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Abstract Iron is an essential element for life on earth, participating in a plethora of cellular processes where one-electron transfer reactions are required. Its essentiality, coupled to its scarcity in aqueous oxidative environments, has compelled living organisms to develop mechanisms that ensure an adequate iron supply, at times with disregard to long-term deleterious effects derived from iron accumulation. However, iron is an intrinsic producer of reactive oxygen species, and increased levels of iron promote neurotoxicity because of hydroxyl radical formation, which results in glutathione consumption, protein aggregation, lipid peroxidation and nucleic acid modification. Neurons from brain areas sensitive to degeneration accumulate iron with age and thus are subjected to an ever increasing oxidative stress with the accompanying cellular damage. The ability of these neurons to survive depends on the adaptive mechanisms developed to cope with the increasing oxidative load. Here, we describe the chemical and thermodynamic peculiarities of iron chemistry in living matter, review the components of iron homeostasis in neurons and elaborate on the mechanisms by which iron homeostasis is lost in Parkinson's disease, Alzheimer's disease and other diseases in which iron accumulation has been demonstrated.

Keywords Iron homeostasis · Mitochondria dysfunction · GSH · Fe–S clusters · Neurodegeneration

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Iron chemistry and toxicity

Iron (atomic number: 26; atomic weight: 55.85) is the 26th element in the periodic table, forming part of the first horizontal triad of transition elements, together with cobalt and nickel. Iron has a maximal oxidation state of 6+, but only the 2+ and 3+ states are common in biological environments. The neutral iron atom has four unpaired electrons in the 3d orbital and two paired electrons in the 4s orbital (Fig. 1). Removal of the 4s electrons gives rise to the 2+ state, while additional removal of one 3d electron gives rise to the 3+ state (Sienko and Plane 1976).

There is a notable difference in solubility between the Fe^{2+} and the Fe^{3+} salts, with the Fe^{3+} salts practically insoluble (Ksp for $Fe(OH)_3$: 2.8×10^{-39}; maximal solubility at pH 7.0: 10^{-17} M), whereas Fe^{2+} salts reach solubilities of 10^{-1} M (Spiro and Salman 1974). Thus, Fe^{2+} is the prevalent species in reductive biological fluids as the intracellular milieu.

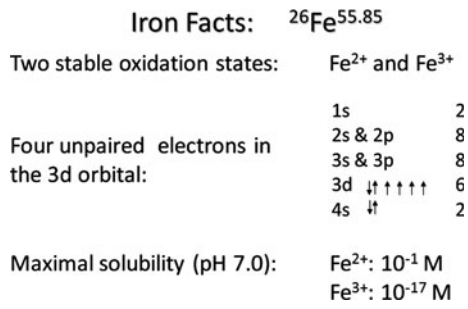


Fig. 1 Iron facts**.** Unpaired electrons in the 3d orbital give rise to the paramagnetic properties of iron and its capacity to exchange 1 electron in oxidation–reduction reactions

Nevertheless, because of oxidation by O_2, iron is continuously cycling between the 2+ and 3+ states.

Iron's capacity to exchange one electron in biological reactions is extraordinary. In the cellular environment, both Fe^{2+} and Fe^{3+} establish coordination complexes with a great diversity of ligands. Iron complexes display a variety of reduction potentials, ranging from very positive to negative values. This property is determined by a basic concept in coordination chemistry, which institutes that the ligand modifies the electron cloud surrounding the metal, thus modifying its reduction potential. This property allows for fine tuning between iron reduction potential and the electron transfer process that iron catalyzes. It is estimated that the predominant reduction potential for iron in the intracellular milieu of the cell is about 0 V (Wood 1988). Given that as the reduction potential nears zero iron more readily exchanges between 2+ and 3+ forms, iron is an extremely flexible element for electron exchange reactions and is widely used in nature.

Iron is an intrinsic reactive oxygen species (ROS) producer. When one or more of its six ligand binding sites is not tightly bound, iron engages in one-electron exchange reactions with the potential of producing free radicals (Graf et al. 1984). Indeed, iron toxicity is the product of this electron-exchange capacity combined with a reductive intracellular environment and the presence of oxygen. Fe^{2+} can react with O_2 to give rise to a superoxide radical which, catalyzed by superoxide dismutase, quickly dismutates to O_2 and H_2O_2. Fe^{2+} also reacts with H_2O_2 to generate the highly reactive hydroxyl free radical (reactions 1–3):

$$2Fe^{2+} + 2O_2 \rightarrow 2Fe^{3+} + 2O_2^{\cdot -} \quad E0 : -0.26\,\mathrm{V}; \quad \Delta G = 25.1\,\mathrm{KJ/mol} \tag{1}$$

$$2O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2 \quad E0 : 0.89\,\mathrm{V}; \quad \Delta G = -85.9\,\mathrm{KJ/mol} \tag{2}$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\cdot}OH \quad E0 : 0.28\,\mathrm{V}; \quad \Delta G = -27.0\,\mathrm{KJ/mol} \tag{3}$$

This set of reactions is known as the Haber–Weiss reactions while reaction 3, the reaction of Fe^{2+} with hydrogen peroxide to produce a hydroxyl radical, is known as the Fenton reaction. Reactions 2 and 3 have strong negative ΔG values and thus drive reaction 1 to the production of a superoxide anion ($O_2^{\bullet -}$). The coupling of reactions 1–3 gives reaction 4:

$$3Fe^{2+} + O_2 + 2H^+ \rightarrow 3Fe^{3+} + OH^- + {}^{\cdot}OH \quad E0 : 0.91\,\mathrm{V}; \quad \Delta G = -87.6\,\mathrm{KJ/mol} \tag{4}$$

The intracellular medium provides plenty of reductant power in the form of ascorbate and reduced glutathione (GSH) to regenerate the ferrous state:

$$2Fe^{3+} + 2GSH \rightarrow 2Fe^{2+} + GSSG + 2H^+ \quad \Delta G = -61.7\,\mathrm{KJ/mol} \tag{5}$$

The balance of reactions 1–5 results in reaction 6:

$$2GSH + O_2 \xrightarrow{Fe^{2+}/Fe^{3+}} GSSG + OH^- + OH^{\cdot} \quad \Delta G = -113.7\,\mathrm{KJ/mol} \tag{6}$$

In summary, in a reductive and O_2-containing environment such as the intracellular milieu, iron promotes the production of a hydroxyl radical at the expense of O_2 and GSH consumption. The in vivo relevance of reaction 6 is accentuated by the consideration that under normal conditions the cytoplasm has millimolar concentrations of GSH and sub-micromolar concentrations of reactive iron (Epsztejn et al. 1997; Kruszewski 2003). It is expected that with age and under iron overload conditions the cell concentration of redox-active iron raises (Zecca et al. 2004; Glickstein et al. 2006) and thus the generation of hydroxyl radicals. In dopaminergic cells, another source of free radicals derives from the non-enzymatic oxidation of dopamine mediated by redox-active iron, resulting in the production of semiquinones and H_2O_2 (Zoccarato et al. 2005).Thus, redox-active iron, both through the Fenton reaction and via dopamine oxidation, is a dangerous pro-oxidant agent.

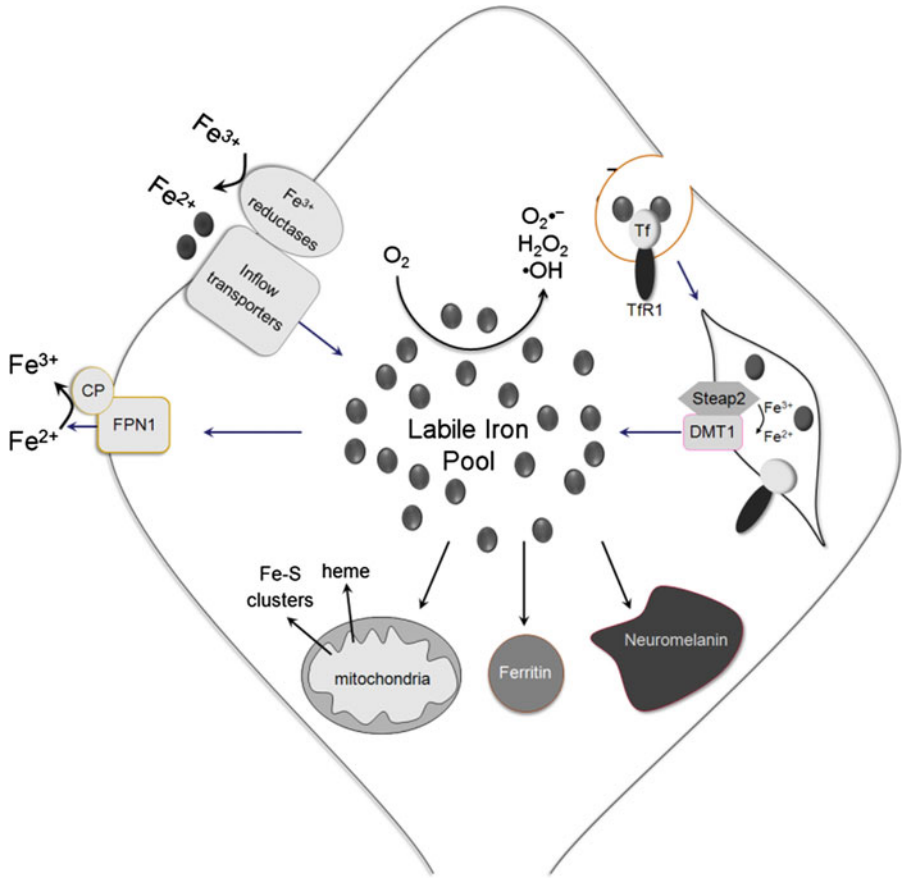


Fig. 2 Molecular components of neuronal iron homeostasis. The scheme includes Tf and its receptor (TfR1); inflow (DMT1, TRPC6, L-type voltage-dependent calcium channels (L-VDCC) and Zip14) and efflux (ferroportin 1, FPN1) membrane transporters; the iron storage protein ferritin; the ferroxidase ceruloplasmin (CP) and the ferrireductase Dcytb that reduces Fe^{3+} to Fe^{2+} previously to inflow transporters. Iron is taken up either through the endocytosis of Fe-containing Tf or transported directly through the plasma membrane. Once in the cytoplasm, iron forms part of the labile iron pool (LIP) from where it distributes to mitochondria, ferritin and neuromelanin, or it is exo-transported by FPN1. In the cellular environment, iron in the LIP is a net ROS producer. Putative Fe^{3+} reductases include Dcytb, its homologous cytochrome b561, SDR2 and Steap2 (see text)

Neuronal iron homeostasis

Iron concentration in cerebrospinal fluid (CSF) ranges between 0.2 and 1.1 μM whereas transferrin (Tf) concentration lies around 0.24 μM (Symons and Gutteridge 1998; Moos and Morgan 1998). Although CSF and the interstitial fluid that bath the neurons are in different compartments, it is likely that the composition of CSF reflects that of the interstitial fluid (Bradbury 1997). Considering that one Tf molecule binds two iron atoms, CSF iron often exceeds the binding capacity of Tf. Hence, non-Tf bound iron (NTBI) uptake is expected to occur in neurons that express divalent metal transporter 1 (DMT1) or other iron uptake transporters. The components of neuronal iron homeostasis are depicted in Fig. 2. NTBI uptake occurs directly at the plasma membrane, mediated by one or more of the transporters shown in Fig. 2. Tf-bound iron uptake initiates with the binding of Tf to the transferrin receptor (TfR), followed by internalization into the endosomal system. Release of iron is mediated by endosomal acidification, Fe^{3+} reduction possibly by Steap2 (Ohgami et al. 2006) and transport into the cytoplasm by endosomal DMT1. Once in the cytoplasm, Fe^{2+} becomes part of the labile or reactive iron pool where it distributes to mitochondria and ferritin or engages in electron exchange reactions (Kakhlon and Cabantchik 2002; Kruszewski 2003).

All the components described in Fig. 2 have been detected in the brain (Moos et al. 2007; Rouault et al. 2009; Haeger et al. 2010). In neurons of the substantia nigra (SN) and in the noradrenergic neurons of the locus coeruleus, iron also distributes to neuromelanin granules (Zecca et al. 2003).

The iron transporters and auxiliary proteins

DMT1 is a Fe^{2+}/H^{+} co-transporter that transports iron into cells (Gunshin et al. 1997). In the brain, DMT1 is expressed in hippocampal pyramidal and granule cells, cerebella granule cells, pyramidal cells of the piriform cortex, SN and the ventral portion of the anterior olfactory nucleus, striatum, cerebellum, hippocampus and thalamus, as well as in vascular cells throughout the brain and ependymal cells in the third ventricle (Gunshin et al. 1997; Williams et al. 2000; Burdo et al. 2001). The pervasive presence of DMT1 in neurons suggests that DMT1 is needed for normal neuronal function (Hidalgo and Núñez 2007; Wright and Baccarelli 2007; Pelizzoni et al. 2011). In support of this notion are our observations that mice trained in the Morris water maze presented increased expression of DMT1 in the hippocampus when compared with untrained mice, and that treatment of hippocampal neurons for a short time (5 min) with NMDA resulted in increased expression of DMT1 for at least 2 days (Haeger et al. 2010).

The mammalian DMT1 gene undergoes alternative splicing generating 4 isoforms, all active in Fe^{2+} transport. The 1A and 1B mRNA DMT1 variants originate from alternative splicing at the 5′ end (exons 1A and 1B), while variants, that contain or do not contain an iron responsive element (IRE), (+)IRE and (−)IRE, respectively, motif in the mRNA, originate from splicing at the 3′ end (exons 16/16A and 17) (Hubert and Hentze 2002; Ludwiczek et al. 2007). The 1B isoforms are expressed in brain and other tissues (Haeger et al. 2010) whereas the 1A isoforms are almost exclusively expressed in intestine (Hubert and Hentze 2002). It is generally accepted that the expression of the (+)IRE isoforms is regulated by the IRE/iron regulatory protein (IRP) system, which post-transcriptionally regulates the expression of iron homeostasis proteins such as TfR1, DMT1, FPN1 and ferritin, in response to the concentration of reactive iron in the cytoplasm (Garrick and Garrick 2009). Knowledge on the transcriptional regulation of DMT1 expression is emerging. The inflammatory cytokine NFκB and nuclear factor Y regulate DMT1(1B) expression in embryonic carcinoma cells (Paradkar and Roth 2006). Hypoxia up-regulates expression of the DMT1(1A) isoform, presumably through activation of HIF-1 (Lis et al. 2005; Wang et al. 2010). HIF-1 also promotes the expression of the 1B isoform by binding to a putative hypoxia-response element sequence in DMT1 exon1B gene (Qian et al. 2011).

Other putative neuronal iron inward transporters are Transient receptor potential cation channel, subfamily C, member 6 (TRPC6), L-type voltage-dependent calcium channels (L-VDCC) and Zrt- and Irt-like protein 13 (Zip14). TRPC6 belongs to a family of store- and receptor-operated calcium channels (Krizaj 2005). TRPC6 was found into the proximal dendrites of Tyrosine hydroxylase (TH)-positive dopaminergic neurons in the SN (Giampa et al. 2007). Although the iron transport capacity of TRPC6 was reported (Mwanjewe and Grover 2004), its role as a putative iron transporter in neurons awaits further evaluation.

Evidence indicates that L-VDCC is partially responsible for iron overload in cardiomyocytes (Oudit et al. 2006). The evidence that L-VDCC channels are a conduit for Fe^{2+} influx into neuronal cells arises from the observation that treatment with the selective L-VDCC channel inhibitor nimodipine prior to an iron challenge provides significant protection against death in cultured neurons. This observation led to the hypothesis that L-VDCCs double as Fe^{2+} channels that can participate in iron overload in neurodegenerative diseases (Gaasch et al. 2007; Lockman et al. 2012).

Zip14 has two-fourth exons, giving rise to ZIP14A and ZIP14B splicing variants (Girijashanker et al. 2008). In mice ZIP14A expression is highest in the duodenum, liver and kidney, whereas ZIP14B expression is high in brain, and testis (Lichten and Cousins 2009). Recent reports have provided compelling evidence demonstrating that Zip14 is a Fe^{2+} transporter. Overexpression of Zip14 in human embryonic kidney 293 (HEK293) cells increases Fe^{2+} uptake that is inhibited both by Zn^{2+} and ZIP14 siRNA (Liuzzi et al. 2006). In mouse models, inflammatory stimuli like lipopolysaccharide (LPS) and turpentine induce upregulation of Zip14 through a mechanism involving IL-6 and induction of iNOS (Liuzzi et al. 2005). The upregulation of ZIP14 by inflammatory stimuli raises

the possibility that this transporter may act as a novel interface between inflammation and tissue iron accumulation.

FPN1 is the only member of the SLC40 family of transporters and the first reported protein that mediates the exit of iron from cells (McKie et al. 2000). FPN1 has a unique role mediating iron exit from enterocytes and macrophages into circulating blood. In enterocytes, FPN1 is responsible for iron efflux during the process of intestinal iron absorption, while in Kupffer cells FPN1 mediates iron export for reutilization by the bone marrow (Devalia et al. 2002). Neither the transport mechanism nor the Fe species transported by FPN1 are known, although the latter is probably Fe^{2+}, the prevalent form of available intracellular iron.

FPN1 is abundantly expressed in the mouse brain, being present in neurons, microglia, astrocytes and oligodendrocytes (Song et al. 2010). Spatiotemporal expression of FPN1 in neurons is variable (Moos and Rosengren Nielsen 2006). In young mice brain (postnatal day 7 to 21) high immunoreactivity is found in the neurons of the striatum and the hippocampus, both in cell bodies and in projection fibers. FPN1 is mildly expressed in the SN pars compacta and the superior colliculus and weakly expressed in the SN pars reticulata (Boserup et al. 2011). In the adult brain, FPN1 immunoreactivity is lower in the projections of the striatum, but no differences have been found in neuronal cell bodies (Moos and Rosengren Nielsen 2006).

Knowledge on the regulation of FPN1 expression is incipient. Most probably, FPN1 expression is regulated at both transcriptional and translational levels (McKie et al. 2000; Knutson et al. 2003). FPN1 has an IRE motif in the 5′-UTR of its mRNA that bestows on FPN1 a ferritin-like response to variations in cell iron, increasing its expression under elevated iron conditions (McKie et al. 2000; Yang et al. 2002; Aguirre et al. 2005).

The ferrireductases

Ferrous iron is the preferred redox state for transport while Fe^{3+} is the predominant redox state in food, so prior to its transport into the cell, Fe^{3+} must be reduced to Fe^{2+}. The reduction of iron is carried out by ferrireductases present either in the plasma membrane or in the endosome/lysosome system. Four membrane ferrireductases involved in iron transport processes have been described: Duodenal cytochrome B (Dcytb), its homologous cytochrome b561, Stromal cell-derived receptor 2 (SDR2) and 6-transmembrane epithelial antigen of the prostate 3 (Steap3) (Vargas et al. 2003; Ohgami et al. 2006). Dcytb, initially described as the ferrioxidase responsible for reduction of non-heme iron in the duodenal lumen during intestinal iron absorption (McKie et al. 2001), is the only recognized member of the iron transport machinery that lacks an IRE in its mRNA. A recent study of the temporal relationship between Dcytb and Hif-2α during hypoxic stimulus in the enterocyte, revealed that both Dcytb and Hif-2α protein expression increase during the first (6–18) h of a hypoxic stimuli, although a significant change in hepcidin expression was evident only after 72 h of hypoxia (Latunde-Dada et al. 2011). As mentioned before, DMT1 and FPN1 exhibit transcriptional regulation by Hif-1α. Thus, the control of iron acquisition through the up-regulation of Dcytb and DMT1 by Hif-1α, coupled to the down-regulation of FPN1 by Hif-2α, may result in a concerted response of increased iron accumulation under hypoxic conditions.

The presence of Dcytb and SDR2 were recently reported in astrocytes, where they may have a limited role in iron accumulation by these cells since the reduction rate for ferric iron was substantially lower than the rate of cellular iron accumulation from 100 μM ferrous ammonium sulphate (Tulpule et al. 2010). Notoriously, there is no report on the presence of plasma membrane Dcytb in neurons. Perhaps the high concentration of ascorbate in the CSF, thereby maintaining NTBI in the 2+ state, makes unnecessary a membrane-bound iron reduction system in brain cells.

The Steap family of ferrireductases comprises the members Steap1, Steap2, Steap3, and Steap4 (Ohgami et al. 2006). Steap3 was described in endosomes of erythroid precursor cells, catalyzing the reduction of Fe^{3+} to Fe^{2+} prior to transport by DMT1 (Ohgami et al. 2005). In situ hybridization studies show that Steap2 is expressed in the brain (Ohgami et al. 2006). Whether Steap2 or other members of the Steap family has a role in iron uptake by neurons remains speculative.

Ferritin

Ferritin, a multimeric protein formed by 24 subunits of H and L monomers, is the only well-characterized

iron-storage protein in living organisms (Liu and Theil 2005). The 24 subunits of ferritin form a hollow cavity that can store as many as 4,500 Fe^{3+} atoms in the form of crystallized diferric oxo-hydroxyl complexes (Harrison and Arosio 1996). Ferritin plays a fundamental role in controlling the size of the cytoplasmic redox-active iron pool (Salgado et al. 2010). Although ferritin is expected to reduce this pool by storing iron, there is a dynamic exchange of iron between its ferritin-bound form and a cytoplasmic form amenable to transport by FPN1 (De Domenico et al. 2006). In addition, ferritin contributes to the pool of redox-active iron every time it is degraded (Mehlhase et al. 2005). H-ferritin, the subunit responsible for Fe^{2+} oxidation, is relatively poorly expressed in melanized dopaminergic neurons of the sustantia nigra pars compacta, as compared to neurons in other parts of the brain, but is strongly expressed in oligodendrocytes (Snyder and Connor 2009).

The SN of Parkinson's disease patients has a low content of ferritin compared to other brain areas (Dexter et al. 1991; Faucheux et al. 2002), and this ferritin has higher iron content when compared with ferritin of age-matched control individuals (Griffiths et al. 1999). The reasons for this low ferritin expression is unknown, but it could be due to increased Iron regulatory protein 1 (IRP1) activity (Faucheux et al. 2002). IRP1 in its active form binds to IRE elements present in the 5′ untranslated region of ferritin mRNA inhibiting its translation. In support of this notion, it was recently reported that inhibition of electron transport chain complex I, a common occurrence in Parkinson's disease (see ahead), also results in activation of IRP1.

Lack of active ferritin can cause neurodegeneration, as found in hereditary neuroferritinopathy, a neurodegenerative disease characterized by the increase of iron and ferritin in the extracellular spaces and cytoplasm of cells in the basal ganglia of affected individuals (Curtis et al. 2001). The clinical symptoms of neuroferritinopathy include severe movement disorders and the presence of nuclear and cytoplasmic ferritin inclusion bodies in glia and neurons throughout the CNS (Vidal et al. 2003). The molecular cause of neuroferritinopathy is nucleotide insertions in the L-ferritin gene, which results in low capacity of the sub-unit to assemble into 24-mer ferritin shell and the formation of heteropolymers with H-subunits that present a reduced capacity to incorporate iron in vitro (Cozzi et al. 2006).

It is noteworthy that uptake as the result of the endocytosis of exogenous ferritin has been acknowledged (Fisher et al. 2007). In the same vein, ferritin has been demonstrated to be an iron source for developing oligodendrocytes (Todorich et al. 2011), but its role as a putative iron donor to neurons has not been firmly established.

Neuromelanin

The most highly pigmented cells in the human brain are the dopaminergic neurons of the SN and the noradrenergic neurons of the locus coeruleus. The pigment is composed of neuromelanin, a polymer formed by oxidized metabolites of dopamine, containing a peptide component of about 15% (Zecca et al. 2002). Parkinson's disease is characterized by the preferential loss of neuromelanin-containing neurons of the SN (Kastner et al. 1992; Zecca et al. 2003). Thus, it is of relevance to assess the function of neuromelanin in the SN under physiological conditions and as a possible pathogenic element.

Neuromelanin avidly binds iron in its 3+ form, presenting high and low affinity binding sites (Double et al. 2003). Given that ferritin is poorly expressed in melanized dopaminergic neurons of the SN, as compared to neurons in other parts of the brain (Snyder and Connor 2009), neuromelanin is the main iron storage moiety in SN neurons. It is believed that the high affinity sites are protective as they sequester iron in a redox-inactive form, whereas iron in the low affinity sites is redox-active (Gerlach et al. 2008). Thus, under physiological conditions iron should safely bind to high affinity sites. For the contrary, when iron concentration increases above the high affinity binding capacity of neuromelanin, as is the case of increased age or pathophysiological conditions, iron binds to low-affinity binding sites in a redox-active form and the neurons become susceptible to oxidative damage.

The inverse relationship between iron and reduced GSH levels

The tripeptide GSH (γ-L-glutamyl-L-cysteinylglycine) is the most abundant and the main antioxidant agent in the central nervous system, where it reaches millimolar concentrations in the cytoplasm (Meister and

Anderson 1983; Dringen et al. 2000). In its redox cycling, GSH is present either in its reduced (GSH) form or its oxidized disulfide (GSSG) form. The ratio GSH/GSSG is a faithful reflection of the redox state of the cell (Schafer and Buettner 2001).

Iron accumulation induces the consumption of GSH and the production of GSSG (Fig. 3) (Núñez et al. 2004). After exposure to increasing concentrations of iron, SH-SY5Y dopaminergic cells show increased levels of ROS (Fig. 3a) and intracellular iron accumulation (Fig. 3b). The cells undergo a biphasic change in intracellular GSH levels, increasing at low (2–5 μM) iron concentrations and decreasing thereafter (Fig. 3c). Indeed, cell exposure to high iron concentrations (20–80 μM) markedly decreases GSH half-cell reduction potential, with the associated loss of cell viability at values more positive than −300 mV (Fig. 3d). These data support the hypothesis that a decrease in GSH levels is a consequence of the increased oxidative load produced by increased ROS and by the consumption of GSH by iron during its redox cycling. Nevertheless, increased iron and decreased GSH may be intertwined in a positive feedback loop, since in dopaminergic neurons the pharmacological reduction of GSH levels results in increased levels of TfR and an increased labile iron pool (Kaur et al. 2009).

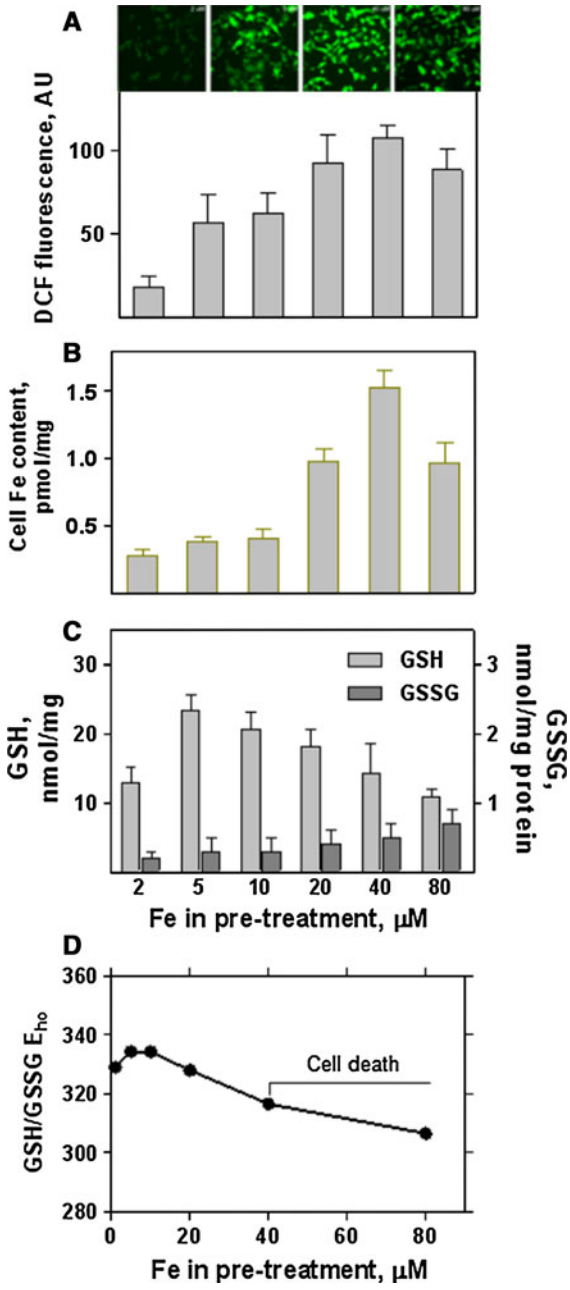
Iron accumulation, ROS and inflammation in Alzheimer's disease

Iron accumulation is a common feature of a number of neurodegenerative disorders of the central nervous system that include Parkinson's disease, Alzheimer's disease, Huntington's disease, Friedreich's ataxia, neuroferritinopathy and Amyotrophic Lateral Sclerosis (Jellinger 1999; Bartzokis et al. 2000; Sayre et al. 2000; Perry et al. 2003; Zecca et al. 2004; Berg and Youdim 2006; Wilson 2006; Weinreb et al. 2011).

Alzheimer's disease (AD) manifests as a dementia of slow progression characterized by the neuropathological findings of senile plaques (SP), extracellular insoluble aggregates of beta-amyloid, and neurofibrillary tangles (NFT), intracellular lesions consisting of paired helical filaments formed of hyperphosphorylated cytoskeletal protein tau (reviewed in (Davison 1987; Reynolds et al. 2007). Most AD cases are sporadic, whereas some rare familial forms are related to mutations in genes linked to the processing of the amyloid precursor protein (Yamada 2004). Available data suggest that SP and NFT represent two stages of response that take place in different brain regions (Braak and Braak 1991). In the neocortex, there is a predominance of SP, whereas in the entorhinal cortex and hippocampus there is a predominance of NFT (Yasha et al. 1997). In this respect, NFT correlates more closely with the clinical symptoms than SP (Duyckaerts 2004). Although the significance of SP aggregates remains ill-defined, current hypotheses propose that oligomeric and intracellular amyloid could mediate neurotoxicity through its ability to disrupt synaptic activity, cause calcium dyshomeostasis and even facilitate hyperphosphorylation of tau (LaFerla et al. 2007). The finding that Aβ-derived diffusible ligands promote tau phosphorylation suggests that tau pathology is secondary (Davis et al. 1999). Nevertheless, a large study that analyzed the prevalence of the amyloid and neurofibrillary lesions as a function of age showed that the NFT precedes SP by several decades (Braak and Braak 1991; Duyckaerts et al. 2009).

One of the more sought after questions that prevails current research in AD relates to the elucidation of the pathophysiological cascade of events leading to neuron dysfunction and death. It has been postulated that Aβ activation of astrocytes and oligodendrocytes upregulates pro-inflammatory cytokine expression and enhances the production of ROS, thus initiating an inflammatory cascade that leads to neuronal death (Johnstone et al. 1999; Meda et al. 2001; Ramirez et al. 2008; von Bernhardi 2007). In fact, a preeminent feature of the disease is the abundance of activated astrocytes and microglia in close proximity to the SPs (Itagaki et al. 1989). There is no certainty on whether inflammation precedes or is a consequence of neurodegeneration. Current evidence favors the hypothesis that microglia dysfunction induced by inflammation and aging-related cellular stress results in the release of further inflammatory stimuli that leads to neuronal dysfunction and death (von Bernhardi 2007).

Oxidative stress has been proposed as contributor to neuronal synaptic dysfunction and loss in AD (Joseph et al. 2005; Mancuso et al. 2007; Shi and Gibson 2007). Specifically, iron accumulation has been reported in brain regions affected by neurodegeneration (Connor and Benkovic 1992; Castellani et al. 2007). This observation has been revealed by post-mortem studies (Zecca et al. 2004) and confirmed by MRI studies (Falangola et al. 2005; Jack et al. 2005).



◀ **Fig. 3** Iron, ROS and GSH relationship**.** Dopaminergic SHSY-5Y cells were seeded and grown for 8 days in standard culture medium and then cultured for 2 days in medium containing 2, 5, 10, 20, 40, or 80 μM Fe prior to the assays. **a** ROS production determined by dichlorofluorescein (DCF) fluorescence. The upper part of the panel shows representative images of cells pre-cultured in 2, 5, 40, or 80 μM Fe. The lower panel shows the quantification of DCF fluorescence intensity in cells pre-cultured in the stated Fe concentrations. **b** Total iron content determined by atomic absorption spectroscopy performed as described (Mura et al. 2006). **c** GSH and GSSG content in cells pre-treated with the stated iron concentrations. **d** GSH/GSSG half-cell reduction potential of cells pre-treated with the stated iron concentrations. For experimental details of figures **a**, **c** and **d** see (Núñez et al., 2004)

There is a correlation between the degrees of iron accumulation and cognitive decline in AD subjects. As with NFT, hippocampal iron accumulation in AD subjects (evaluated by gradient-echo MRI) correlates well with cognitive decline assessed by the mini-mental state examination performance (Ding et al. 2009). In the hippocampus of AD subjects, iron is localized in NFT-containing neurons as well as in neuritic processes surrounding SP (Quintana et al. 2006). Concurrent with iron localization, a significant increase in products of lipoperoxidation (4-hydroxy-nonenal) has been also described in neurons containing NFT and neuritic processes in the periphery of SP (Markesbery and Lovell 1998; Sayre et al. 2000). Recent studies have demonstrated that molecules with the ability to bind iron are effective in slowing disease progression in AD models and patients.

Available evidence suggests that Tf-bound iron would not participate in iron accretion in AD. In quantitative terms, Tf is globally decreased in the brain of AD subjects and instead of being in its regular location in oligodendrocytes, it appears to be sequestered in SP (Connor et al. 1992); Tf receptor is highly expressed in the neocortex and hippocampus of AD brains but localized to capillary endothelium with only a weak expression in neurons (Jefferies et al. 1996). Thus, NTBI uptake could be the primary mechanism by which neurons acquire iron. Considering that iron accumulates in the brain with age (Bartzokis et al. 1997; Barnham and Bush 2008), that major neurodegenerative disorders such as AD and Parkinson's disease are characterized by elevated tissue iron (Thompson et al. 2001; Collingwood et al. 2005; Collingwood et al. 2008; Barnham and Bush 2008) and that excess iron is a strong promoter of oxidative stress, it is of relevance to investigate with more detail the potential role of iron homeostasis dysregulation in microglia and neurons in the inflammatory cascade discussed above.

Iron accumulation, ROS and inflammation in Parkinson's disease

A large body of evidence indicates that in Parkinson's disease iron accumulates in the dopaminergic neurons

of the SN pars compacta (Youdim et al. 1989; Hirsch et al. 1991; Gorell et al. 1995; Vymazal et al. 1999). Due to iron's capacity to catalyze the formation of free radicals, most likely iron accumulation contributes to the progression of events leading to neuronal death.

Considerable advances in the comprehension of the events that lead to neuronal death have been obtained with the use of the parkinsonian toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Langston et al. 1983). MPTP is a potent inhibitor of complex I of the mitochondrial electron transport chain, through which inhibition produces the death of dopaminergic neuron of the SN (Schapira et al. 1990; Schapira and Gegg 2011).

Neuronal death caused by MPTP or 6-hydroxydopamine (another inhibitor of complex I) intoxication is prevented by the pharmacologic or genetic chelation of iron (Kaur et al. 2003; Shachar et al. 2004; Youdim et al. 2004; Youdim and Buccafusco 2005; Zheng et al. 2010) or by dysfunction of the iron transporter DMT1 (Salazar et al. 2008). A recent study in mesencephalic dopaminergic neurons shows that low (0.25–0.5 μM) concentrations of MPP+, the active metabolite of MPTP, induces neuritic tree collapse without loss of cell viability (Gómez et al. 2011). This collapse was effectively prevented by decreasing iron supply or by the addition of antioxidants. Thus, it seems plausible that increased intracellular iron and ROS are involved in the early steps of dopaminergic neuron dysfunction, prior to cell death. At later times, a vicious cycle of iron accumulation, complex I dysfunction and ROS increase may result in uncontrolled oxidative damage and cell death.

ROS have a negative effect on complex I activity. Experiments with isolated synaptosomal mitochondria revealed that low concentrations of H_2O_2 decrease complex I activity by 10%. This relatively minor effect of H_2O_2 was additive to partial inhibition of complex I induced by low concentrations (5 nM–1 μM) of rotenone (Chinopoulos and Adam-Vizi 2001). Similarly, sub-mitochondrial particles exposed to $O_2^{\bullet -}$, H_2O_2, or $^{\bullet}OH$ presented decreased activity of NADH dehydrogenase, a marker of complex I activity (Zhang et al. 1990). Thus, an initial inhibition of complex I could generate a positive loop between ROS generation and further complex I inhibition.

Early post-mortem studies revealed decreased levels of GSH in degenerating SN of Parkinson's disease patients (Perry et al. 1982; Sofic et al. 1988; Sian et al. 1994), implicating that GSH depletion may play a major role in the neurodegenerative process. The question arises whether GSH depletion is an early event during the progression of the disease or a reflection of increased oxidative stress resulting from increased ROS and iron accumulation. Chronic submaximal inhibition of GSH synthesis in N27 dopaminergic cells produces 50% inhibition of mitochondrial electron transport chain complex I without causing cell death (Chinta and Andersen 2006). Thus, decreased level of GSH per se could inhibit mitochondrial function. Under this view, inhibition of complex I by decreased GSH levels results in increased electron leakage from the electron transport chain, increased ROS and iron accumulation. The question remains as to which of the three processes, decreased GSH levels, inhibition of complex I activity or iron accumulation, initiates the oxidative spiral. A reasonable assumption is that if one of them develops the other two will follow.

Fe–S cluster synthesis and its relevance to Parkinson's disease

Iron–sulfur (Fe–S) clusters are small inorganic cofactors formed by tetrahedral coordination of iron atoms with sulfur groups. The Fe–S clusters most commonly found in eukaryotes are 2Fe–2S and 4Fe–4S. Fe–S clusters are cofactors for proteins involved in many cellular processes, including electron transport, enzymatic catalysis and regulation and DNA synthesis (Lill and Muhlenhoff 2008). The proteins that contain Fe–S clusters in eukaryotes are present in mitochondria, endoplasmic reticulum, cytoplasm and nuclei (Lill et al. 2006; Sheftel et al. 2010).

The mitochondrion plays a central role in the generation and biology of Fe–S clusters since it holds the assembly machinery responsible for their synthesis (Lill et al. 2006). Fe–S cluster synthesis also occurs in the cytoplasm, albeit at a minor scale (Ye and Rouault 2010). Iron is transported inside the mitochondria by the transporter mitoferrin (Shaw et al. 2006). In eukaryotes, the Fe–S cluster assembly machinery of mitochondria comprises the cysteine desulfurase, Nfs1, which provides sulfur and Isu1, a protein that serves as a molecular scaffold for the assembly of the Fe–S cluster. Synthesis of the transiently bound Fe–S cluster on the Isu scaffold proteins requires reduced (ferrous) iron and the input of electrons, presumably to

generate sulfide anion from cysteine (Ye and Rouault 2010). The assembled cluster is transferred from the scaffold to recipient apoproteins. Finally, synthesized Fe–S clusters are either transferred to mitochondrial apo-proteins or exported into the cytoplasm by the Fe–S cluster export machinery. This machinery involves the ABC-B7 transporter, the antioxidant GSH and the sulfhydryl oxidase Erv1, which is also involved in protein import (Lill et al. 2006; Sheftel et al. 2010).

Mitochondria contain numerous Fe–S proteins essential for their function. Complex I has eight Fe–S clusters, succinate dehydrogenase (complex II) has three Fe–S clusters and ubiquinone: cytochrome c oxidoreductase (complex III) has one [2Fe–2S] cluster. Additionally, mitochondrial aconitase contains a 4Fe–4S cluster in its catalysis center (Beinert et al. 1983). Proteins involved in purine metabolism in the cytoplasm like xanthine oxidase and phosphoribosyl pyrophosphate amido-transferase contain two [2Fe–2S] and one [4Fe–4S] cluster, respectively (Unciuleac et al. 2004; Martelli et al. 2007). In the nucleus, proteins involved in DNA repair and replication contain Fe–S clusters (Sheftel et al. 2010).

Recent data from our laboratory indicate that inhibition of complex I by rotenone results in decreased synthesis of Fe–S clusters, as shown by the decreased activity of Fe–S cluster-containing enzymes such as cytoplasmic aconitase, mitochondrial aconitase, xanthine oxidase and glutamyl phosphoribosyltransferase as well as the activation of cytoplasmic IRP1 (Mena et al. 2011). As mentioned above, disassembly of the 4Fe–4S cluster in IRP1 alters the active site accessibility and determines IRP1 binding to target mRNAs (Wallander et al. 2006; Cairo and

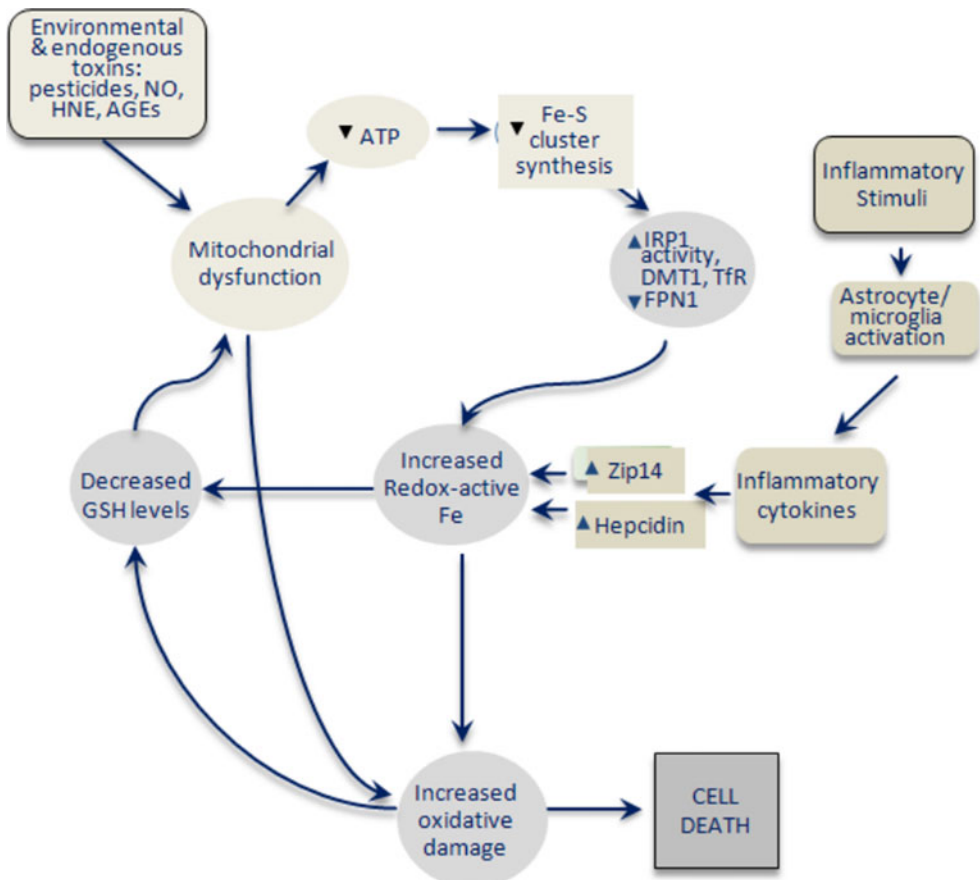


Fig. 4 A positive feedback loop resulting in uncontrolled oxidative load**.** Mitochondrial dysfunction results in decreased Fe–S cluster synthesis which leads to activation of IRP1, increased DMT1 and decreased FPN1 synthesis, iron accumulation, increased oxidative stress and increased GSH consumption. Decreased GSH produces further complex I inhibition. *NO* nitric oxide, *HNE* 4-hydroxynonenal, *AGEs* advanced glycation end products

Recalcati 2007). We think that decreased activity of complex I results, via decreased Fe–S cluster synthesis and the consequent activation of IRP1, in a false "low iron" signal that activates the iron uptake system. In consequence, diminished Fe–S cluster synthesis could play a fundamental role in the accumulation of iron observed in Parkinson's disease.

Defects in mitochondrial electron transport in other neurodegenerative diseases such as Alzheimer's disease, Huntington disease and Friedreich's ataxia (Brennan et al. 1985; Parker et al. 1994; Lodi et al. 1999), have raised the hypothesis that mitochondrial dysfunction is a common cause for a number of neurodegenerative and non-neurodegenerative diseases (Schapira 2006).

A positive feedback loop in the death of neurons in Parkinson's disease

We propose that inhibition of mitochondrial complex I by endogenous and/or exogenous toxins, and inflammatory processes produced by trauma or other causes, result in a vicious cycle of increased oxidative stress, increased iron accumulation and decreased GSH content (Fig. 4). In this scheme, neuronal death linked to complex I dysfunction is brought about by a positive feedback loop in which complex I inhibition results in decreased Fe–S cluster synthesis, IRP1 activation, increased DMT1 and TfR expression and iron accumulation. Complex I dysfunction and increased cellular iron result in decreased GSH levels. Both increased oxidative stress and low GSH levels further inhibit complex I activity. Another input to this cycle is contributed by inflammatory cytokines that induce hepcidin synthesis, which by inducing FPN1 degradation results in increased glial and neuronal iron content. Inflammatory cytokines also transcriptionally regulate DMT1, FPN1 and Zip 14 synthesis, and activate IRPs (see text). Central to this scheme is the deregulation of iron homeostasis since iron chelators effectively block cell death (Zhu et al. 2007; Kupershmidt et al. 2011; Weinreb et al. 2011).

Conclusions

A number of neurodegenerative diseases such as Parkinson's, Alzheimer's and Huntington, present diminished activity of mitochondrial complex I, iron accumulation, oxidative stress and inflammation. It is possible that the initiation of any one of these processes will initiate and enhance the others through the generation of a positive feedback loop that will produce apoptotic neuronal death. Intervention of this positive loop should result in prolonged life of the affected neurons. Still unanswered is the question of why SN pars compacta neurons are so particular prone to this deregulation.

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References

Aguirre P, Mena N, Tapia V, Arredondo M, Núñez MT (2005) Iron homeostasis in neuronal cells: a role for IREG1. BMC Neurosci 6:3

Barnham KJ, Bush AI (2008) Metals in Alzheimer's and Parkinson's diseases. Curr Opin Chem Biol 12(2):222–228

Bartzokis G, Beckson M, Hance DB, Marx P, Foster JA, Marder SR (1997) MR evaluation of age-related increase of brain iron in young adult and older normal males. Magn Reson Imaging 15(1):29–35

Bartzokis G, Sultzer D, Cummings J, Holt LE, Hance DB, Henderson VW, Mintz J (2000) In vivo evaluation of brain iron in Alzheimer disease using magnetic resonance imaging. Arch Gen Psychiatry 57(1):47–53

Beinert H, Emptage MH, Dreyer JL, Scott RA, Hahn JE, Hodgson KO, Thomson AJ (1983) Iron-sulfur stoichiometry and structure of iron-sulfur clusters in three-iron proteins: evidence for [3Fe–4S] clusters. Proc Natl Acad Sci USA 80(2):393–396

Berg D, Youdim MB (2006) Role of iron in neurodegenerative disorders. Top Magn Reson Imaging 17(1):5–17

Boserup MW, Lichota J, Haile D, Moos T (2011) Heterogenous distribution of ferroportin-containing neurons in mouse brain. Biometals 24(2):357–375

Braak H, Braak E (1991) Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 82(4):239–259

Bradbury MWB (1997) Transport of Iron in the blood-brain-Ccrebrospinal fluid system. J Neurochem 69(2):443–454

Brennan WA Jr, Bird ED, Aprille JR (1985) Regional mitochondrial respiratory activity in Huntington's disease brain. J Neurochem 44(6):1948–1950

Burdo JR, Menzies SL, Simpson IA, Garrick LM, Garrick MD, Dolan KG, Haile DJ, Beard JL, Connor JR (2001) Distribution of divalent metal transporter 1 and metal transport protein 1 in the normal and Belgrade rat. J Neurosci Res 66(6):1198–1207

Cairo G, Recalcati S (2007) Iron-regulatory proteins: molecular biology and pathophysiological implications. Expert Rev Mol Med 9(33):1–13

Castellani RJ, Moreira PI, Liu G, Dobson J, Perry G, Smith MA, Zhu X (2007) Iron: the Redox-active center of oxidative stress in Alzheimer disease. Neurochem Res 32(10):1640–1645

Chinopoulos C, Adam-Vizi V (2001) Mitochondria deficient in complex I activity are depolarized by hydrogen peroxide in nerve terminals: relevance to Parkinson's disease. J Neurochem 76(1):302–306

Chinta SJ, Andersen JK (2006) Reversible inhibition of mitochondrial complex I activity following chronic dopaminergic glutathione depletion in vitro: implications for Parkinson's disease. Free Radic Biol Med 41(9):1442–1448

Collingwood JF, Mikhaylova A, Davidson M, Batich C, Streit WJ, Terry J, Dobson J (2005) In situ characterization and mapping of iron compounds in Alzheimer's disease tissue. J Alzheimers Dis 7(4):267–272

Collingwood JF, Chong RK, Kasama T, Cervera-Gontard L, Dunin-Borkowski RE, Perry G, Posfai M, Siedlak SL, Simpson ET, Smith MA, Dobson J (2008) Three-dimensional tomographic imaging and characterization of iron compounds within Alzheimer's plaque core material. J Alzheimers Dis 14(2):235–245

Connor JR, Benkovic SA (1992) Iron regulation in the brain: histochemical, biochemical, and molecular considerations. Ann Neurol 32(Suppl):S51–S61

Connor JR, Snyder BS, Beard JL, Fine RE, Mufson EJ (1992) Regional distribution of iron and iron-regulatory proteins in the brain in aging and Alzheimer's disease. J Neurosci Res 31(2):327–335

Cozzi A, Santambrogio P, Corsi B, Campanella A, Arosio P, Levi S (2006) Characterization of the l-ferritin variant 460InsA responsible of a hereditary ferritinopathy disorder. Neurobiol Dis 23(3):644–652

Curtis AR, Fey C, Morris CM, Bindoff LA, Ince PG, Chinnery PF, Coulthard A, Jackson MJ, Jackson AP, McHale DP, Hay D, Barker WA, Markham AF, Bates D, Curtis A, Burn J (2001) Mutation in the gene encoding ferritin light polypeptide causes dominant adult-onset basal ganglia disease. Nat Genet 28(4):350–354

Davis DG, Schmitt FA, Wekstein DR, Markesbery WR (1999) Alzheimer neuropathologic alterations in aged cognitively normal subjects. J Neuropathol Exp Neurol 58(4):376–388

Davison AN (1987) Pathophysiology of ageing brain. Gerontology 33(3–4):129–135

De Domenico I, Vaughn MB, Li L, Bagley D, Musci G, Ward DM, Kaplan J (2006) Ferroportin-mediated mobilization of ferritin iron precedes ferritin degradation by the proteasome. EMBO J 25(22):5396–5404

Devalia V, Carter K, Walker AP, Perkins SJ, Worwood M, May A, Dooley JS (2002) Autosomal dominant reticuloendothelial iron overload associated with a 3-base pair deletion in the ferroportin 1 gene (SLC11A3). Blood 100(2):695–697

Dexter DT, Carayon A, Javoy-Agid F, Agid Y, Wells FR, Daniel SE, Lees AJ, Jenner P, Marsden CD (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

Ding B, Chen KM, Ling HW, Sun F, Li X, Wan T, Chai WM, Zhang H, Zhan Y, Guan YJ (2009) Correlation of iron in the hippocampus with MMSE in patients with Alzheimer's disease. J Magn Reson Imaging 29(4):793–798

Double KL, Gerlach M, Schunemann V, Trautwein AX, Zecca L, Gallorini M, Youdim MB, Riederer P, Ben-Shachar D (2003) Iron-binding characteristics of neuromelanin of the human substantia nigra. Biochem Pharmacol 66(3):489–494

Dringen R, Gutterer JM, Hirrlinger J (2000) Glutathione metabolism in brain metabolic interaction between astrocytes and neurons in the defense against reactive oxygen species. Eur J Biochem 267(16):4912–4916

Duyckaerts C (2004) Looking for the link between plaques and tangles. Neurobiol Aging 25(6):735–739 (discussion 743–736)

Duyckaerts C, Delatour B, Potier MC (2009) Classification and basic pathology of Alzheimer disease. Acta Neuropathol 118(1):5–36

Epsztejn S, Kakhlon O, Glickstein H, Breuer W, Cabantchik I (1997) Fluorescence analysis of the labile iron pool of mammalian cells. Anal Biochem 248(1):31–40

Falangola MF, Lee SP, Nixon RA, Duff K, Helpern JA (2005) Histological co-localization of iron in Abeta plaques of PS/APP transgenic mice. Neurochem Res 30(2):201–205

Faucheux BA, Martin ME, Beaumont C, Hunot S, Hauw JJ, Agid Y, Hirsch EC (2002) Lack of up-regulation of ferritin is associated with sustained iron regulatory protein-1 binding activity in the substantia nigra of patients with Parkinson's disease. J Neurochem 83(2):320–330

Fisher J, Devraj K, Ingram J, Slagle-Webb B, Madhankumar AB, Liu X, Klinger M, Simpson IA, Connor JR (2007) Ferritin: a novel mechanism for delivery of iron to the brain and other organs. Am J Physiol Cell Physiol 293(2):C641–C649

Gaasch JA, Geldenhuys WJ, Lockman PR, Allen DD, Van der Schyf CJ (2007) Voltage-gated calcium channels provide an alternate route for iron uptake in neuronal cell cultures. Neurochem Res 32(10):1686–1693

Garrick MD, Garrick LM (2009) Cellular iron transport. Biochim Biophys Acta 1790(5):309–325

Gerlach M, Riederer P, Double KL (2008) Neuromelanin-bound ferric iron as an experimental model of dopaminergic neurodegeneration in Parkinson's disease. Parkinsonism Relat Disord 14(Suppl 2):S185–S188

Giampa C, DeMarch Z, Patassini S, Bernardi G, Fusco FR (2007) Immunohistochemical localization of TRPC6 in the rat substantia nigra. Neurosci Lett 424(3):170–174

Girijashanker K, He L, Soleimani M, Reed JM, Li H, Liu Z, Wang B, Dalton TP, Nebert DW (2008) Slc39a14 gene encodes ZIP14, a metal/bicarbonate symporter: similarities to the ZIP8 transporter. Mol Pharmacol 73(5):1413–1423

Glickstein H, El RB, Link G, Breuer W, Konijn AM, Hershko C, Nick H, Cabantchik ZI (2006) Action of chelators in iron-loaded cardiac cells: accessibility to intracellular labile iron and functional consequences. Blood 108(9):3195–3203

Gómez FJ, Aguirre P, Gonzalez-Billault C, Núñez MT (2011) Iron mediates neuritic tree collapse in mesencephalic neurons treated with 1-methyl-4-phenylpyridinium (MPP+). J Neural Transm 118(3):421–431

Gorell JM, Ordidge RJ, Brown GG, Deniau JC, Buderer NM, Helpern JA (1995) Increased iron-related MRI contrast in

the substantia nigra in Parkinson's disease. Neurology 45(6):1138–1143

Graf E, Mahoney JR, Bryant RG, Eaton JW (1984) Iron-catalyzed hydroxyl radical formation. Stringent requirement for free iron coordination site. J Biol Chem 259(6):3620–3624

Griffiths PD, Dobson BR, Jones GR, Clarke DT (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

Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, Nussberger S, Gollan JL, Hediger MA (1997) Cloning and characterization of a mammalian proton-coupled metal-ion transporter. Nature 388(6641):482–488

Haeger P, Alvarez A, Leal N, Adasme T, Núñez MT, Hidalgo C (2010) Increased hippocampal expression of the divalent metal transporter 1 (DMT1) mRNA variants 1B and +IRE and DMT1 protein after NMDA-receptor stimulation or spatial memory training. Neurotoxic Res 17(3):238–247

Harrison PM, Arosio P (1996) The ferritins: molecular properties, iron storage function and cellular regulation. Biochim Biophys Acta 1275(3):161–203

Hidalgo C, Núñez MT (2007) Calcium, iron and neuronal function. IUBMB Life 59(4–5):280–285

Hirsch EC, Brandel JP, Galle P, Javoy-Agid F, Agid Y (1991) Iron and aluminum increase in the substantia nigra of patients with Parkinson's disease: an X-ray microanalysis. J Neurochem 56(2):446–451

Hubert N, Hentze MW (2002) Previously uncharacterized isoforms of divalent metal transporter (DMT)-1: implications for regulation and cellular function. Proc Natl Acad Sci USA 99(19):12345–12350

Itagaki S, McGeer PL, Akiyama H, Zhu S, Selkoe D (1989) Relationship of microglia and astrocytes to amyloid deposits of Alzheimer disease. J Neuroimmunol 24(3): 173–182

Jack CR Jr, Wengenack TM, Reyes DA, Garwood M, Curran GL, Borowski BJ, Lin J, Preboske GM, Holasek SS, Adriany G, Poduslo JF (2005) In vivo magnetic resonance microimaging of individual amyloid plaques in Alzheimer's transgenic mice. J Neurosci 25(43):10041–10048

Jefferies WA, Food MR, Gabathuler R, Rothenberger S, Yamada T, Yasuhara O, McGeer PL (1996) Reactive microglia specifically associated with amyloid plaques in Alzheimer's disease brain tissue express melanotransferrin. Brain Res 712(1):122–126

Jellinger KA (1999) The role of iron in neurodegeneration: prospects for pharmacotherapy of Parkinson's disease. Drugs Aging 14(2):115–140

Johnstone M, Gearing AJ, Miller KM (1999) A central role for astrocytes in the inflammatory response to beta-amyloid; chemokines, cytokines and reactive oxygen species are produced. J Neuroimmunol 93(1–2):182–193

Joseph JA, Shukitt-Hale B, Casadesus G, Fisher D (2005) Oxidative stress and inflammation in brain aging: nutritional considerations. Neurochem Res 30(6–7):927–935

Kakhlon O, Cabantchik ZI (2002) The labile iron pool: characterization, measurement, and participation in cellular processes(1). Free Radic Biol Med 33(8):1037–1046

Kastner A, Hirsch EC, Lejeune O, Javoy-Agid F, Rascol O, Agid Y (1992) Is the vulnerability of neurons in the substantia nigra of patients with Parkinson's disease related to their neuromelanin content? J Neurochem 59(3):1080–1089

Kaur D, Yantiri F, Rajagopalan S, Kumar J, Mo JQ, Boonplueang R, Viswanath V, Jacobs R, Yang L, Beal MF, DiMonte D, Volitaskis I, Ellerby L, Cherny RA, Bush AI, Andersen JK (2003) Genetic or pharmacological iron chelation prevents MPTP-induced neurotoxicity in vivo: a novel therapy for Parkinson's disease. Neuron 37(6):899–909

Kaur D, Lee D, Ragapolan S, Andersen JK (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(5):593–598

Knutson MD, Vafa MR, Haile DJ, Wessling-Resnick M (2003) Iron loading and erythrophagocytosis increase ferroportin 1 (FPN1) expression in J774 macrophages. Blood 102(12): 4191–4197

Krizaj D (2005) Compartmentalization of calcium entry pathways in mouse rods. Eur J Neurosci 22(12):3292–3296

Kruszewski M (2003) Labile iron pool: the main determinant of cellular response to oxidative stress. Mutat Res 531(1–2): 81–92

Kupershmidt L, Weinreb O, Amit T, Mandel S, Bar-Am O, Youdim MB (2011) Novel molecular targets of the neuroprotective/neurorescue multimodal iron chelating drug M30 in the mouse brain. Neuroscience 189:345–358

LaFerla FM, Green KN, Oddo S (2007) Intracellular amyloid-beta in Alzheimer's disease. Nat Rev Neurosci 8(7):499–509

Langston JW, Ballard P, Tetrud JW, Irwin I (1983) Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. Science 219(4587):979–980

Latunde-Dada GO, Xiang L, Simpson RJ, McKie AT (2011) Duodenal cytochrome b (Cybrd 1) and HIF-2alpha expression during acute hypoxic exposure in mice. Eur J Nutr 50(8):699–704

Lichten LA, Cousins RJ (2009) Mammalian zinc transporters: nutritional and physiologic regulation. Annu Rev Nutr 29:153–176

Lill R, Muhlenhoff U (2008) Maturation of iron-sulfur proteins in eukaryotes: mechanisms, connected processes, and diseases. Annu Rev Biochem 77:669–700

Lill R, Dutkiewicz R, Elsasser HP, Hausmann A, Netz DJ, Pierik AJ, Stehling O, Urzica E, Muhlenhoff U (2006) Mechanisms of iron-sulfur protein maturation in mitochondria, cytosol and nucleus of eukaryotes. Biochim Biophys Acta 1763(7):652–667

Lis A, Paradkar PN, Singleton S, Kuo HC, Garrick MD, Roth JA (2005) Hypoxia induces changes in expression of isoforms of the divalent metal transporter (DMT1) in rat pheochromocytoma (PC12) cells. Biochem Pharmacol 69(11): 1647–1655

Liu X, Theil EC (2005) Ferritins: dynamic management of biological iron and oxygen chemistry. Acc Chem Res 38(3):167–175

Liuzzi JP, Lichten LA, Rivera S, Blanchard RK, Aydemir TB, Knutson MD, Ganz T, Cousins RJ (2005) Interleukin-6 regulates the zinc transporter Zip14 in liver and contributes to the hypozincemia of the acute-phase response. Proc Natl Acad Sci USA 102(19):6843–6848

Liuzzi JP, Aydemir F, Nam H, Knutson MD, Cousins RJ (2006) Zip14 (Slc39a14) mediates non-transferrin-bound iron uptake into cells. Proc Natl Acad Sci USA 103(37):13612–13617

Lockman JA, Geldenhuys WJ, Bohn KA, Desilva SF, Allen DD, Van der Schyf CJ (2012) Differential effect of nimodipine in attenuating iron-induced toxicity in brain- and blood-brain barrier-associated cell types. Neurochem Res 37(1): 134–142

Lodi R, Cooper JM, Bradley JL, Manners D, Styles P, Taylor DJ, Schapira AH (1999) Deficit of in vivo mitochondrial ATP production in patients with Friedreich ataxia. Proc Natl Acad Sci USA 96(20):11492–11495

Ludwiczek S, Theurl I, Muckenthaler MU, Jakab M, Mair SM, Theurl M, Kiss J, Paulmichl M, Hentze MW, Ritter M, Weiss G (2007) Ca2+ channel blockers reverse iron overload by a new mechanism via divalent metal transporter-1. Nat Med 13(4):448–454

Mancuso C, Scapagini G, Curro D, Giuffrida Stella AM, De Marco C, Butterfield DA, Calabrese V (2007) Mitochondrial dysfunction, free radical generation and cellular stress response in neurodegenerative disorders. Front Biosci 12:1107–1123

Markesbery WR, Lovell MA (1998) Four-hydroxynonenal, a product of lipid peroxidation, is increased in the brain in Alzheimer's disease. Neurobiol Aging 19(1):33–36

Martelli A, Wattenhofer-Donze M, Schmucker S, Bouvet S, Reutenauer L, Puccio H (2007) Frataxin is essential for extramitochondrial Fe–S cluster proteins in mammalian tissues. Hum Mol Genet 16(22):2651–2658

McKie AT, Marciani P, Rolfs A, Brennan K, Wehr K, Barrow D, Miret S, Bomford A, Peters TJ, Farzaneh F, Hediger MA, Hentze MW, Simpson RJ (2000) A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation. Mol Cell 5(2):299–309

McKie AT, Barrow D, Latunde-Dada GO, Rolfs A, Sager G, Mudaly E, Mudaly M, Richardson C, Barlow D, Bomford A, Peters TJ, Raja KB, Shirali S, Hediger MA, Farzaneh F, Simpson RJ (2001) An iron-regulated ferric reductase associated with the absorption of dietary iron. Science 291(5509):1755–1759

Meda L, Baron P, Scarlato G (2001) Glial activation in Alzheimer's disease: the role of Abeta and its associated proteins. Neurobiol Aging 22(6):885–893

Mehlhase J, Sandig G, Pantopoulos K, Grune T (2005) Oxidation-induced ferritin turnover in microglial cells: role of proteasome. Free Radic Biol Med 38(2):276–285

Meister A, Anderson ME (1983) Glutathione. Annu Rev Biochem 52:711–760

Mena NP, Bulteau AL, Salazar J, Hirsch EC, Núñez MT (2011) Effect of mitochondrial complex I inhibition on Fe–S cluster protein activity. Biochem Biophys Res Commun 409(2):241–246

Moos T, Morgan EH (1998) Evidence for low molecular weight, non-transferrin-bound iron in rat brain and cerebrospinal fluid. J Neurosci Res 54(4):486–494

Moos T, Rosengren Nielsen T (2006) Ferroportin in the postnatal rat brain: implications for axonal transport and neuronal export of iron. Semin Pediatr Neurol 13(3):149–157

Moos T, Rosengren Nielsen T, Skjorringe T, Morgan EH (2007) Iron trafficking inside the brain. J Neurochem 103(5): 1730–1740

Mura C, Delgado R, Aguirre P, Bacigalupo J, Nuñez MT (2006) SHSY5Y neuroblastoma cells survival to iron challenge results in a quiescent and functional cell population. J Neurochem 98(1):11–19

Mwanjewe J, Grover AK (2004) Role of transient receptor potential canonical 6 (TRPC6) in non-transferrin-bound iron uptake in neuronal phenotype PC12 cells. Biochem J 378(Pt 3):975–982

Núñez MT, Gallardo V, Muñoz P, Tapia V, Esparza A, Salazar J, Speisky H (2004) Progressive iron accumulation induces a biphasic change in the glutathione content of neuroblastoma cells. Free Radic Biol Med 37(7):953–960

Ohgami RS, Campagna DR, Greer EL, Antiochos B, McDonald A, Chen J, Sharp JJ, Fujiwara Y, Barker JE, Fleming MD (2005) Identification of a ferrireductase required for efficient transferrin-dependent iron uptake in erythroid cells. Nat Genet 37(11):1264–1269

Ohgami RS, Campagna DR, McDonald A, Fleming MD (2006) The Steap proteins are metalloreductases. Blood 108(4): 1388–1394

Oudit GY, Trivieri MG, Khaper N, Liu PP, Backx PH (2006) Role of L-type Ca2+ channels in iron transport and iron-overload cardiomyopathy. J Mol Med (Berl) 84(5):349–364

Paradkar PN, Roth JA (2006) Post-translational and transcriptional regulation of DMT1 during P19 embryonic carcinoma cell differentiation by retinoic acid. Biochem J 394(Pt 1):173–183

Parker WD Jr, Parks J, Filley CM, Kleinschmidt-DeMasters BK (1994) Electron transport chain defects in Alzheimer's disease brain. Neurology 44(6):1090–1096

Pelizzoni I, Macco R, Morini MF, Zacchetti D, Grohovaz F, Codazzi F (2011) Iron handling in hippocampal neurons: activity-dependent iron entry and mitochondria-mediated neurotoxicity. Aging Cell 10(1):172–183

Perry TL, Godin DV, Hansen S (1982) Parkinson's disease: a disorder due to nigral glutathione deficiency? Neurosci Lett 33(3):305–310

Perry G, Taddeo MA, Petersen RB, Castellani RJ, Harris PL, Siedlak SL, Cash AD, Liu Q, Nunomura A, Atwood CS, Smith MA (2003) Adventiously-bound redox active iron and copper are at the center of oxidative damage in Alzheimer disease. Biometals 16(1):77–81

Qian ZM, Wu XM, Fan M, Yang L, Du F, Yung WH, Ke Y (2011) Divalent metal transporter 1 is a hypoxia-inducible gene. J Cell Physiol 226(6):1596–1603

Quintana C, Bellefqih S, Laval JY, Guerquin-Kern JL, Wu TD, Avila J, Ferrer I, Arranz R, Patino C (2006) Study of the localization of iron, ferritin, and hemosiderin in Alzheimer's disease hippocampus by analytical microscopy at the subcellular level. J Struct Biol 153(1):42–54

Ramirez G, Rey S, von Bernhardi R (2008) Proinflammatory stimuli are needed for induction of microglial cell-mediated AbetaPP_{244-C} and Abeta-neurotoxicity in hippocampal cultures. J Alzheimers Dis 15(1):45–59

Reynolds MR, Berry RW, Binder LI (2007) Nitration in neurodegeneration: deciphering the "Hows" "nYs". Biochemistry 46(25):7325–7336

Rouault TA, Zhang DL, Jeong SY (2009) Brain iron homeostasis, the choroid plexus, and localization of iron transport proteins. Metab Brain Dis 24(4):673–684

Salazar J, Mena N, Hunot S, Prigent A, Alvarez-Fischer D, Arredondo M, Duyckaerts C, Sazdovitch V, Zhao L, Garrick LM, Núñez MT, Garrick MD, Raisman-Vozari R, Hirsch EC (2008) Divalent metal transporter 1 (DMT1) contributes to neurodegeneration in animal models of Parkinson's disease. Proc Natl Acad Sci USA 105(47): 18578–18583

Salgado JC, Olivera-Nappa A, Gerdtzen ZP, Tapia V, Theil EC, Conca C, Núñez MT (2010) Mathematical modeling of the dynamic storage of iron in ferritin. BMC Syst Biol 4:147

Sayre LM, Perry G, Atwood CS, Smith MA (2000) The role of metals in neurodegenerative diseases. Cell Mol Biol (Noisy-le-grand) 46(4):731–741

Schafer FQ, Buettner GR (2001) Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic Biol Med 30(11): 1191–1212

Schapira AH (2006) Mitochondrial disease. Lancet 368(9529): 70–82

Schapira AH, Gegg M (2011) Mitochondrial contribution to Parkinson's disease pathogenesis. Parkinsons Dis 2011: 159160

Schapira AH, Cooper JM, Dexter D, Clark JB, Jenner P, Marsden CD (1990) Mitochondrial complex I deficiency in Parkinson's disease. J Neurochem 54(3):823–827

Shachar DB, Kahana N, Kampel V, Warshawsky A, Youdim MB (2004) Neuroprotection by a novel brain permeable iron chelator, VK-28, against 6-hydroxydopamine lession in rats. Neuropharmacology 46(2):254–263

Shaw GC, Cope JJ, Li L, Corson K, Hersey C, Ackermann GE, Gwynn B, Lambert AJ, Wingert RA, Traver D, Trede NS, Barut BA, Zhou Y, Minet E, Donovan A, Brownlie A, Balzan R, Weiss MJ, Peters LL, Kaplan J, Zon LI, Paw BH (2006) Mitoferrin is essential for erythroid iron assimilation. Nature 440(7080):96–100

Sheftel A, Stehling O, Lill R (2010) Iron-sulfur proteins in health and disease. Trends Endocrinol Metab 21(5):302–314

Shi Q, Gibson GE (2007) Oxidative stress and transcriptional regulation in Alzheimer disease. Alzheimer Dis Assoc Disord 21(4):276–291

Sian J, Dexter DT, Lees AJ, Daniel S, Agid Y, Javoy-Agid F, Jenner P, Marsden CD (1994) Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia. Ann Neurol 36(3):348–355

Sienko MJ, Plane RA (1976) Chemistry, 5th edn. McGraw-Hill, New York

Snyder AM, Connor JR (2009) Iron, the substantia nigra and related neurological disorders. Biochim Biophys Acta 1790(7):606–614

Sofic E, Riederer P, Heinsen H, Beckmann H, Reynolds GP, Hebenstreit G, Youdim MB (1988) Increased iron (III) and total iron content in post mortem substantia nigra of parkinsonian brain. J Neural Transm 74(3):199–205

Song N, Wang J, Jiang H, Xie J (2010) Ferroportin 1 but not hephaestin contributes to iron accumulation in a cell model of Parkinson's disease. Free Radic Biol Med 48(2):332–341

Spiro TG, Salman P (1974) Inorganic chemistry. In: Jacobs A, Worwood M (eds) Iron in biochemistry and medicine. Academic Press, New York, pp 1–28

Symons MCR, Gutteridge JMC (1998) Free radicals and iron: chemistry, biology, and medicine. Oxford University Press, Oxford

Thompson KJ, Shoham S, Connor JR (2001) Iron and neurodegenerative disorders. Brain Res Bull 55(2):155–164

Todorich B, Zhang X, Connor JR (2011) H-ferritin is the major source of iron for oligodendrocytes. Glia 59(6):927–935

Tulpule K, Robinson SR, Bishop GM, Dringen R (2010) Uptake of ferrous iron by cultured rat astrocytes. J Neurosci Res 88(3):563–571

Unciuleac M, Warkentin E, Page CC, Boll M, Ermler U (2004) Structure of a xanthine oxidase-related 4-hydroxybenzoyl-CoA reductase with an additional [4Fe–4S] cluster and an inverted electron flow. Structure 12(12):2249–2256

Vargas JD, Herpers B, McKie AT, Gledhill S, McDonnell J, van den Heuvel M, Davies KE, Ponting CP (2003) Stromal cell-derived receptor 2 and cytochrome b561 are functional ferric reductases. Biochim Biophys Acta 1651(1–2):116–123

Vidal R, Delisle MB, Rascol O, Ghetti B (2003) Hereditary ferritinopathy. J Neurol Sci 207(1–2):110–111

von Bernhardi R (2007) Glial cell dysregulation: a new perspective on Alzheimer disease. Neurotoxic Res 12(4):215–232

Vymazal J, Righini A, Brooks RA, Canesi M, Mariani C, Leonardi M, Pezzoli G (1999) T1 and T2 in the brain of healthy subjects, patients with Parkinson disease, and patients with multiple system atrophy: relation to iron content. Radiology 211(2):489–495

Wallander ML, Leibold EA, Eisenstein RS (2006) Molecular control of vertebrate iron homeostasis by iron regulatory proteins. Biochim Biophys Acta 1763(7):668–689

Wang D, Wang LH, Zhao Y, Lu YP, Zhu L (2010) Hypoxia regulates the ferrous iron uptake and reactive oxygen species level via divalent metal transporter 1 (DMT1) Exon1B by hypoxia-inducible factor-1. IUBMB Life 62(8):629–636

Weinreb O, Amit T, Mandel S, Youdim MB (2011) Novel therapeutic approach for neurodegenerative pathologies: multitarget iron-chelating drugs regulating hypoxia-inducible factor 1 signal transduction pathway. Neurodegener Dis. [Epub ahead of print]

Williams K, Wilson MA, Bressler J (2000) Regulation and developmental expression of the divalent metal-ion transporter in the rat brain. Cell Mol Biol (Noisy-le-grand) 46(3):563–571

Wilson RB (2006) Iron dysregulation in Friedreich ataxia. Semin Pediatr Neurol 13(3):166–175

Wood PM (1988) The potential diagram for oxygen at pH 7. Biochem J 253(1):287–289

Wright RO, Baccarelli A (2007) Metals and neurotoxicology. J Nutr 137(12):2809–2813

Yamada M (2004) Cerebral amyloid angiopathy and gene polymorphisms. J Neurol Sci 226(1–2):41–44

Yang F, Liu XB, Quinones M, Melby PC, Ghio A, Haile DJ (2002) Regulation of reticuloendothelial iron transporter MTP1 (Slc11a3) by inflammation. J Biol Chem 277(42): 39786–39791

Yasha TC, Shankar L, Santosh V, Das S, Shankar SK (1997) Histopathological & immunohistochemical evaluation of ageing changes in normal human brain. Indian J Med Res 105:141–150

Ye H, Rouault TA (2010) Erythropoiesis and iron sulfur cluster biogenesis. Adv Hematol 2010

Youdim MB, Buccafusco JJ (2005) Multi-functional drugs for various CNS targets in the treatment of neurodegenerative disorders. Trends Pharmacol Sci 26(1):27–35

Youdim MB, Ben-Shachar D, Riederer P (1989) Is Parkinson's disease a progressive siderosis of substantia nigra resulting in iron and melanin induced neurodegeneration? Acta Neurol Scand Suppl 126:47–54

Youdim MB, Stephenson G, Ben-Shachar D (2004) Ironing iron out in Parkinson's disease and other neurodegenerative diseases with iron chelators: a lesson from 6-hydroxydopamine and iron chelators, desferal and VK-28. Ann N Y Acad Sci 1012:306–325

Zecca L, Tampellini D, Gatti A, Crippa R, Eisner M, Sulzer D, Ito S, Fariello R, Gallorini M (2002) The neuromelanin of human substantia nigra and its interaction with metals. J Neural Transm 109(5–6):663–672

Zecca L, Zucca FA, Wilms H, Sulzer D (2003) Neuromelanin of the substantia nigra: a neuronal black hole with protective and toxic characteristics. Trends Neurosci 26(11):578–580

Zecca L, Youdim MB, Riederer P, Connor JR, Crichton RR (2004) Iron, brain ageing and neurodegenerative disorders. Nat Rev Neurosci 5(11):863–873

Zhang Y, Marcillat O, Giulivi C, Ernster L, Davies KJ (1990) The oxidative inactivation of mitochondrial electron transport chain components and ATPase. J Biol Chem 265(27):16330–16336

Zheng H, Youdim MB, Fridkin M (2010) Site-activated chelators targeting acetylcholinesterase and monoamine oxidase for Alzheimer's therapy. ACS Chem Biol 5(6):603–610

Zhu W, Xie W, Pan T, Xu P, Fridkin M, Zheng H, Jankovic J, Youdim MB, Le W (2007) Prevention and restoration of lactacystin-induced nigrostriatal dopamine neuron degeneration by novel brain-permeable iron chelators. FASEB J 21(14):3835–3844

Zoccarato F, Toscano P, Alexandre A (2005) Dopamine-derived dopaminochrome promotes H(2)O(2) release at mitochondrial complex I: stimulation by rotenone, control by Ca(2+), and relevance to Parkinson disease. J Biol Chem 280(16):15587–15594