IRON, BRAIN AGEING AND NEURODEGENERATIVE DISORDERS

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Abstract | There is increasing evidence that iron is involved in the mechanisms that underlie many neurodegenerative diseases. Conditions such as neuroferritinopathy and Friedreich ataxia are associated with mutations in genes that encode proteins that are involved in iron metabolism, and as the brain ages, iron accumulates in regions that are affected by Alzheimer's disease and Parkinson's disease. High concentrations of reactive iron can increase oxidative-stress induced neuronal vulnerability, and iron accumulation might increase the toxicity of environmental or endogenous toxins. By studying the accumulation and cellular distribution of iron during ageing, we should be able to increase our understanding of these neurodegenerative disorders and develop new therapeutic strategies.

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Iron is an essential cofactor for many proteins that are involved in the normal function of neuronal tissue, such as the non-haem iron enzyme tyrosine hydroxylase, which is required for dopamine synthesis. However, there is increasing evidence that iron accumulation in the brain can cause a vast range of disorders of the CNS. It has also become apparent that iron progressively accumulates in the brain with age, and that iron-induced oxidative stress can cause neurodegeneration.

We can distinguish two classes of iron-related neurodegenerative disorder — those that result from iron accumulation in specific brain regions, and those that result from defects in iron metabolism and/or homeostasis. They frequently involve protein modification, misfolding and aggregation, leading to the formation of the intracellular inclusion bodies that are the post-mortem hallmark of many neurodegenerative diseases. As life expectancy increases, we expect to see a corresponding increase in the occurrence of iron-related neurodegenerative diseases, and it is, therefore, timely to review our current knowledge of the relationship between iron and neurodegeneration.

Iron metabolism and homeostasis

The importance of adequate amounts of iron for health and well-being in humans is well established. It is involved in oxygen transport, storage and activation, electron transport and many important metabolic processes (for a general review, see REF. 1). Iron metabolism in humans is conservative: 1–2 mg of iron is absorbed per day, and the same amount is excreted. Global iron homeostasis is regulated at the level of iron absorption from the gastrointestinal tract. This involves a series of molecular interactions between proteins that include the product of the HAEMOCHROMATOSIS gene (HFE), transferrin (Tf), the transferrin receptor (TfR) and iron regulatory proteins (IRPs) in the crypts of Lieberkühn, which determine the amount of iron that is allowed to cross the enterocyte and enter the bloodstream[1]. HFE, Tf and TfR interact with each other, whereas IRPs interact with the mRNAs of TfR and ferritin. Recent investigations into the role of humoral factors in programming the enterocyte indicated that the main molecules involved are the antimicrobial peptide hepcidin[2,3], and haemojuvelin (HFE2) — the recently identified product of the gene for a juvenile form of haemochromatosis[4].

Most cells take up iron from diferric transferrin ($Tf\text{-}Fe_2$) by way of the TfR and the transferrin-to-cell cycle[5] (FIG. 1). After binding and internalization of the $Tf\text{-}Fe_2$–TfR complex, the iron is released from Tf in an acidic, ENDOSOMAL COMPARTMENT, and is transported across the endosomal membrane into the cytosol by

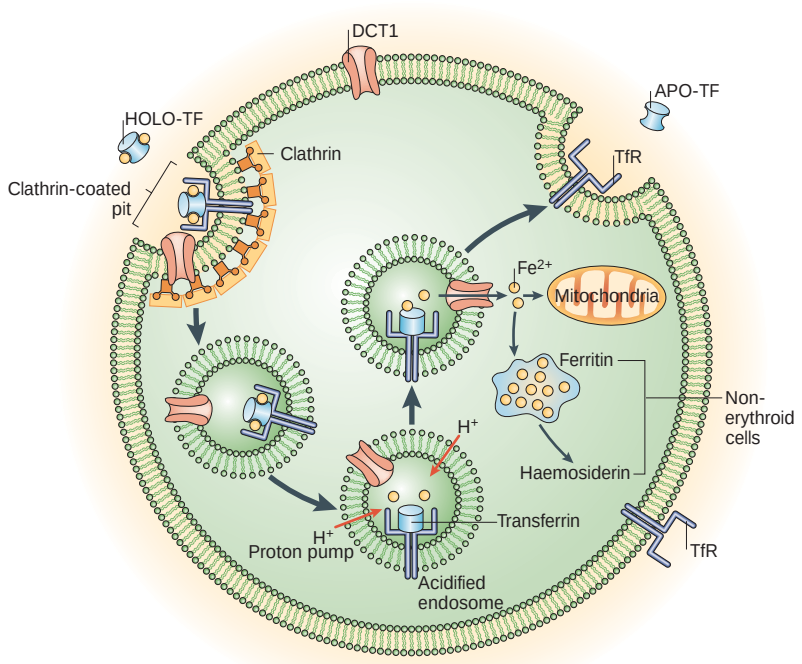

Figure 1 | **The Tf cycle.** Holotransferrin (HOLO-TF) binds to the transferrin receptor (TfR) at the cell surface. These complexes localize to clathrin-coated pits, which invaginate to initiate endocytosis. Specialized endosomes form, which are acidified by a proton pump. When the required acidic pH is reached, iron is released from transferrin (Tf) and is co-transported, with the protons, out of the endosomes by the divalent cation transporter DCT1. Apotransferrin (APO-TF) is returned to the cell membrane bound to TfR, where, at neutral pH, they dissociate to participate in further rounds of iron delivery. The iron can be targeted to the mitochondria. In non-erythroid cells, iron is stored in the form of ferritin and haemosiderin.

HAEMOCHROMATOSIS
An iron-overload disorder, in which an excessive amount of iron is absorbed from the diet. The iron accumulates in various organs, including the liver, pancreas and heart, which can lead to severe organ damage.

ENDOSOMAL COMPARTMENT
A system of organelles that carry materials that have been ingested by endocytosis, and pass them to lysosomes for degradation, or recycle them to the cell surface.

MICROGLIA
Phagocytic immune cells in the brain that engulf and remove cells that have undergone apoptosis.

SUBSTANTIA NIGRA
A part of the midbrain that contains dopamine-producing neurons, the axons of which innervate the striatum and thereby control body movements.

LOCUS COERULEUS
A nucleus of the brainstem that is the main supplier of noradrenaline to the brain.

the divalent cation transporter DCT1 (REF. 6). It then either passes into the mitochondria, to supply iron for haem and iron–sulphur cluster biosynthesis, or is stored in the cytosolic iron-storage protein ferritin. The iron-free transferrin (apotransferrin) is transported, bound to TfR, back to the plasma membrane, where it is released into the circulation to undergo further rounds of iron mobilization and delivery. Some cells, such as liver cells, have a second TfR, TfR2 (REF. 7).

The storage protein ferritin is a 24-subunit protein shell enclosing a hollow interior, in which large amounts of iron are stored in a soluble, non-toxic but bioavailable form, essentially as the mineral phase ferrihydrite[1]. Mammalian ferritins contain variable amounts of two types of polypeptide chain, heavy (H) and light (L). The ferritin subunits have different functions and are encoded by different chromosomes[1]. H chains have a ferroxidase centre, which can catalyse the rapid oxidation of Fe^{2+} to Fe^{3+}, whereas L chains are thought to function in the nucleation of the mineral core within the protein shell. L-rich ferritins are associated with iron storage, whereas H-chain ferritins are associated with responses to stress.

In most cells, changes in iron status (iron overload or depletion) lead to compensating changes in the IRP/iron regulatory element (IRE) system of translational control of iron homeostasis (FIG. 2). When iron is in excess, IRPs are in their inactive form and do not bind to the IREs on the mRNAs of ferritin and TfR. Consequently, the storage protein is synthesized and the TfR mRNA is degraded by nucleases. However, when iron concentrations are limiting, the IRPs bind to the IREs on the mRNAs. Under these conditions, synthesis of the TfR is assured by binding of the IRPs to the IREs in the 3′-untranslated region (UTR), thereby protecting the mRNA from degradation, and IRPs bind to the corresponding IRE in the 5′-UTR in ferritin mRNA to prevent initiation of protein synthesis. Transcriptional control is also involved in regulating the expression of ferritin and TfR, as well as other proteins of iron metabolism, such as cytochrome *b* reductase 1 (DCYTB) (ferric reductase of the apical surface of enterocytes), DCT1 (which is involved in transport of Fe(II) across the apical membrane of enterocytes and of iron out of the endosomal compartment after its release from Tf), and the iron-regulated transporter IREG1 (which is present at the basolateral membrane of enterocytes and is also implicated in the release of iron from macrophages)[1,8,9].

The brain has several characteristics that make it unique among organs with regard to iron metabolism. First, it resides behind a vascular barrier, which limits its access to plasma iron. There is a transport mechanism in the blood–brain barrier (BBB) that moves iron across the endothelial cells and into the brain[10]. However, little is known about the mechanism of iron release into the brain or the regulation of the transport mechanism. Insights into this transport mechanism could be crucial for understanding how an excess of iron can accumulate in the brain in many neurodegenerative diseases. Second, the concentration of iron in various regions of the brain varies greatly. Regions of the brain that are associated with motor functions (extrapyramidal regions) tend to have more iron than non-motor-related regions[11], which might explain why movement disorders are commonly associated with iron imbalance. It is known that at birth these regions have little iron, however, with ageing they accumulate significant amounts, probably through axonal transport. The amount of iron that is present exceeds the amount that is required by the various iron-dependent processes (calculated to be about 5–10% of brain iron). So, the rest of the iron must have some other function.

In the brain, the most common cell type to stain for iron under normal conditions is the oligodendrocyte[12]. However, neurons and MICROGLIA, as well as oligodendrocytes, express ferritin, indicating that all of these cell types have the capacity to store iron. Interestingly, the relative abundance of H- and L-ferritin depends on the cell type[13] — neurons express mostly H-ferritin, microglia express mostly L-ferritin and oligodendrocytes express similar amounts of both subunits. Overall, the levels of H- and L-ferritin in neurons are much lower than in oligodendrocytes[14,15]. Very little ferritin expression is seen in astrocytes, indicating that these cells provide little iron storage.

Large amounts of iron are sequestered in neuromelanin granules in the dopaminergic neurons of the SUBSTANTIA NIGRA and the noradrenergic neurons of the LOCUS COERULEUS[16]. Neuromelanin is synthesized by the oxidation of excess cytosolic catechols that are

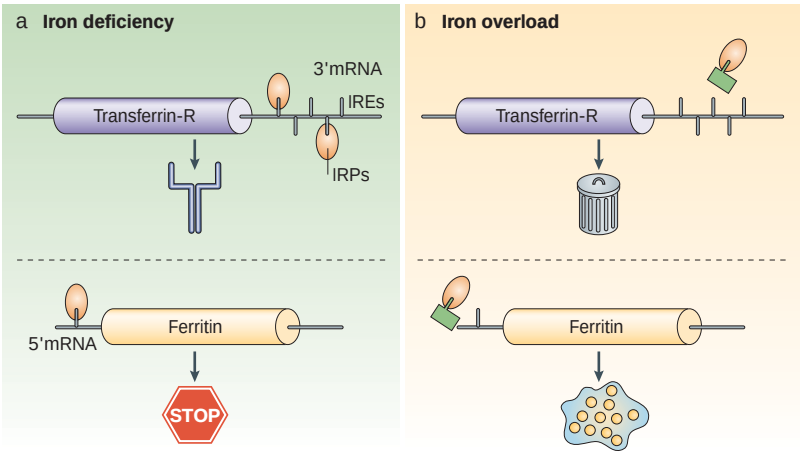

Figure 2 | **Translational regulation of the transferrin receptor and ferritin production.** Production of the transferrin receptor (TfR) and ferritin is regulated at the level of mRNA by iron regulatory proteins (IRPs), which bind to iron response elements (IREs) on the 3′- and 5′- untranslated regions of their respective mRNAs[1]. **a** | In iron deficiency, the IRPs bind to the IREs, protecting the TfR mRNA from nuclease digestion and preventing the synthesis of ferritin. **b** | When iron is abundant, the modified IRP no longer binds to the IREs — in IRP1 the IRE binding site is blocked by a 4Fe–4S cluster (green rectangle), whereas in IRP2 the protein is targeted for destruction in the proteasome — allowing TfR mRNA to be destroyed and allowing the expression of ferritin.

not accumulated in synaptic vesicles by vesicular monoamine transporter-2 (REF. 17). Neuromelanin binds iron avidly, forming stable octahedral complexes that contain high-spin oxy-hydroxide iron (III) clusters (for a review, see REF. 18). Neuromelanin is a complex molecule, the structure of which is arranged as a multilayer system, where each layer is a polymer that is composed of melanic groups bound to aliphatic and peptide chains. This melanic group contains benzothiazine and dihydroxyindole units (ratio 1:3). The dihydroxyindole units are responsible for the strong chelating ability for iron compared to other metals[16,19].

The iron transport protein Tf was originally thought to be synthesized only by oligodendrocytes in the brain[20], although evidence of Tf mRNA has recently been found in neurons in the human substantia nigra[21]. CHOROID PLEXUS epithelial cells and other cells outside the brain that make Tf also secrete it, but it does not seem to be secreted by oligodendrocytes[22]. Three proteins that can transport iron have been shown to bind in the brain. TfRs appear predominantly on neurons, and Tf binding is seen exclusively in grey matter. Lactoferrin reportedly binds to neuromelanin cells, and, recently, ferritin binding has been shown in white matter tracts[21,23].

In summary, the brain is unique among organs because of its non-uniformity of iron distribution, both regionally and cellularly, and because of the apparent selectivity in cellular responsibilities for iron storage and mobilization, and presumably usage and methods of uptake.

Iron accumulation, invasion and reactivity

It is generally accepted that iron accumulates in the brain as a function of age. However, this process is quite specific and involves the accumulation of iron-containing molecules in certain cells, particularly in brain regions that are preferentially targeted in neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD).

Concentrations of non-haem iron (mostly ferritin) increase in the PUTAMEN, motor cortex, prefrontal cortex, sensory cortex and thalamus during the first 30–35 years of life, and variable changes are observed in older individuals[24]. Recent studies have shown that levels of H-ferritin in older individuals (67–88 years of age) were higher than in younger controls (27–66 years) in the frontal cortex, CAUDATE NUCLEUS, putamen, substantia nigra and GLOBUS PALLIDUS. In the case of L-ferritin, this increase is observed only in the substantia nigra and globus pallidus[25,26].

In the parieto-occipital lobe of the cortex, the expression of haem oxygenase-1 and ferritin increases with age, whereas in the hippocampus, only haem oxygenase-1 expression increases. Haem oxygenase-1 is found in both glia and neurons, whereas ferritin is only found in glia. The increase in haem oxygenase-1 might contribute to an increased susceptibility to oxidative stress in older people[27].

Several lines of evidence indicate that in the brains of older individuals iron homeostasis is accomplished more efficiently in the locus coeruleus than in the substantia nigra. A linear increase in iron concentration occurs with age in the substantia nigra, whereas in the locus coeruleus the iron concentration is lower, and remains constant throughout life. In the substantia nigra, H- and L-ferritin concentrations increase with age, whereas in the locus coeruleus both remain lower and invariant[15,26]. In individuals over 80 years of age, many extraneuronal iron deposits are present in the substantia nigra, especially in oligodendrocytes, but few iron deposits are observed in the locus coeruleus. Iron deposits are found in neurons of the substantia nigra that do not contain neuromelanin, but no iron deposits are seen in neuromelanin-containing neurons in either the substantia nigra or the locus coeruleus (FIG. 3). The concentration of the neuromelanin–iron complex in neurons of the substantia nigra and locus coeruleus increases linearly during life, but the slope of the accumulation curve in the substantia nigra is steeper than in the locus coeruleus[15].

When older individuals (60–90 years of age), are compared with younger subjects (28–49 years), more iron staining is observed in the microglia and astrocytes of the cortex, cerebellum, hippocampus, basal ganglia and amygdala, and ferritin immunoreactivity is also stronger in these cells. Oligodendrocytes contain the largest amount of iron, ferritin and Tf, but this content remains constant during ageing[28]. Iron accumulation in microglia might stimulate the activation of these cells in the neuroinflammatory processes that contribute to AD and PD.

All of the above data were from post-mortem tissue, but iron concentrations can also be assessed by non-invasive methods, such as MRI, which has revealed an age-related increase in the non-haem iron concentration in the nucleus caudatus, putamen and globus pallidus[29–31].

CHOROID PLEXUS
A site of production of cerebrospinal fluid in the adult brain. It is formed by the invagination of ependymal cells into the ventricles, which become richly vascularized.

PUTAMEN/CAUDATE NUCLEUS
Two of the components of the striatum, a subpallidal structure that also includes the nucleus accumbens and the olfactory tubercle.

GLOBUS PALLIDUS
The medial part of the lentiform nucleus, which is one of the components of the basal ganglia.

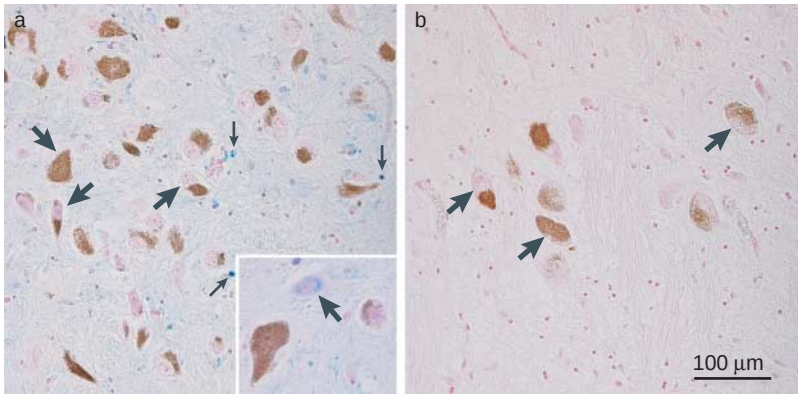

Figure 3 | **Iron deposits in the ageing brain.** Iron histochemistry with modified Perls' staining of human substantia nigra (**a**) and locus coeruleus (**b**) from a normal male subject aged 88. Neuromelanin in dopaminergic neurons of the substantia nigra and noradrenaline neurons of the locus coeruleus are seen as brown granules, and iron deposits are stained blue. Neuromelanin-containing neurons in both the substantia nigra (large arrows in **a**) and locus coeruleus (large arrows in **b**) do not stain blue for iron. In the substantia nigra, there are many iron-positive cells, most of which are oligodendrocytes with cytoplasmic iron deposits (small arrows), and in the locus coeruleus, there are very few oligodendrocytes with light iron staining. Iron deposits are also present in the cytoplasm of non-pigmented neurons of the substantia nigra, as shown at higher magnification in the inset in panel **a** (arrow). Iron deposits can be observed in the whole substantia nigra parenchyma, with the exception of pigmented neurons, but are completely absent in locus coeruleus parenchyma. Reproduced, with permission, from REF. 15 © (2004) National Academy of Sciences USA.

The increased iron concentrations in certain brain regions could result from the altered vascularization that is observed during ageing and in neurodegenerative diseases[32–34]. The role of vascular factors in AD and the common features shared by AD and vascular dementia have recently been discussed[35]. The increase in iron concentrations in neurons, astrocytes and microglia, which normally have low iron contents up to middle age, is typically present in regions such as the cortex, hippocampus and substantia nigra, which are particularly susceptible to the neuropathological changes that characterize AD and PD[36]. This iron 'invasion' and 'perennial occupation' might directly damage these cells or perturb the cellular environment, making it more susceptible to toxins and activation processes of a pathogenic type (for example, inflammation, factor release, morphological change or apoptosis). Moreover, during brain ageing, iron is partially converted from its stable and soluble form (ferritin) into haemosiderin and other oxyhydroxide derivatives that contain iron at higher reactivity (for a review, see REF. 1). So the prelude of the pathogenic role of iron in brain ageing can be summarized in this triad: iron accumulation, invasion and increased reactivity.

Iron and neurological disease

Parkinson's disease. Several authors have reported an increase in total iron concentration in the substantia nigra in the most severe cases of PD, but no changes in milder cases[37–39] (for a review, see REF. 40). Other studies have reported no increase in total iron concentration in the substantia nigra of patients with PD, probably because of methodology issues and the different disease stages of the patients[41,42]. An increase in iron content in the lateral globus pallidus in comparison to the medial globus pallidus was found, and this might be indicative of retrograde degeneration of dopaminergic neurons in PD[43,44]. There is also a significant inverse relationship between dopamine concentration and iron concentrations in the putamen, but not in the substantia nigra, of patients with PD, supporting the concept of a retrograde degenerative process[45].

Iron (III) deposits were found in microglia, oligodendrocytes, astrocytes located close to neurons, pigmented neurons and in the rim of LEWY BODIES in the substantia nigra pars compacta of patients with PD. A similar picture of iron accumulation in glia and neurons was found in the putamen and pallidum of the same patients. Numbers of ferritin-loaded microglia are increased in the substantia nigra of individuals with PD, and reactive microglia are often associated with degenerating and neuromelanin-loaded dopaminergic cells[36] (FIG. 4). In control patients with presymptomatic PD, which is indicated neuropathologically by neuronal loss and the presence of Lewy bodies, there was no evidence of total iron increase[46]. However, even in this case, iron mismanagement might have occurred through intracellular (for example, from cytosol to mitochondria) and intercellular (for example, from oligodendrocytes to neurons) translocation.

Various methods[16,47] have provided compelling evidence for the accumulation of iron in pigmented neurons — in particular, in the form of a neuromelanin–iron complex (for a review, see REF. 40). Synthetic dopamine–melanin and neuromelanin both bind iron to a high degree and in a saturation-dependent manner[48,49]. Neuronal cytoplasmic iron is seen at low concentrations in the brains of patients with PD[50], because neuromelanin is a strong iron chelator and scavenges cytoplasmic iron. The neuromelanin–iron complex activates microglia *in vitro*, leading to the release of neurotoxic compounds such as tumour necrosis factor-α, interleukin-6 and nitric oxide. In PD, although different toxic or genetic mechanisms can initiate neuronal damage in the substantia nigra, the neuromelanin that is released by dying neurons can induce release of neurotoxic microglial factors, potentially leading to aggravation of neurodegeneration[51,52].

MRI can be used to detect an augmented brain iron content in living patients with PD that correlates with measurements from post-mortem tissues[53,54]. Transcranial sonography, a newly developed technique that enables two-dimensional visualization of the brain parenchyma through the intact skull, shows an increased area of echogenicity in the substantia nigra of patients with PD, which correlates with iron content[55].

An increase in redox-active iron associated with neuromelanin occurs in substantia nigra neurons in PD. This increase is higher in patients that have the most severe neuronal loss, and it is not found in the immediate vicinity of melanized neurons, providing further evidence for a central role for neuromelanin in modulating iron reactivity[56]. PD is also associated with increased haem oxygenase-1 immunoreactivity in the neuropil of the substantia nigra, and intense immunostaining in Lewy bodies in affected dopaminergic neurons[57]. In patients with PD, the expression of the divalent cation transporter DCT1 is augmented in substantia nigra dopaminergic neurons[58].

LEWY BODIES
Intraneuronal inclusion bodies that form one of the pathological hallmarks of Parkinson's disease. They consist of a dense granular core that is surrounded by a halo of radiating filaments. Their main protein components include α-synuclein and ubiquitin.

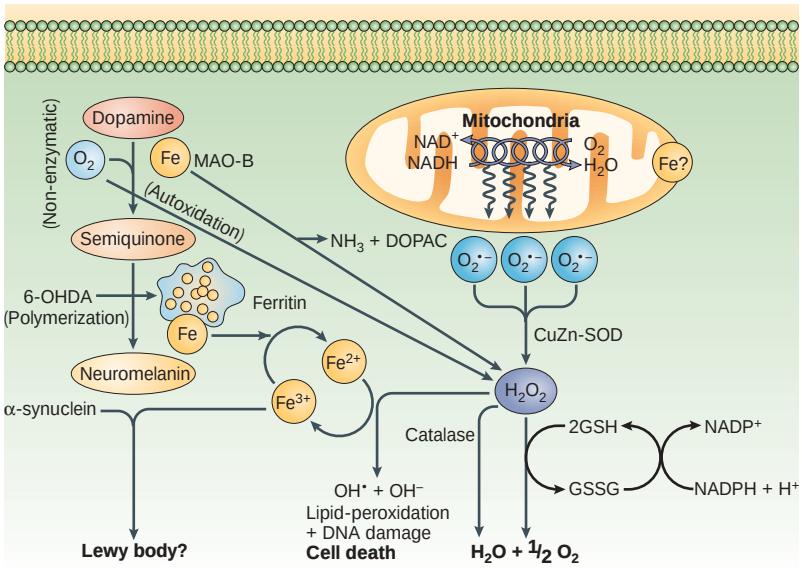

Figure 4 | **Iron and oxidative stress hypothesis of Parkinson's disease.** This scheme summarizes pathochemical findings in Parkinson's disease and attempts to explain possible synergistic cascades, such as developing cell-death mechanisms. The model is based on post-mortem findings, which indicate reduced mitochondrial complex I activity; loss of reduced glutathione (GSH); increased iron concentrations; an increase in oxidative stress markers in the substantia nigra; an increase in dopamine turnover and loss of dopamine in the striatum; α-synuclein pathology, and Lewy-body generation and membranal degeneration in neurons. Similar mechanisms involving iron-induced and hydrogen peroxide-induced oxidative stress have been put forward for other neurodegenerative diseases. CuZn-SOD, copper and zinc-containing superoxide dismutase; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide, MAO-B, monoamine oxidase B; NAD, nicotinamide adenine dinucleotide; $OH^\bullet$, hydroxyl radical; $.O_2^{\bullet-}$, superoxide radical anion; OH^-, hydroxide anion; 6-OHDA, 6-hydroxydopamine. Figure drawn using information from REF. 116.

The mechanism that underlies increased iron concentrations in the substantia nigra is not known. It seems to be independent of the Tf/TfR system, as there is a significant decrease in TfR density on melanized neurons of the substantia nigra of patients with PD[59].

The main iron-storage protein, ferritin, shows a significantly increased iron load in PD[43]. Other studies showed an increase of lactoferrin receptors on substantia nigra neurons and microvessels, and an increase of lactoferrin content in neurons of patients with PD[60,61]. However, this generalized increase of the lactoferrin system could be secondary to iron accumulation in the substantia nigra. In the substantia nigra of patients with PD, an upregulation of ferritin expression in response to iron increase has been reported[36] but other studies did not support this finding[25,44,62].

Elevated iron concentrations in the substantia nigra might result from mutations in genes that are relevant to iron transport and binding. One example is the G258S Tf polymorphism — a higher frequency of the G allele was found in patients for whom the onset of PD occurred after 60 years of age, in conjunction with a negative family history[63]. Another potential source of increased iron is from peripheral influx through a disturbed or open BBB in the substantia nigra. To investigate this possibility, Leenders[64] recently used radiolabelled verapamil hydrochloride and positron-emission tomography (PET) in patients with PD and age-matched healthy controls. Verapamil is a specific substrate for the P-glycoprotein (Pgp) multidrug resistance system in the cell membrane. Pgp functions as an efflux pump, and verapamil does not cross the BBB. They found a high level of uptake of verapamil in the mesencephalon of patients with PD but no uptake in controls. Therefore, the Pgp system of the BBB might not work well in certain brain regions in PD, thereby rendering the brain accessible to serum iron.

What mechanisms might underlie iron-induced cell damage in PD? Mutations in α-synuclein cause a form of familial PD, and wild-type α-synuclein is an important component of Lewy bodies. Abnormal filamentous aggregates of misfolded α-synuclein protein are the main components of Lewy bodies with iron deposits in the rim, perhaps indicating a pathogenic role for α-synuclein in PD. So far, little is known about the importance of α-synuclein in the nigral–dopaminergic pathway in either normal or pathological situations. In mice, α-synuclein is highly expressed in the nigrostriatal pathway and substantia nigra, and together with iron, is upregulated following treatment with the neurotoxin MPTP (*N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)[65]. The consequence of increased cellular iron is degradation of IRP2 through the UBIQUITIN pathway[66]. Radical scavengers, such as R-apomorphine, and the iron chelators deferoxamine mesylate (Desferal, Novartis) and epigallocatechin gallate (EGCG), induce neuroprotection and reverse these effects[66].

Iron contributes to the enhanced generation of REACTIVE OXYGEN SPECIES (ROS), and increases oxidative stress and protein aggregation, including the aggregation of α-synuclein, and aggregations that lead to the formation of advanced glycation end products[67–69]. The studies of Hashimoto[70] indicate that iron-catalysed oxidative reactions mediated by cytochrome *c*/hydrogen peroxide might be crucially involved in promoting α-synuclein aggregation, a process that is inhibited by desferrioxamine. In the presence of iron and free-radical generators, such as dopamine or hydrogen peroxide, BE-M17 human neuroblastoma cells that overexpress wild-type, A53T or A30P α-synuclein produce intracellular aggregates that contain α-synuclein and ubiquitin[67]. This is prevented by the action of desferrioxamine. Such aggregates disturb the cytosolic environment and interact with vesicles and their dopamine transporters and intraneuronal mitochondria, and these disturbances might result in activation of cell-death cascades. However, the question of whether α-synuclein is neurotoxic or neuroprotective is still open, as the formation of Lewy bodies and accumulation of α-synuclein could also be a compensatory process to enable the neuron to protect itself.

Alzheimer's disease. Accumulation of iron in the brain, particularly in cells that are associated with neuritic plaques, is a consistent observation in AD and has been extensively investigated[71]. In the brains of patients with AD, iron accumulation occurs without the normal age-related increase in ferritin[72], thereby increasing the risk of oxidative stress[73]. Recent evidence indicates that ageing and AD are associated with a decline in myelin, and this

UBIQUITIN
A molecule that is attached to lysine residues of other proteins, often as a tag for their rapid cellular degradation by the proteasome.

REACTIVE OXYGEN SPECIES
(ROS) Oxygen radicals that are produced by the mitochondrial respiratory chain. In excess, they can cause intracellular and mitochondrial damage, which promotes cell death.

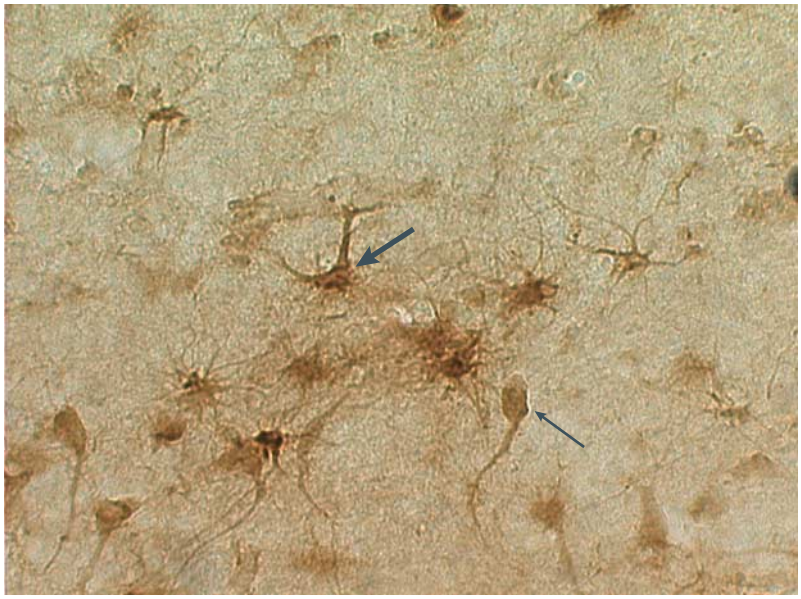

Figure 5 | **Haemochromatosis protein staining of reactive astrocytes in brains of patients with Alzheimer's disease.** Brain tissue from a patient with Alzheimer's disease was immunostained for the haemochromatosis (HFE) protein. In this micrograph from the superior temporal gyrus, a number of immunoreactive astrocytes are visible. These cells appear brown in the micrograph, and the cell bodies and short branching processes are visible. Some of the astrocytes (for example, that indicated by the large arrow) are associated with blood vessels. In addition to the astrocytes, a number of dystrophic neurons (for example, that indicated by the small arrow) can also be seen in the microscopic field.

could be related to the iron content of the myelin[74]. Iron might also have a direct impact on plaque formation through its effects on amyloid precursor protein (APP) processing[75]. Furthermore, the ability of α-secretase to cleave APP can be modulated by iron[76]. Iron seems to promote both deposition of amyloid-β (Aβ) and oxidative stress, which is associated with the plaques[77–79], although some have argued that by binding iron, Aβ might, in fact, protect the surrounding neurons from oxidative stress[80]. Therefore, understanding the cellular mechanisms for regulating iron could be fundamentally important for understanding the biological basis of AD. Chelators for metals such as iron that have redox potential are being developed and considered for the treatment of AD (see below)[81–84].

A link between congenital iron overload (haemochromatosis, or HFE1) and AD has recently become apparent. The HFE protein is found in blood vessels in the brain and the choroid plexus, and is expressed by cells associated with neuritic plaques[85]. HFE is also expressed by reactive astrocytes in the brains of patients with AD (FIG. 5), as well as by neurons. The pattern of neuronal staining for HFE in AD might indicate that HFE is induced by stress, and neurons that stain for TAU are also HFE-positive[86]. Mutations in the HFE gene that are associated with this iron-overload disorder[87] — typically C282Y and H63D — are found at a higher carrier frequency in people of European ancestry than phenylketonuria and cystic fibrosis combined. The presence of the HFE mutation in AD strongly supports the idea that iron imbalance in the brain contributes to AD, and its prevalence indicates that it could be an important risk factor for AD[88]. HFE mutations are associated with increased oxidative stress and severity of disease, as assessed neuropathologically[89,90], providing further support for the idea that iron underlies the increase in oxidative stress that promotes neurodegeneration in AD.

The transferrin subtype C2 was also found with increased frequency in patients with AD when compared with age-matched controls[91–93]. Patients with AD who are homozygous for the apolipoprotein E isoform E4 (APOE4) allele are twice as likely to have a TfC2 allele than those without, or with only a single copy of, APOE4 (REF. 91,94). The presence of the C2 variant plus an HFE mutation increased the risk of AD five-fold, and this risk was increased even more in the presence of APOE4 (REF. 95). There is no significant difference between TfC2 and other Tf variants in their ability to bind iron[92], so the impact of the TfC2 mutation on AD is probably not mediated through the ability of Tf to deliver iron to the brain or to neurons. The combined data on Tf and HFE mutations clearly indicate that genetic alterations specific to iron-management proteins can increase the risk of AD, and they provide strong evidence that iron mismanagement in the brain can contribute to AD. To date, there is an ongoing investigation into the relationship between HFE mutations and AD, as some studies have found an association with AD and others have not.

Other neurological diseases. Iron, particularly its accumulation, has been implicated in a series of other neurological diseases[96] and some of these are discussed briefly here.

Iron and copper are intimately linked through the blue, multicopper protein of plasma, ceruloplasmin[97]. In congenital aceruloplasminaemia — a condition that is characterized by extrapyramidal symptoms, ataxia and progressive CNS and retinal neurodegeneration — mutations in the ceruloplasmin gene cause the protein to be absent from the plasma[98–100]. Although this does not affect copper transport, severe iron loading is found in parenchymal tissues including the brain, particularly in the basal ganglia. The implications are that ceruloplasmin is required for iron mobilization from cells[101].

Friedreich's ataxia is the most common of the early-onset inherited ataxias, and it is characterized by degeneration of the large sensory neurons and spinocerebellar tracts, and cardiomyopathy[102]. The disease is caused by a substantial reduction in the concentration of the mitochondrial protein frataxin, which is provoked by a large GAA triplet-repeat expansion in the first intron of the frataxin gene, resulting in a reduction in frataxin expression by transcription inhibition[103]. This leads to increased mitochondrial iron content, which seems to reflect the vital role of frataxin in iron–sulphur cluster biosynthesis[104,105]. Some success has been achieved in retarding the disease-associated cardiomyopathy using the ubiquinone analogue 2,3-dimethoxy-5-methyl-6-(10-hydroxydecyl)-1,4-benzoquinone[106] (Idebenone, Takeda Chemical Industries).

TAU
A neuronal protein that binds to microtubules, promoting their assembly and stability. It is also a component of neurofibrillary tangles, which are one of the pathological hallmarks of Alzheimer's disease.

Neuroferritinopathy is a dominantly inherited, late-onset basal-ganglia disease, which is caused by a single adenine insertion into the gene for L-chain ferritin. This is thought to alter the carboxyl terminus of the protein[107]. Iron deposition in the basal ganglia, abnormal aggregates of ferritin and iron in the globus pallidus and substantia nigra, together with low serum ferritin concentrations, are found in affected patients[108]. The clinical symptoms include choreoathetosis, DYSTONIA, spasticity and rigidity. Another mutation — a 2-base-pair insertion in exon 4 of the L-chain ferritin gene that causes changes in the amino-acid sequence and length of this polypeptide — was also described. Patients with neuroferritinopathy had abnormal ferritin accumulation in neurons and glia of the striatum and cerebellar cortex, along with severe neuronal loss. Clinically, they were affected by movement disorders and cognitive decline[109].

Mutations in the gene that codes for pantothenate kinase 2 (PANK2) have been shown to be the main genetic defects associated with neurodegeneration with brain iron accumulation (NBIA — formerly known as Hallervorden–Spatz syndrome)[110,111]. This autosomal recessive disease, which is characterized by dystonia and pigmentary retinopathy in children and speech or neuropsychiatric defects in adults, has a characteristic MRI pattern in the globus pallidus, known as 'the eye of the tiger' because of its appearance[111]. Pantothenate kinase is necessary for coenzyme-A biosynthesis, and it is targeted to mitochondria. It is proposed that accumulation of cysteine, which chelates iron, causes oxidative stress and leads to the accumulation of iron in the basal ganglia.

Iron deficiency during development can result in life-long cognitive and motor impairment[112]. Restless legs syndrome (RLS) has been identified as a neurological disorder in adults that seems to be associated with decreased iron in the substantia nigra and a possible defect in IRP1 (REF. 21). The symptoms of RLS are responsive to dopaminergic agonists[113].

Animal models and therapeutic approaches

Recent genetic analyses of iron overload that is confined to the brain, together with biochemical studies, have placed iron at the centre of research into neurodegenerative diseases (for reviews, see REFS 84,114–116). These genetic mutations will help us to delineate the molecular mechanisms through which misregulation of iron metabolism can lead to neurodegeneration. Abnormal accumulation of iron is thought to participate in the induction of toxic ROS, which can attack neurons and induce neurodegeneration[117]. Adult mice with a knockout of the *Irp2* gene accumulate iron and ferritin in white matter tracts and the striatum, and these animals exhibit a movement disorder consisting of ataxia, tremor and bradykinesia[118]. Iron chelators and free-radical scavengers that cross the BBB might be able to mobilize iron out of the brain and prevent the damage that is caused by ROS.

Neurotoxins such as 6-hydroxydopamine, MPTP, L-methampetamine and kainate have been used in animals to generate models to determine the mechanism of neurodegeneration in PD and Huntington's disease. These molecules initiate neuronal death through a mechanism that involves oxidative stress, which results from accumulation of the divalent metal transporter DMT1 and iron at sites where neurodegeneration occurs[116,119,120]. This leads to depletion of reduced glutathione (GSH) and accumulation of cytotoxic ROS, most notably the hydroxyl radical that is generated by the interaction of iron and hydrogen peroxide[45,121,122]. However, genomic and proteomic profiling of neurotoxin-induced neurodegeneration have indicated more complex mechanistic features that involve a cascade of 'domino' events, among which are oxidative stress, inflammatory processes and iron misregulation[123].

An important neuropathological feature of neurotoxin-induced neurodegeneration is an increase in iron concentrations, which has been observed in the substantia nigra pars compacta of 6-hydroxydopamine- and MPTP-lesioned animals (rats, mice and monkeys)[119,124,125], and in hippocampal neurons of kainite-treated rats and mice[120,126]. It has not been possible to determine whether the accumulated iron is in the labile ionic pool (free form), which can participate in the Fenton reaction with hydrogen peroxide to generate the reactive hydroxyl radical. It has been suggested that this process cannot take place unless GSH peroxidase, the main enzyme that is responsible for breaking down hydrogen peroxide in the brain, is inactive[127]. The reduction in GSH (the rate limiting co-factor of GSH peroxidase), which occurs in PD[37] and in response to 6-hydroxydopamine and MPTP, together with increased iron accumulation and reactive hydroxyl-radical generation, might be taken as evidence for the presence of free iron. Indeed, it is the free form of iron that initiates depletion of cellular GSH[127]. If cells are depleted of GSH, the release of iron is accompanied by oxidative stress. GSH depletion by antimetabolites does not induce ferritin-bound iron release or accumulation. Therefore, the fate of the cell in which iron release and accumulation occurs seems to depend on intracellular GSH.

The mechanism by which neurotoxins increase iron concentrations is not known, but it must be associated with an alteration in the brain-iron-uptake process, as in normal circumstances serum iron does not cross the BBB. Indeed, kainate increases expression of DMT1 in the mouse hippocampus[120]. It remains to be seen whether 6-hydroxydopamine and MPTP have similar effects.

There is evidence that increased iron participates in the neurodegeneration process from studies where intraventricular pretreatment with a brain-impermeable prototype iron chelator, desferrioxamine, was shown to protect against 6-hydroxydopamine and MPTP in rats and mice, respectively[119]. These results have now been substantiated with systemic injection of a brain-permeable iron chelator, VK-28 (Varinel Inc.) (REF. 83), and the copper–iron chelator iodochlorhydroxyquin[128] (Clioquinol, Prana Biotechnology Limited

DYSTONIA
A movement disorder that is characterized by abnormal muscle tone.

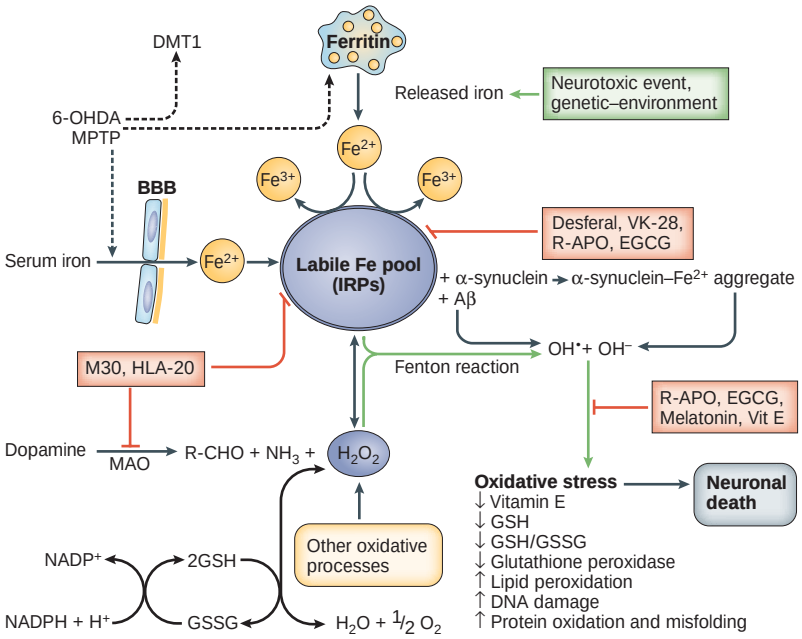

Figure 6 | **Iron-induced neurodegeneration in Parkinson's disease and its prevention.** Neurodegeneration can result from iron transport across the blood brain barrier (BBB), release from ferritin, the generation of reactive hydroxyl radicals (OH•) and induction of oxidative stress in substantia nigra pars compacta dopamine neurons. Iron is transported across the BBB by the divalent metal transporter (DMT1). Serum iron cannot cross the BBB if it is formed normally. Neurotoxins such as kainate, 6-hydroxydopamine (6-OHDA) and MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) induce accumulation of iron at sites where they initiate neurodegeneration. This might result either from increased uptake of iron through DMT1, or its release from ferritin. Iron is thought to enter a labile pool (ionic iron), where it is accessible to participate in the Fenton reaction with hydrogen peroxide (H_2O_2), which is generated by dopamine metabolism by MAO (monoamine oxidase), auto-oxidation and other oxidative processes. The Fenton reaction produces reactive hydroxyl radicals (OH•). The resulting effect is oxidative stress as a consequence of depletion of cellular antioxidants (vitamin E, reduced glutathione (GSH)), reduction of GSH/GSSG (oxidized glutathione) and possibly glutathione peroxidase activity, and increased membrane lipid peroxidation, DNA damage and protein oxidation and misfolding. Labile iron can also cause aggregation of α-synuclein and amyloid-β (Aβ) to form toxic aggregates, which, in turn, can initiate OH• generation, causing oxidative stress. Neuroprotective agents that can be used to prevent iron-induced neurodegeneration include M30 and HLA-20 (bifunctional iron chelator-MAO inhibitors): desferal, VK-28, R-APO (R-apomorphine) and EGCG (epigallocatechin gallate) (iron chelators); R-APO, EGCG, melatonin and Vit E (vitamin E) (radical scavengers). IRP2, iron regulatory protein 2 (REFS 37,84,114,116).

(US and Japan)/P.N. Gerolymatos S.A. (Europe and other territories)) in mice and rats in response to 6-hydroxydopamine and MPTP, respectively. These results might explain why antioxidant-radical scavengers, such a vitamin E, lipoic acid, melatonin, ebselen and the polyphenol EGCG, are protective against neurotoxins[129–131]. In the MPTP model, iron chelation by pharmacological means or a ferritin transgene is protective against MPTP neurotoxicity[128], and nutritional iron deficiency protects rats against kainate and 6-hydroxydopamine[126].

Evidence to link abnormal metal (iron, copper and zinc) metabolism and handling with AD pathology has repeatedly been reported. However, unlike the animal models for PD, where the role of iron in dopaminergic neurodegeneration has been investigated, there have been few such studies with transgenic AD models. Nevertheless, iron has taken centre stage in AD as a consequence of the studies by Rogers and Lahiri[132]. The presence of an IRE in the 5′-UTR of the APP transcript provided the first molecular biological support for the current model that APP is a metalloprotein. The involvement of metals in the plaques of patients with AD, and the demonstration of metal-dependent translation of APP mRNA, together with the role of iron in PD, have encouraged the development of chelators as a new therapeutic strategy for the treatment of these and other neurodegenerative disorders that involve iron misregulation.

Metal chelation as a neuroprotective strategy

Genetic and biochemical manipulation of iron have indicated that iron has a pivotal role in neurotoxicity and neurodegeneration in animal models of neurodegenerative diseases. This is supported by the discovery of genetic and non-genetic misregulation of iron metabolism in these disorders. The neuroprotection that is seen with iron chelators in the animal models indicates that iron-chelation therapy could be a viable neuroprotective approach for treating PD, AD and other neurological disorders that are associated with abnormal iron metabolism in the brain[117].

Chelation has the potential to prevent iron-induced ROS, oxidative stress and aggregation of α-synuclein and Aβ, and the limited *in vitro* and *in vivo* neuroprotective studies that have been carried out so far seem to support this idea. This approach is not unprecedented: the copper chelator D-penicillamine (Cuparamine, Merck) has been used successfully for the removal of copper in WILSON'S DISEASE, and chronic desferal treatment for aceruloplasminaemia was shown by functional MRI to remove iron and improve the neurological aspects of the disorder[131]. Some success has also been reported with the copper chelator clioquinol in AD[82]. Unfortunately, however, clioquinol is highly toxic, and desferal has poor penetration across the BBB.

Non-toxic lipophilic brain-permeable iron chelators offer potential therapeutic benefits for progressive neurodegenerative diseases. Compounds such as VK-28 (REF. 83) and the bifunctional iron chelators HLA-20 and M30 (REFS 84,133), which possess the propargylamine monoamine oxidase inhibitory and neuroprotective moiety of the PD-therapeutic drug N-propargyl-1-(R)aminoindan[134] (Rasagiline, Teva Pharmaceutical Industries Ltd) (FIG. 6), offer a potential therapeutic solution to the neurotoxicity that is induced by divalent metal misregulation. Preliminary studies in animals[83,119,128] have not shown any interference with neurotransmitter metabolism or nicotinamide adenine dinucleotide (NADH) oxidoreductase (complex I) activity, but the use of iron chelators must be carefully controlled to avoid toxic effects[135]. One possible non-toxic approach for treating AD and PD with metal chelators could make use of the polyphenols EGCG and curcumin (a constituent of turmeric). Both compounds have antioxidant, iron chelating and anti-inflammatory activies, are neuroprotective in animal models of PD and AD, and also regulate the processing of APP through a non-amyloidogenic pathway[131,136].

WILSON'S DISEASE
A genetic disorder that causes excessive copper accumulation in the liver and brain, resulting in hepatitis and psychiatric and neurological symptoms.

Conclusions

During ageing, the total iron concentration increases in some brain regions that are targeted by degenerative diseases such as AD, PD and Huntington's disease. This inability to regulate iron homeostasis generates a surplus of reactive iron, which invades cells such as astrocytes, microglia and neurons. Understanding the timing of iron mismanagement in relation to the progression of neuronal loss would provide important information on pathogenesis, and would raise the possibility of monitoring iron changes as a marker of disease progression, and perhaps even pre-clinical diagnosis in conditions where iron misregulation is an early event. It is possible that iron accumulation is purely a consequence of neuronal loss and substitution by cells with higher iron content, or a breakdown of the BBB that allows more iron to access the brain.

More data is needed on the age trend of iron concentrations in different brain regions and the cellular distribution of iron molecules in the same individuals. This is a necessary baseline to understand disease-related changes that involve iron. Information on iron is available only in a few brain regions from different individuals, and complete maps of individual patients are not available. Moreover, data on iron in some areas that are seriously impaired in AD and PD are still missing. The relaxation rate values of MRI studies have been correlated with approximate ferritin accumulation from a 1958 study that was carried out using a non-validated method[24]. Other *in vivo* imaging techniques, such as transcranial ultrasonography, could, with adequate technological improvement, provide good descriptions of iron accumulation in the substantia nigra and basal ganglia[55]. *In vivo* studies with PET using ^{52}Fe molecules that cross the BBB could describe physiological iron pathways and altered iron metabolism in the living human brain[137]. These imaging techniques could enable the early detection of iron misregulation/accumulation and pre-clinical diagnosis of neurodegenerative diseases.

More information on proteins and other molecules that are involved in iron metabolism is also needed. The development of non-toxic and more selective iron chelators could allow the removal of potentially harmful iron deposits in older people. More *in vitro* and *in vivo* studies should be done to investigate how iron misregulation/accumulation synergizes with common endogenous and environmental toxins, because this is probably the most frequent pathogenic mechanism in sporadic AD and PD. Iron misregulation/accumulation alone can kill neurons only in genetic disorders where iron imbalance occurs rapidly and is extensive. However, in more than 95% of AD and PD cases, the amount of iron build-up is relatively low and the neurotoxic mechanism might involve a combination of iron and other toxins.

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Competing interests statement

The authors declare competing financial interests: see Web version for details.

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