis, D., Moir, R.D., Masters, C.L., McLean, C., Tanzi, R.E., Cappai, R., Barnham, K.J., Ciccotosto, G.D., Rogers, J.T., Bush, A.I., 2010. Iron-export ferroxidase activity of beta-amyloid precursor protein is inhibited by zinc in Alzheimer's disease. *Cell* 142, 857-867.
- Esteves, A.R., Swerdlow, R.H., Cardoso, S.M., 2014. LRRK2, a puzzling protein: insights into Parkinson's disease pathogenesis. *Exp Neurol* 261, 206-216.
- Faucheux, B.A., Hauw, J.J., Agid, Y., Hirsch, E.C., 1997. The density of [125I]-transferrin binding sites on perikarya of melanized neurons of the substantia nigra is decreased in Parkinson's disease. *Brain Res* 749, 170-174.
- Faucheux, B.A., Hirsch, E.C., Villares, J., Selimi, F., Mouatt-Prigent, A., Javoy-Agid, F., Hauw, J.J., Agid, Y., 1993. Distribution of 125I-ferrotransferrin binding sites in the mesencephalon of control subjects and patients with Parkinson's disease. *J Neurochem* 60, 2338-2341.
- Fidai, I., Wachnowsky, C., Cowan, J.A., 2016. Glutathione-complexed [2Fe-2S] clusters function in Fe-S cluster storage and trafficking. *J Biol Inorg Chem* 21, 887-901.

- Fitzmaurice, P.S., Ang, L., Guttman, M., Rajput, A.H., Furukawa, Y., Kish, S.J., 2003. Nigral glutathione deficiency is not specific for idiopathic Parkinson's disease. *Mov Disord* 18, 969-976.
- Foehring, R.C., Zhang, X.F., Lee, J.C., Callaway, J.C., 2009. Endogenous calcium buffering capacity of substantia nigral dopamine neurons. *J Neurophysiol* 102, 2326-2333.
- Freedman, J.H., Ciriolo, M.R., Peisach, J., 1989. The role of glutathione in copper metabolism and toxicity. *J Biol Chem* 264, 5598-5605.
- Friedmann Angeli, J.P., Schneider, M., Proneth, B., Tyurina, Y.Y., Tyurin, V.A., Hammond, V.J., Herbach, N., Aichler, M., Walch, A., Eggenhofer, E., Basavarajappa, D., Radmark, O., Kobayashi, S., Seibt, T., Beck, H., Neff, F., Esposito, I., Wanke, R., Forster, H., Yefremova, O., Heinrichmeyer, M., Bornkamm, G.W., Geissler, E.K., Thomas, S.B., Stockwell, B.R., O'Donnell, V.B., Kagan, V.E., Schick, J.A., Conrad, M., 2014. Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. *Nat Cell Biol* 16, 1180-1191.
- Gaballah, H.H., Zakaria, S.S., Elbatsh, M.M., Tahoona, N.M., 2016. Modulatory effects of resveratrol on endoplasmic reticulum stress-associated apoptosis and oxido-inflammatory markers in a rat model of rotenone-induced Parkinson's disease. *Chem Biol Interact* 251, 10-16.
- Gal, S., Zheng, H., Fridkin, M., Youdim, M.B., 2010. Restoration of nigrostriatal dopamine neurons in post-MPTP treatment by the novel multifunctional brain-permeable iron chelator-monoamine oxidase inhibitor drug, M30. *Neurotox Res* 17, 15-27.
- Gan, L., Vargas, M.R., Johnson, D.A., Johnson, J.A., 2012. Astrocyte-specific overexpression of Nrf2 delays motor pathology and synuclein aggregation throughout the CNS in the alpha-synuclein mutant (A53T) mouse model. *J Neurosci* 32, 17775-17787.
- Gardner, P.R., Fridovich, I., 1991. Superoxide sensitivity of the Escherichia coli aconitase. *J Biol Chem* 266, 19328-19333.
- Garrido, M., Tereshchenko, Y., Zhevtsova, Z., Taschenberger, G., Bahr, M., Kugler, S., 2011. Glutathione depletion and overproduction both initiate degeneration of nigral dopaminergic neurons. *Acta Neuropathol* 121, 475-485.
- Gehrmann, J., Banati, R.B., Cuzner, M.L., Kreutzberg, G.W., Newcombe, J., 1995. Amyloid precursor protein (APP) expression in multiple sclerosis lesions. *Glia* 15, 141-151.
- German, D.C., Manaye, K.F., White, C.L., 3rd, Woodward, D.J., McIntire, D.D., Smith, W.K., Kalara, R.N., Mann, D.M., 1992. Disease-specific patterns of locus coeruleus cell loss. *Ann Neurol* 32, 667-676.
- Giannoccaro, M.P., La Morgia, C., Rizzo, G., Carelli, V., 2017. Mitochondrial DNA and primary mitochondrial dysfunction in Parkinson's disease. *Mov Disord* 32, 346-363.
- Gladyshev, V.N., Liu, A., Novoselov, S.V., Krysan, K., Sun, Q.A., Kryukov, V.M., Kryukov, G.V., Lou, M.F., 2001. Identification and characterization of a new mammalian glutaredoxin (thioltransferase), Grx2. *J Biol Chem* 276, 30374-30380.
- Goldberg, J.A., Guzman, J.N., Estep, C.M., Ilijic, E., Kondapalli, J., Sanchez-Padilla, J., Surmeier, D.J., 2012. Calcium entry induces mitochondrial oxidant stress in vagal neurons at risk in Parkinson's disease. *Nat Neurosci* 15, 1414-1421.
- Gonzalez-Cuyar, L.F., Perry, G., Miyajima, H., Atwood, C.S., Riveros-Angel, M., Lyons, P.F., Siedlak, S.L., Smith, M.A., Castellani, R.J., 2008. Redox active iron accumulation in aceruloplasminemia. *Neuropathology* 28, 466-471.

- Griffiths, P.D., Dobson, B.R., Jones, G.R., Clarke, D.T., 1999. Iron in the basal ganglia in Parkinson's disease. An in vitro study using extended X-ray absorption fine structure and cryo-electron microscopy. *Brain* 122 (Pt 4), 667-673.
- Grolez, G., Moreau, C., Sablonniere, B., Garcon, G., Devedjian, J.C., Meguig, S., Gele, P., Delmaire, C., Bordet, R., Defebvre, L., Cabantchik, I.Z., Devos, D., 2015. Ceruloplasmin activity and iron chelation treatment of patients with Parkinson's disease. *BMC Neurol* 15, 74.
- Guiney, S.J., Adlard, P.A., Bush, A.I., Finkelstein, D.I., Ayton, S., 2017. Ferroptosis and cell death mechanisms in Parkinson's disease. *Neurochem Int*.
- Guzman, J.N., Sanchez-Padilla, J., Wokosin, D., Kondapalli, J., Ilijic, E., Schumacker, P.T., Surmeier, D.J., 2010. Oxidant stress evoked by pacemaking in dopaminergic neurons is attenuated by DJ-1. *Nature* 468, 696-700.
- Halliday, G.M., Ophof, A., Broe, M., Jensen, P.H., Kettle, E., Fedorow, H., Cartwright, M.I., Griffiths, F.M., Shepherd, C.E., Double, K.L., 2005. Alpha-synuclein redistributes to neuromelanin lipid in the substantia nigra early in Parkinson's disease. *Brain* 128, 2654-2664.
- Hambright, W.S., Fonseca, R.S., Chen, L., Na, R., Ran, Q., 2017. Ablation of ferroptosis regulator glutathione peroxidase 4 in forebrain neurons promotes cognitive impairment and neurodegeneration. *Redox Biol* 12, 8-17.
- Hara, H., Ohta, M., Adachi, T., 2006. Apomorphine protects against 6-hydroxydopamine-induced neuronal cell death through activation of the Nrf2-ARE pathway. *J Neurosci Res* 84, 860-866.
- Hare, D.J., Double, K.L., 2016. Iron and dopamine: a toxic couple. *Brain* 139, 1026-1035.
- Hare, D.J., Lei, P., Ayton, S., Roberts, B.R., Grimm, R., George, J.L., Bishop, D.P., Beavis, A.D., Donovan, S.J., McColl, G., Volitakis, I., Masters, C.L., Adlard, P.A., Cherny, R.A., Bush, A.I., Finkelstein, D.I., Doble, P.A., 2014. An iron-dopamine index predicts risk of parkinsonian neurodegeneration in the substantia nigra pars compacta. *Chem Sci* 5, 2160-2169.
- Hatori, Y., Clasen, S., Hasan, N.M., Barry, A.N., Lutsenko, S., 2012. Functional partnership of the copper export machinery and glutathione balance in human cells. *J Biol Chem* 287, 26678-26687.
- Hatori, Y., Yan, Y., Schmidt, K., Furukawa, E., Hasan, N.M., Yang, N., Liu, C.N., Sockanathan, S., Lutsenko, S., 2016. Neuronal differentiation is associated with a redox-regulated increase of copper flow to the secretory pathway. *Nat Commun* 7, 10640.
- Hauser, R.A., Lyons, K.E., McClain, T., Carter, S., Perlmutter, D., 2009. Randomized, double-blind, pilot evaluation of intravenous glutathione in Parkinson's disease. *Mov Disord* 24, 979-983.
- He, Y., Lee, T., Leong, S.K., 1999. Time-course and localization of transferrin receptor expression in the substantia nigra of 6-hydroxydopamine-induced parkinsonian rats. *Neuroscience* 91, 579-585.
- Hellman, N.E., Kono, S., Mancini, G.M., Hoogeboom, A.J., De Jong, G.J., Gitlin, J.D., 2002. Mechanisms of copper incorporation into human ceruloplasmin. *J Biol Chem* 277, 46632-46638.
- Heo, H., Ahn, J.B., Lee, H.H., Kwon, E., Yun, J.W., Kim, H., Kang, B.C., 2017. Neurometabolic profiles of the substantia nigra and striatum of MPTP-intoxicated common marmosets: An in vivo proton MRS study at 9.4 T. *NMR Biomed* 30.

- Hirrlinger, J., Dringen, R., 2009. The cytosolic redox state of astrocytes: Maintenance, regulation and functional implications for metabolite trafficking. *Brain Res Rev* 63, 177-188.
- Hirsch, E.C., 2006. Altered regulation of iron transport and storage in Parkinson's disease. *J Neural Transm Suppl*, 201-204.
- Hochstrasser, H., Bauer, P., Walter, U., Behnke, S., Spiegel, J., Csoti, I., Zeiler, B., Bornemann, A., Pahnke, J., Becker, G., Riess, O., Berg, D., 2004. Ceruloplasmin gene variations and substantia nigra hyperechogenicity in Parkinson disease. *Neurology* 63, 1912-1917.
- Hochstrasser, H., Tomiuk, J., Walter, U., Behnke, S., Spiegel, J., Kruger, R., Becker, G., Riess, O., Berg, D., 2005. Functional relevance of ceruloplasmin mutations in Parkinson's disease. *FASEB J* 19, 1851-1853.
- Hoff, K.G., Culler, S.J., Nguyen, P.Q., McGuire, R.M., Silberg, J.J., Smolke, C.D., 2009. In vivo fluorescent detection of Fe-S clusters coordinated by human GRX2. *Chem Biol* 16, 1299-1308.
- Horowitz, M.P., Milanese, C., Di Maio, R., Hu, X., Montero, L.M., Sanders, L.H., Tapias, V., Sepe, S., van Cappellen, W.A., Burton, E.A., Greenamyre, J.T., Mastroberardino, P.G., 2011. Single-cell redox imaging demonstrates a distinctive response of dopaminergic neurons to oxidative insults. *Antioxid Redox Signal* 15, 855-871.
- Hou, W., Xie, Y., Song, X., Sun, X., Lotze, M.T., Zeh, H.J., 3rd, Kang, R., Tang, D., 2016. Autophagy promotes ferroptosis by degradation of ferritin. *Autophagy* 12, 1425-1428.
- Hung, L.W., Villemagne, V.L., Cheng, L., Sherratt, N.A., Ayton, S., White, A.R., Crouch, P.J., Lim, S., Leong, S.L., Wilkins, S., George, J., Roberts, B.R., Pham, C.L., Liu, X., Chiu, F.C., Shackleford, D.M., Powell, A.K., Masters, C.L., Bush, A.I., O'Keefe, G., Culvenor, J.G., Cappai, R., Cherny, R.A., Donnelly, P.S., Hill, A.F., Finkelstein, D.I., Barnham, K.J., 2012. The hypoxia imaging agent CuII(atism) is neuroprotective and improves motor and cognitive functions in multiple animal models of Parkinson's disease. *J Exp Med* 209, 837-854.
- Ikawa, M., Okazawa, H., Kudo, T., Kuriyama, M., Fujibayashi, Y., Yoneda, M., 2011. Evaluation of striatal oxidative stress in patients with Parkinson's disease using [62Cu]ATSM PET. *Nucl Med Biol* 38, 945-951.
- Ito, K., Eguchi, Y., Imagawa, Y., Akai, S., Mochizuki, H., Tsujimoto, Y., 2017. MPP⁺ induces necrostatin-1- and ferrostatin-1-sensitive necrotic death of neuronal SH-SY5Y cells. *Cell Death Discov* 3, 17013.
- Jaffrey, S.R., Cohen, N.A., Rouault, T.A., Klausner, R.D., Snyder, S.H., 1994. The iron-responsive element binding protein: a target for synaptic actions of nitric oxide. *Proc Natl Acad Sci U S A* 91, 12994-12998.
- Jakel, R.J., Kern, J.T., Johnson, D.A., Johnson, J.A., 2005. Induction of the protective antioxidant response element pathway by 6-hydroxydopamine in vivo and in vitro. *Toxicological Sciences* 87, 176-186.
- Jakel, R.J., Townsend, J.A., Kraft, A.D., Johnson, J.A., 2007. Nrf2-mediated protection against 6-hydroxydopamine. *Brain Res* 1144, 192-201.
- Jeong, S.Y., David, S., 2003. Glycosylphosphatidylinositol-anchored ceruloplasmin is required for iron efflux from cells in the central nervous system. *J Biol Chem* 278, 27144-27148.
- Jeong, S.Y., David, S., 2006. Age-related changes in iron homeostasis and cell death in the cerebellum of ceruloplasmin-deficient mice. *J Neurosci* 26, 9810-9819.

- Jha, N., Jurma, O., Lalli, G., Liu, Y., Pettus, E.H., Greenamyre, J.T., Liu, R.M., Forman, H.J., Andersen, J.K., 2000. Glutathione depletion in PC12 results in selective inhibition of mitochondrial complex I activity. Implications for Parkinson's disease. *J Biol Chem* 275, 26096-26101.
- Jiang, W.D., Liu, Y., Hu, K., Jiang, J., Li, S.H., Feng, L., Zhou, X.Q., 2014. Copper exposure induces oxidative injury, disturbs the antioxidant system and changes the Nrf2/ARE (CuZnSOD) signaling in the fish brain: protective effects of myo-inositol. *Aquat Toxicol* 155, 301-313.
- Kabiraj, P., Valenzuela, C.A., Marin, J.E., Ramirez, D.A., Mendez, L., Hwang, M.S., Varela-Ramirez, A., Fenelon, K., Narayan, M., Skouta, R., 2015. The neuroprotective role of ferrostatin-1 under rotenone-induced oxidative stress in dopaminergic neuroblastoma cells. *Protein J* 34, 349-358.
- Kagan, V.E., Mao, G., Qu, F., Angeli, J.P., Doll, S., Croix, C.S., Dar, H.H., Liu, B., Tyurin, V.A., Ritov, V.B., Kapralov, A.A., Amoscato, A.A., Jiang, J., Anthonymuthu, T., Mohammadyani, D., Yang, Q., Proneth, B., Klein-Seetharaman, J., Watkins, S., Bahar, I., Greenberger, J., Mallampalli, R.K., Stockwell, B.R., Tyurina, Y.Y., Conrad, M., Bayir, H., 2017. Oxidized arachidonic and adrenic PEs navigate cells to ferroptosis. *Nat Chem Biol* 13, 81-90.
- Kaidery, N.A., Banerjee, R., Yang, L., Smirnova, N.A., Hushpulian, D.M., Liby, K.T., Williams, C.R., Yamamoto, M., Kensler, T.W., Ratan, R.R., Sporn, M.B., Beal, M.F., Gazaryan, I.G., Thomas, B., 2013. Targeting Nrf2-mediated gene transcription by extremely potent synthetic triterpenoids attenuate dopaminergic neurotoxicity in the MPTP mouse model of Parkinson's disease. *Antioxid Redox Signal* 18, 139-157.
- Kaur, D., Lee, D., Ragapalan, S., 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.
- Kaur, D., Yantiri, F., Rajagopalan, S., Kumar, J., Mo, J.Q., Boonplueang, R., Viswanath, V., Jacobs, R., Yang, L., Beal, M.F., DiMonte, D., Volitaskis, I., Ellerby, L., Cherny, R.A., Bush, A.I., Andersen, J.K., 2003. Genetic or pharmacological iron chelation prevents MPTP-induced neurotoxicity in vivo: a novel therapy for Parkinson's disease. *Neuron* 37, 899-909.
- Kelly, S.C., He, B., Perez, S.E., Ginsberg, S.D., Mufson, E.J., Counts, S.E., 2017. Locus coeruleus cellular and molecular pathology during the progression of Alzheimer's disease. *Acta Neuropathol Commun* 5, 8.
- Khatri, D.K., Juvekar, A.R., 2016. Neuroprotective effect of curcumin as evinced by abrogation of rotenone-induced motor deficits, oxidative and mitochondrial dysfunctions in mouse model of Parkinson's disease. *Pharmacol Biochem Behav* 150-151, 39-47.
- Kumar, C., Igarria, A., D'Autreaux, B., Planson, A.G., Junot, C., Godat, E., Bachhawat, A.K., Delaunay-Moisan, A., Toledano, M.B., 2011. Glutathione revisited: a vital function in iron metabolism and ancillary role in thiol-redox control. *Embo J* 30, 2044-2056.
- Langston, J.W., Ballard, P., Tetrad, J.W., Irwin, I., 1983. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* 219, 979-980.
- Lastres-Becker, I., Garcia-Yague, A.J., Scannevin, R.H., Casarejos, M.J., Kugler, S., Rabano, A., Cuadrado, A., 2016. Repurposing the NRF2 Activator Dimethyl Fumarate as Therapy Against Synucleinopathy in Parkinson's Disease. *Antioxid Redox Signal* 25, 61-77.

- Lee, D.W., Kaur, D., Chinta, S.J., Rajagopalan, S., Andersen, J.K., 2009a. A disruption in iron-sulfur center biogenesis via inhibition of mitochondrial dithiol glutaredoxin 2 may contribute to mitochondrial and cellular iron dysregulation in mammalian glutathione-depleted dopaminergic cells: implications for Parkinson's disease. *Antioxid Redox Signal* 11, 2083-2094.
- Lee, D.W., Rajagopalan, S., Siddiq, A., Gwiazda, R., Yang, L., Beal, M.F., Ratan, R.R., Andersen, J.K., 2009b. Inhibition of prolyl hydroxylase protects against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity: model for the potential involvement of the hypoxia-inducible factor pathway in Parkinson disease. *J Biol Chem* 284, 29065-29076.
- Lei, P., Ayton, S., Appukuttan, A.T., Volitakis, I., Adlard, P.A., Finkelstein, D.I., Bush, A.I., 2015. Clioquinol rescues Parkinsonism and dementia phenotypes of the tau knockout mouse. *Neurobiol Dis*.
- Lei, P., Ayton, S., Finkelstein, D.I., Spoerri, L., Ciccotosto, G.D., Wright, D.K., Wong, B.X., Adlard, P.A., Cherny, R.A., Lam, L.Q., Roberts, B.R., Volitakis, I., Egan, G.F., McLean, C.A., Cappai, R., Duce, J.A., Bush, A.I., 2012. Tau deficiency induces parkinsonism with dementia by impairing APP-mediated iron export. *Nat Med* 18, 291-295.
- Liang, C.L., Nelson, O., Yazdani, U., Pasbakhsh, P., German, D.C., 2004. Inverse relationship between the contents of neuromelanin pigment and the vesicular monoamine transporter-2: human midbrain dopamine neurons. *J Comp Neurol* 473, 97-106.
- Liang, C.L., Sinton, C.M., German, D.C., 1996. Midbrain dopaminergic neurons in the mouse: co-localization with Calbindin-D28K and calretinin. *Neuroscience* 75, 523-533.
- Liddell, J.R., 2015. Targeting mitochondrial metal dyshomeostasis for the treatment of neurodegeneration. *Neurodegener Dis Manag* 5, 345-364.
- Liddell, J.R., 2017. Interplay between Nrf2 and NF- κ B in Neuroinflammatory Diseases. *J Clin Cell Immunol* 8, 489.
- Liddell, J.R., Lehtonen, S., Duncan, C., Keksa-Goldsteine, V., Levonen, A.L., Goldsteins, G., Malm, T., White, A.R., Koistinaho, J., Kanninen, K.M., 2016. Pyrrolidine dithiocarbamate activates the Nrf2 pathway in astrocytes. *J Neuroinflammation* 13, 49.
- Lin, M.T., Beal, M.F., 2006. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature* 443, 787-795.
- Lopez-Fabuel, I., Martin-Martin, L., Resch-Beusher, M., Azkona, G., Sanchez-Pernaute, R., Bolanos, J.P., 2017. Mitochondrial respiratory chain disorganization in Parkinson's disease-relevant PINK1 and DJ1 mutants. *Neurochem Int*.
- Lv, E., Deng, J., Yu, Y., Wang, Y., Gong, X., Jia, J., Wang, X., 2015. Nrf2-ARE signals mediated the anti-oxidative action of electroacupuncture in an MPTP mouse model of Parkinson's disease. *Free Radic Res* 49, 1296-1307.
- Lv, Z., Jiang, H., Xu, H., Song, N., Xie, J., 2011. Increased iron levels correlate with the selective nigral dopaminergic neuron degeneration in Parkinson's disease. *J Neural Transm* 118, 361-369.
- Mahlknecht, P., Krismer, F., Poewe, W., Seppi, K., 2017. Meta-analysis of dorsolateral nigral hyperintensity on magnetic resonance imaging as a marker for Parkinson's disease. *Mov Disord*.
- Mailloux, R.J., Xuan, J.Y., Beauchamp, B., Jui, L., Lou, M., Harper, M.E., 2013. Glutaredoxin-2 is required to control proton leak through uncoupling protein-3. *J Biol Chem* 288, 8365-8379.

- Malm, T.M., Iivonen, H., Goldsteins, G., Keksa-Goldsteine, V., Ahtoniemi, T., Kanninen, K., Salminen, A., Auriola, S., Van Groen, T., Tanila, H., Koistinaho, J., 2007. Pyrrolidine dithiocarbamate activates Akt and improves spatial learning in APP/PS1 mice without affecting beta-amyloid burden. *J Neurosci* 27, 3712-3721.
- Mann, V.M., Cooper, J.M., Daniel, S.E., Srai, K., Jenner, P., Marsden, C.D., Schapira, A.H., 1994. Complex I, iron, and ferritin in Parkinson's disease substantia nigra. *Ann Neurol* 36, 876-881.
- Maryon, E.B., Molloy, S.A., Kaplan, J.H., 2013. Cellular glutathione plays a key role in copper uptake mediated by human copper transporter 1. *Am J Physiol Cell Physiol* 304, C768-779.
- Masilamoni, G.J., Groover, O., Smith, Y., 2017. Reduced noradrenergic innervation of ventral midbrain dopaminergic cell groups and the subthalamic nucleus in MPTP-treated parkinsonian monkeys. *Neurobiol Dis* 100, 9-18.
- Mastroberardino, P.G., Hoffman, E.K., Horowitz, M.P., Betarbet, R., Taylor, G., Cheng, D., Na, H.M., Gutekunst, C.A., Gearing, M., Trojanowski, J.Q., Anderson, M., Chu, C.T., Peng, J., Greenamyre, J.T., 2009. A novel transferrin/TfR2-mediated mitochondrial iron transport system is disrupted in Parkinson's disease. *Neurobiol Dis* 34, 417-431.
- Mazumder, B., Sampath, P., Fox, P.L., 2006. Translational control of ceruloplasmin gene expression: beyond the IRE. *Biol Res* 39, 59-66.
- McMahon, M., Itoh, K., Yamamoto, M., Chanas, S.A., Henderson, C.J., McLellan, L.I., Wolf, C.R., Cavin, C., Hayes, J.D., 2001. The Cap'n'Collar basic leucine zipper transcription factor Nrf2 (NF-E2 p45-related factor 2) controls both constitutive and inducible expression of intestinal detoxification and glutathione biosynthetic enzymes. *Cancer Res* 61, 3299-3307.
- McNeill, A., Pandolfo, M., Kuhn, J., Shang, H., Miyajima, H., 2008. The neurological presentation of ceruloplasmin gene mutations. *Eur Neurol* 60, 200-205.
- Mena, N.P., Bulteau, A.L., Salazar, J., Hirsch, E.C., Nunez, M.T., 2011. Effect of mitochondrial complex I inhibition on Fe-S cluster protein activity. *Biochem Biophys Res Commun* 409, 241-246.
- Mena, N.P., Garcia-Beltran, O., Lourido, F., Urrutia, P.J., Mena, R., Castro-Castillo, V., Cassels, B.K., Nunez, M.T., 2015. The novel mitochondrial iron chelator 5-((methylamino)methyl)-8-hydroxyquinoline protects against mitochondrial-induced oxidative damage and neuronal death. *Biochem Biophys Res Commun* 463, 787-792.
- Mercer, S.W., La Fontaine, S., Warr, C.G., Burke, R., 2016. Reduced glutathione biosynthesis in *Drosophila melanogaster* causes neuronal defects linked to copper deficiency. *J Neurochem* 137, 360-370.
- Mischley, L.K., Leverenz, J.B., Lau, R.C., Polissar, N.L., Neradilek, M.B., Samii, A., Standish, L.J., 2015. A randomized, double-blind phase I/IIa study of intranasal glutathione in Parkinson's disease. *Mov Disord* 30, 1696-1701.
- Mitra, S., Elliott, S.J., 2009. Oxidative disassembly of the [2Fe-2S] cluster of human Grx2 and redox regulation in the mitochondria. *Biochemistry* 48, 3813-3815.
- Mizuno, Y., Ohta, S., Tanaka, M., Takamiya, S., Suzuki, K., Sato, T., Oya, H., Ozawa, T., Kagawa, Y., 1989. Deficiencies in complex I subunits of the respiratory chain in Parkinson's disease. *Biochem Biophys Res Commun* 163, 1450-1455.
- Mochizuki, H., Imai, H., Endo, K., Yokomizo, K., Murata, Y., Hattori, N., Mizuno, Y., 1994. Iron accumulation in the substantia nigra of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced hemiparkinsonian monkeys. *Neurosci Lett* 168, 251-253.

- Morris, C.M., Edwardson, J.A., 1994. Iron histochemistry of the substantia nigra in Parkinson's disease. *Neurodegeneration* 3, 277-282.
- Mortiboys, H., Furnston, R., Bronstad, G., Aasly, J., Elliott, C., Bandmann, O., 2015. UDCA exerts beneficial effect on mitochondrial dysfunction in LRRK2(G2019S) carriers and in vivo. *Neurology* 85, 846-852.
- Muhlenhoff, U., Hoffmann, B., Richter, N., Rietzschel, N., Spantgar, F., Stehling, O., Uzarska, M.A., Lill, R., 2015. Compartmentalization of iron between mitochondria and the cytosol and its regulation. *Eur J Cell Biol* 94, 292-308.
- Munoz, Y., Carrasco, C.M., Campos, J.D., Aguirre, P., Nunez, M.T., 2016. Parkinson's Disease: The Mitochondria-Iron Link. *Parkinson's Disease* 2016, 7049108.
- Nakamura, K., 2013. alpha-Synuclein and mitochondria: partners in crime? *Neurotherapeutics* 10, 391-399.
- Nakaso, K., Nakamura, C., Sato, H., Imamura, K., Takeshima, T., Nakashima, K., 2006. Novel cytoprotective mechanism of anti-parkinsonian drug deprenyl: PI3K and Nrf2-derived induction of antioxidative proteins. *Biochem Biophys Res Commun* 339, 915-922.
- Oakley, A.E., Collingwood, J.F., Dobson, J., Love, G., Perrott, H.R., Edwardson, J.A., Elstner, M., Morris, C.M., 2007. Individual dopaminergic neurons show raised iron levels in Parkinson disease. *Neurology* 68, 1820-1825.
- Oestreicher, E., Sengstock, G.J., Riederer, P., Olanow, C.W., Dunn, A.J., Arendash, G.W., 1994. Degeneration of nigrostriatal dopaminergic neurons increases iron within the substantia nigra: a histochemical and neurochemical study. *Brain Res* 660, 8-18.
- Oide, T., Yoshida, K., Kaneko, K., Ohta, M., Arima, K., 2006. Iron overload and antioxidative role of perivascular astrocytes in aceruloplasminemia. *Neuropathol Appl Neurobiol* 32, 170-176.
- Olivieri, S., Conti, A., Iannaccone, S., Cannistraci, C.V., Campanella, A., Barbariga, M., Codazzi, F., Pelizzoni, I., Magnani, G., Pesca, M., Franciotta, D., Cappa, S.F., Alessio, M., 2011. Ceruloplasmin oxidation, a feature of Parkinson's disease CSF, inhibits ferroxidase activity and promotes cellular iron retention. *J Neurosci* 31, 18568-18577.
- Ozkan, A., Parlak, H., Tanriover, G., Dilmac, S., Ulker, S.N., Birsen, L., Agar, A., 2016. The protective mechanism of docosahexaenoic acid in mouse model of Parkinson: The role of hemeoxygenase. *Neurochem Int*.
- Pardo, C.A., Xu, Z., Borchelt, D.R., Price, D.L., Sisodia, S.S., Cleveland, D.W., 1995. Superoxide dismutase is an abundant component in cell bodies, dendrites, and axons of motor neurons and in a subset of other neurons. *Proc Natl Acad Sci U S A* 92, 954-958.
- Parker, W.D., Jr., Boyson, S.J., Parks, J.K., 1989. Abnormalities of the electron transport chain in idiopathic Parkinson's disease. *Ann Neurol* 26, 719-723.
- Parker, W.D., Jr., Parks, J.K., Swerdlow, R.H., 2008. Complex I deficiency in Parkinson's disease frontal cortex. *Brain Res* 1189, 215-218.
- ParkinsonStudyGroup, 2013. Phase II safety, tolerability, and dose selection study of isradipine as a potential disease-modifying intervention in early Parkinson's disease (STEADY-PD). *Mov Disord* 28, 1823-1831.
- Patel, B.N., Dunn, R.J., Jeong, S.Y., Zhu, Q., Julien, J.P., David, S., 2002. Ceruloplasmin regulates iron levels in the CNS and prevents free radical injury. *J Neurosci* 22, 6578-6586.

- Pearce, R.K., Owen, A., Daniel, S., Jenner, P., Marsden, C.D., 1997. Alterations in the distribution of glutathione in the substantia nigra in Parkinson's disease. *J Neural Transm* 104, 661-677.
- Perry, T.L., Yong, V.W., 1986. Idiopathic Parkinson's disease, progressive supranuclear palsy and glutathione metabolism in the substantia nigra of patients. *Neurosci Lett* 67, 269-274.
- Popescu, B.F., George, M.J., Bergmann, U., Garachtchenko, A.V., Kelly, M.E., McCrea, R.P., Luning, K., Devon, R.M., George, G.N., Hanson, A.D., Harder, S.M., Chapman, L.D., Pickering, I.J., Nichol, H., 2009. Mapping metals in Parkinson's and normal brain using rapid-scanning x-ray fluorescence. *Phys Med Biol* 54, 651-663.
- Ramsey, C.P., Glass, C.A., Montgomery, M.B., Lindl, K.A., Ritson, G.P., Chia, L.A., Hamilton, R.L., Chu, C.T., Jordan-Sciutto, K.L., 2007. Expression of Nrf2 in neurodegenerative diseases. *J Neuropathol Exp Neurol* 66, 75-85.
- Riederer, P., Sofic, E., Rausch, W.D., Schmidt, B., Reynolds, G.P., Jellinger, K., Youdim, M.B., 1989. Transition metals, ferritin, glutathione, and ascorbic acid in parkinsonian brains. *J Neurochem* 52, 515-520.
- Rios, C., Alvarez-Vega, R., Rojas, P., 1995. Depletion of copper and manganese in brain after MPTP treatment of mice. *Pharmacol Toxicol* 76, 348-352.
- Roberts, B.R., Lim, N.K., McAllum, E.J., Donnelly, P.S., Hare, D.J., Doble, P.A., Turner, B.J., Price, K.A., Chun Lim, S., Paterson, B.M., Hickey, J.L., Rhoads, T.W., Williams, J.R., Kanninen, K.M., Hung, L.W., Liddell, J.R., Grubman, A., Monty, J.F., Llanos, R.M., Kramer, D.R., Mercer, J.F., Bush, A.I., Masters, C.L., Duce, J.A., Li, Q.X., Beckman, J.S., Barnham, K.J., White, A.R., Crouch, P.J., 2014. Oral Treatment with CuII(atsm) Increases Mutant SOD1 In Vivo but Protects Motor Neurons and Improves the Phenotype of a Transgenic Mouse Model of Amyotrophic Lateral Sclerosis. *J Neurosci* 34, 8021-8031.
- Rocha, N.P., de Miranda, A.S., Teixeira, A.L., 2015. Insights into Neuroinflammation in Parkinson's Disease: From Biomarkers to Anti-Inflammatory Based Therapies. *Biomed Res Int* 2015, 628192.
- Rodriguez-Manzanique, M.T., Tamarit, J., Belli, G., Ros, J., Herrero, E., 2002. Grx5 is a mitochondrial glutaredoxin required for the activity of iron/sulfur enzymes. *Mol Biol Cell* 13, 1109-1121.
- Rogers, J.T., Randall, J.D., Cahill, C.M., Eder, P.S., Huang, X., Gunshin, H., Leiter, L., McPhee, J., Sarang, S.S., Utsuki, T., Greig, N.H., Lahiri, D.K., Tanzi, R.E., Bush, A.I., Giordano, T., Gullans, S.R., 2002. An iron-responsive element type II in the 5'-untranslated region of the Alzheimer's amyloid precursor protein transcript. *J Biol Chem* 277, 45518-45528.
- Rotig, A., de Lonlay, P., Chretien, D., Foury, F., Koenig, M., Sidi, D., Munnich, A., Rustin, P., 1997. Aconitase and mitochondrial iron-sulphur protein deficiency in Friedreich ataxia. *Nat Genet* 17, 215-217.
- Schapira, A.H., Cooper, J.M., Dexter, D., Clark, J.B., Jenner, P., Marsden, C.D., 1990. Mitochondrial complex I deficiency in Parkinson's disease. *J Neurochem* 54, 823-827.
- Schapira, A.H., Cooper, J.M., Dexter, D., Jenner, P., Clark, J.B., Marsden, C.D., 1989. Mitochondrial complex I deficiency in Parkinson's disease. *Lancet* 1, 1269.
- Schmidt, H., Arendt, O., Brown, E.B., Schwaller, B., Eilers, J., 2007. Parvalbumin is freely mobile in axons, somata and nuclei of cerebellar Purkinje neurones. *J Neurochem* 100, 727-735.

- Schweitzer, K.J., Brussel, T., Leitner, P., Kruger, R., Bauer, P., Voitalla, D., Tomiuk, J., Gasser, T., Berg, D., 2007. Transcranial ultrasound in different monogenetic subtypes of Parkinson's disease. *J Neurol* 254, 613-616.
- Sechi, G., Deledda, M.G., Bua, G., Satta, W.M., Deiana, G.A., Pes, G.M., Rosati, G., 1996. Reduced intravenous glutathione in the treatment of early Parkinson's disease. *Prog Neuropsychopharmacol Biol Psychiatry* 20, 1159-1170.
- Shi, Z.H., Nie, G., Duan, X.L., Rouault, T., Wu, W.S., Ning, B., Zhang, N., Chang, Y.Z., Zhao, B.L., 2010. Neuroprotective mechanism of mitochondrial ferritin on 6-hydroxydopamine-induced dopaminergic cell damage: implication for neuroprotection in Parkinson's disease. *Antioxid Redox Signal* 13, 783-796.
- Sian, J., Dexter, D.T., Lees, A.J., Daniel, S., Agid, Y., Javoy-Agid, F., Jenner, P., Marsden, C.D., 1994a. Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia. *Ann Neurol* 36, 348-355.
- Sian, J., Dexter, D.T., Lees, A.J., Daniel, S., Jenner, P., Marsden, C.D., 1994b. Glutathione-related enzymes in brain in Parkinson's disease. *Ann Neurol* 36, 356-361.
- Sofic, E., Lange, K.W., Jellinger, K., Riederer, P., 1992. Reduced and oxidized glutathione in the substantia nigra of patients with Parkinson's disease. *Neurosci Lett* 142, 128-130.
- Sofic, E., Paulus, W., Jellinger, K., Riederer, P., Youdim, M.B., 1991. Selective increase of iron in substantia nigra zona compacta of parkinsonian brains. *J Neurochem* 56, 978-982.
- Sofic, E., Riederer, P., Heinsen, H., Beckmann, H., Reynolds, G.P., Hebenstreit, G., Youdim, M.B., 1988. Increased iron (III) and total iron content in post mortem substantia nigra of parkinsonian brain. *J Neural Transm* 74, 199-205.
- Song, M.O., Mattie, M.D., Lee, C.H., Freedman, J.H., 2014. The role of Nrf1 and Nrf2 in the regulation of copper-responsive transcription. *Exp Cell Res* 322, 39-50.
- Srivastava, S., Blower, P.J., Aubdool, A.A., Hider, R.C., Mann, G.E., Siow, R.C., 2016. Cardioprotective effects of Cu(II)ATSM in human vascular smooth muscle cells and cardiomyocytes mediated by Nrf2 and DJ-1. *Sci Rep* 6, 7.
- Stehling, O., Wilbrecht, C., Lill, R., 2014. Mitochondrial iron-sulfur protein biogenesis and human disease. *Biochimie* 100, 61-77.
- Surmeier, D.J., Obeso, J.A., Halliday, G.M., 2017. Selective neuronal vulnerability in Parkinson disease. *Nat Rev Neurosci* 18, 101-113.
- Surmeier, D.J., Schumacker, P.T., Guzman, J.D., Ilijic, E., Yang, B., Zampese, E., 2016. Calcium and Parkinson's disease. *Biochem Biophys Res Commun*.
- Surmeier, D.J., Sulzer, D., 2013. The pathology roadmap in Parkinson disease. *Prion* 7, 85-91.
- Swanson, C.R., Du, E., Johnson, D.A., Johnson, J.A., Emborg, M.E., 2013. Neuroprotective Properties of a Novel Non-Thiazolinedione Partial PPAR-gamma Agonist against MPTP. *PPAR Res* 2013, 582809.
- Takanashi, M., Mochizuki, H., Yokomizo, K., Hattori, N., Mori, H., Yamamura, Y., Mizuno, Y., 2001. Iron accumulation in the substantia nigra of autosomal recessive juvenile parkinsonism (ARJP). *Parkinsonism Relat Disord* 7, 311-314.
- Tanner, C.M., Kamel, F., Ross, G.W., Hoppin, J.A., Goldman, S.M., Korell, M., Marras, C., Bhudhikanok, G.S., Kasten, M., Chade, A.R., Comyns, K., Richards, M.B., Meng, C., Priestley, B., Fernandez, H.H., Cambi, F., Umbach, D.M., Blair, A., Sandler, D.P., Langston, J.W., 2011. Rotenone, paraquat, and Parkinson's disease. *Environ Health Perspect* 119, 866-872.

- Temlett, J.A., Landsberg, J.P., Watt, F., Grime, G.W., 1994. Increased iron in the substantia nigra compacta of the MPTP-lesioned hemiparkinsonian African green monkey: evidence from proton microprobe elemental microanalysis. *J Neurochem* 62, 134-146.
- Todorovic, M., Newman, J.R., Shan, J., Bentley, S., Wood, S.A., Silburn, P.A., Mellick, G.D., 2015. Comprehensive assessment of genetic sequence variants in the antioxidant 'master regulator' NRF2 in idiopathic Parkinson's disease. *PLoS One* 10, e0128030.
- Truban, D., Hou, X., Caulfield, T.R., Fiesel, F.C., Springer, W., 2017. PINK1, Parkin, and Mitochondrial Quality Control: What can we Learn about Parkinson's Disease Pathobiology? *J Parkinsons Dis* 7, 13-29.
- von Otter, M., Bergstrom, P., Quattrone, A., De Marco, E.V., Annesi, G., Soderkvist, P., Wettinger, S.B., Drozdik, M., Bialecka, M., Nissbrandt, H., Klein, C., Nilsson, M., Hammarsten, O., Nilsson, S., Zetterberg, H., 2014. Genetic associations of Nrf2-encoding NFE2L2 variants with Parkinson's disease - a multicenter study. *BMC Med Genet* 15, 131.
- Wang, Y.Q., Chang, S.Y., Wu, Q., Gou, Y.J., Jia, L., Cui, Y.M., Yu, P., Shi, Z.H., Wu, W.S., Gao, G., Chang, Y.Z., 2016. The Protective Role of Mitochondrial Ferritin on Erastin-Induced Ferroptosis. *Front Aging Neurosci* 8, 308.
- Wingert, R.A., Galloway, J.L., Barut, B., Foott, H., Fraenkel, P., Axe, J.L., Weber, G.J., Dooley, K., Davidson, A.J., Schmid, B., Paw, B.H., Shaw, G.C., Kingsley, P., Palis, J., Schubert, H., Chen, O., Kaplan, J., Zon, L.I., 2005. Deficiency of glutaredoxin 5 reveals Fe-S clusters are required for vertebrate haem synthesis. *Nature* 436, 1035-1039.
- Wong, B.X., Tsatsanis, A., Lim, L.Q., Adlard, P.A., Bush, A.I., Duce, J.A., 2014. beta-Amyloid Precursor Protein Does Not Possess Ferroxidase Activity but Does Stabilize the Cell Surface Ferrous Iron Exporter Ferroportin. *PLoS One* 9, e114174.
- Wu, H., Lin, L., Giblin, F., Ho, Y.S., Lou, M.F., 2011. Glutaredoxin 2 knockout increases sensitivity to oxidative stress in mouse lens epithelial cells. *Free Radic Biol Med* 51, 2108-2117.
- Wu, H., Yu, Y., David, L., Ho, Y.S., Lou, M.F., 2014. Glutaredoxin 2 (Grx2) gene deletion induces early onset of age-dependent cataracts in mice. *J Biol Chem* 289, 36125-36139.
- Xiao, H., Lv, F., Xu, W., Zhang, L., Jing, P., Cao, X., 2011. Deprenyl prevents MPP(+)-induced oxidative damage in PC12 cells by the upregulation of Nrf2-mediated NQO1 expression through the activation of PI3K/Akt and Erk. *Toxicology* 290, 286-294.
- Yang, W.S., SriRamaratnam, R., Welsch, M.E., Shimada, K., Skouta, R., Viswanathan, V.S., Cheah, J.H., Clemons, P.A., Shamji, A.F., Clish, C.B., Brown, L.M., Girotti, A.W., Cornish, V.W., Schreiber, S.L., Stockwell, B.R., 2014. Regulation of ferroptotic cancer cell death by GPX4. *Cell* 156, 317-331.
- Ye, H., Jeong, S.Y., Ghosh, M.C., Kovtunovych, G., Silvestri, L., Ortillo, D., Uchida, N., Tisdale, J., Camaschella, C., Rouault, T.A., 2010. Glutaredoxin 5 deficiency causes sideroblastic anemia by specifically impairing heme biosynthesis and depleting cytosolic iron in human erythroblasts. *J Clin Invest* 120, 1749-1761.
- Yong, V.W., Perry, T.L., Krisman, A.A., 1986. Depletion of glutathione in brainstem of mice caused by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine is prevented by antioxidant pretreatment. *Neurosci Lett* 63, 56-60.

- You, L.H., Li, F., Wang, L., Zhao, S.E., Wang, S.M., Zhang, L.L., Zhang, L.H., Duan, X.L., Yu, P., Chang, Y.Z., 2015. Brain iron accumulation exacerbates the pathogenesis of MPTP-induced Parkinson's disease. *Neuroscience* 284, 234-246.
- You, L.H., Li, Z., Duan, X.L., Zhao, B.L., Chang, Y.Z., Shi, Z.H., 2016. Mitochondrial ferritin suppresses MPTP-induced cell damage by regulating iron metabolism and attenuating oxidative stress. *Brain Res* 1642, 33-42.
- Zecca, L., Gallorini, M., Schunemann, V., Trautwein, A.X., Gerlach, M., Riederer, P., Vezzoni, P., Tampellini, D., 2001. Iron, neuromelanin and ferritin content in the substantia nigra of normal subjects at different ages: consequences for iron storage and neurodegenerative processes. *J Neurochem* 76, 1766-1773.
- Zecca, L., Stroppolo, A., Gatti, A., Tampellini, D., Toscani, M., Gallorini, M., Giaveri, G., Arosio, P., Santambrogio, P., Fariello, R.G., Karatekin, E., Kleinman, M.H., Turro, N., Hornykiewicz, O., Zucca, F.A., 2004. The role of iron and copper molecules in the neuronal vulnerability of locus coeruleus and substantia nigra during aging. *Proc Natl Acad Sci U S A* 101, 9843-9848.
- Zucca, F.A., Segura-Aguilar, J., Ferrari, E., Munoz, P., Paris, I., Sulzer, D., Sarna, T., Casella, L., Zecca, L., 2015. Interactions of iron, dopamine and neuromelanin pathways in brain aging and Parkinson's disease. *Prog Neurobiol*.

Figure legends

Figure 1. Interplay between mitochondrial dysfunction, iron accumulation, diminished copper content and depleted glutathione levels in Parkinson's disease. The loss of complex I activity evident in PD and in response to toxins including rotenone, MPTP/MPP⁺, 6-hydroxydopamine and paraquat induces oxidative stress. This depletes glutathione levels, which are required to maintain copper uptake, and Fe-S cluster assembly and binding to iron regulatory protein 1 directly and via mitochondrial glutaredoxins. Impaired Fe-S cluster assembly impairs complex I activity and results in aberrant iron regulatory protein 1 signalling and a false iron starvation phenotype, resulting in iron accumulation. This exacerbates oxidative stress and loss of mitochondrial function, depleting glutathione and diminishing copper uptake. Lack of copper decreases Nrf2 signalling and thereby glutathione, and inhibits activity of cuproproteins including complex IV in mitochondria and amyloid precursor protein and ceruloplasmin, the latter diminishing iron export and exacerbating iron accumulation. 6OHDA, 6-hydroxydopamine; APP, amyloid precursor protein; Fpn, ferroportin; Grx2, glutaredoxin 2; Grx5, glutaredoxin 5; IRP1, iron regulatory protein 1; ISC, Fe-S cluster; MPP⁺, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

Table 1: Regional biochemical characteristics

	Substantia nigra		Ventral tegmental area	Locus coeruleus
	Pars compacta (SNc)	Pars reticulata		
<i>In normal conditions</i>				
Presence of tyrosine hydroxylase	High	Low	High	High
Presence of neuromelanin	High	Low	High, less than SNc (Liang et al., 2004)	High
Predominant neurotransmitter	Dopamine	GABA	Dopamine	Noradrenaline
Iron content	High	Very high	Low	Low
Copper content	High	Lower than SNc		High
Basal mitochondrial stress	High	Low	Low	High
<i>In Parkinson's disease</i>				
Neuronal loss	Highest	Low	Low, late	High
Iron		Accumulates	Unchanged	Accumulates
Copper		Depleted		Depleted
Ctrl expression	High	Low		
SOD1 expression	High	Low		
Glutathione		Depleted		
α -synuclein pathology	Late	Late	Little	Early
Sensitivity to mitochondrial toxins	High	Low	Low	High
Degeneration in other diseases	Multiple system atrophy, progressive supranuclear palsy			Alzheimer's, Down syndrome

Highlights

Distinct characteristics demarcate vulnerable brain regions in Parkinson's disease
PD involves disruption of mitochondrial function, iron, copper and glutathione levels
Perturbation of any one of these provokes the pathological impairment of the others
Hence these core pathological changes in PD are mechanistically linked