The iron brain: Post-mortem and in vivo imaging of iron in brain diseases

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Introduction

The brain produces our thoughts, stores our memories, and make us feel and experience; it is the most complex organ of the human body. As our brain ages, people start to experience cognitive decline and the risk of developing a neurodegenerative disease or dementia is increasing. However, the brain is not uniformly affected by the ageing process, nor are individuals. The biological mechanisms behind these diseases are complex and as yet not understood. Over the last decades, many unifying hypotheses were brought up on common mechanisms underlying neurodegenerative diseases, ranging from disturbances in protein aggregation, vascular function and energy metabolism or faults in the brain inflammatory response, to name a few.

Accumulation of iron in the brain is also one of these phenomena occurring in many neurodegenerative diseases. Iron deposition has recently gained attention as a potential driving factor in brain ageing and neurodegeneration. This is largely due to empirical evidence from a novel technique allowing the quantification of iron *in vivo* in the human brain: susceptibility-based magnetic resonance imaging (MRI). Using this technique it was shown that iron is essential for normal brain development and brain function, but also accumulates with age and disease. Iron accumulation is associated with disease state and speed of progression. However, there is still a large gap between the MRI observations and the interpretation of these observed changes in terms of the microscopic and molecular nature of iron in the brain, let alone the mechanistic role of iron in the processes of ageing and disease.

In this introductory chapter iron homeostasis and iron accumulation during ageing and neurodegeneration will be explained in more detail. Then, the potential of susceptibility-based MRI, such as T_2^*-weighted MRI, as a proxy and promising imaging

biomarker for iron is described. Important gaps in knowledge about iron imaging with MRI and the role in neurodegeneration will be introduced. Finally, the overall aim of the thesis and a short introduction of the various chapters will be given.

1.1. IRON IN THE BRAIN

Iron plays a crucial role in an abundance of cellular processes in the brain, including oxygen transport, mitochondrial respiration, and the synthesis of myelin, DNA, and neurotransmitters. Both iron excess and deficiency are detrimental and can lead to impaired brain functioning [1, 2]. Therefore, brain iron homeostasis is tightly regulated to provide optimum conditions for cell function and to prevent the brain from toxic effects caused by excessive concentrations of free iron.

Within the brain, the homeostatic balance of iron is regulated through several processes comprising iron transport, uptake, storage and export (Fig. 1.1). Iron transport across the blood-brain barrier (BBB) in physiological conditions relies on uptake of transferrin, a high-affinity iron-binding protein [3], and is regulated by the transferrin receptor 1 (TFR) [4, 5]. As TFR is highly expressed on the luminal side of endothelial cells of the blood-brain barrier, entry of iron into the brain is controlled by binding of transferrin-bound iron to the TFR [4]. Subsequently, iron is released into the extracellular compartment, allowing uptake by other cells. Iron is continuously moving between neurons, microglia, astrocytes and oligodendrocytes, depending on these cells needs [1]. The exact mechanisms of iron import and release, however, are not fully understood. Both neurons and microglia express TFR, facilitating iron import through a transferrin-TFR complex [6]. Import of iron through a non-transferrin-bound-iron transport also exists and is facilitated by divalent metal transporter 1 (DMT1) [5]. Astrocytes are ideally positioned for iron uptake, because the perivascular end-foot processes ensheath the blood-brain barrier [7]. As astrocytes are devoid of TFR, iron uptake is probably regulated through DMT1 and exported through ceruloplasmin, a ferroxidase capable of oxidizing ferrous iron to ferric iron [8].

Iron can be stored in ferritin in a soluble, non-reactive form [9, 10]. Ferritin is predominantly found in oligodendrocytes, which import large amounts of iron for axon myelination via the ferritin receptor Tim2 [11]. However, iron stored in ferritin is also found in neurons, astrocytes and microglia. The major exporter of iron out the cell is the transmembrane protein ferroportin, which predominantly facilitates the export of iron in the form of ferrous iron [12]. Finally, hepcidin controls iron export by binding to ferroportin resulting in internalization and degradation of the receptor-ligand complex [13].

In healthy aging, the iron levels are increased in the substantia nigra, striatum and cortices [14–16], and are thought to be a consequence of increased blood-brain bar-

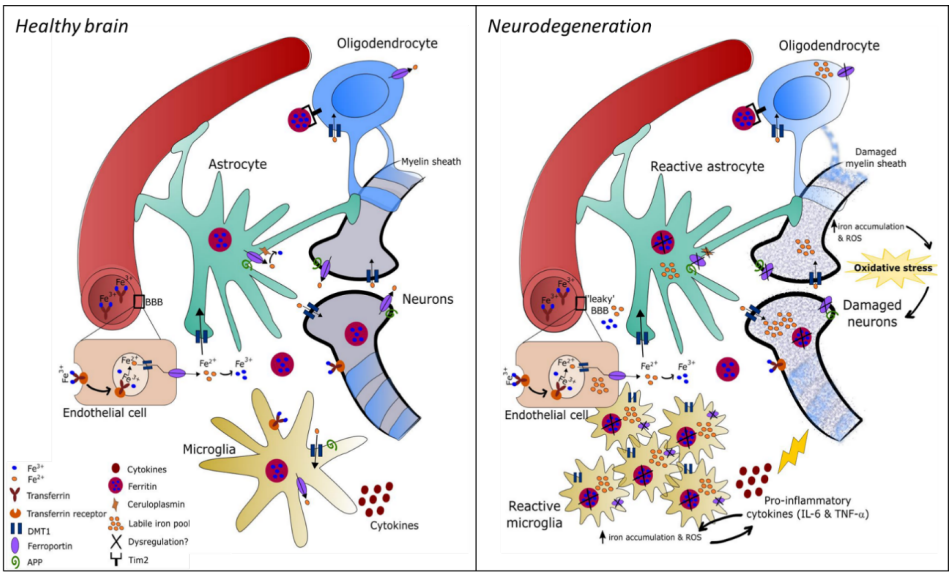

Figure 1.1: **Iron homeostasis in a healthy brain and iron dysregulation during neurodegeneration.** Figure adapted from Daglas et al. 2018 [24]

rier permeability, inflammation, and iron dyshomeostasis [17, 18]. Interestingly, the amount of iron accumulation does not only vary among brain regions, but also among cell types. Neurons, astrocytes and microglia are known to accumulate iron during life, whereas iron levels in oligodendrocytes remain stable [19]. When the storage capacity of proteins or other iron-binding molecules is reached, iron levels in the labile iron pool can increase consisting of increased availability of chelatable and redox-active iron [20]. A well-known consequence of excess iron is oxidative stress by the generation of reactive oxygen species (ROS). ROS can further induce iron release from iron storage proteins, which can lead to further ROS production via the Fenton reaction [21]. Eventually, iron toxicity can lead to apoptosis and ferroptosis, an iron-specific form of non-apoptotic cell death [22, 23].

1.2. IRON AND NEURODEGENERATION

Iron accumulates in disease-specific regions to a much greater extent than during healthy ageing, as is observed in many neurodegenerative diseases including Alzheimer's disease (AD) [25, 26], Huntington's disease (HD) [27], Parkinson's disease [28, 29], frontotemporal dementia [30], corticobasal degeneration, and progressive supranuclear palsy [28, 31, 32]. The potential of iron accumulation as a contributing factor to neurodegeneration in these diseases is demonstrated by studies investigat-

ing a class of diseases termed neurodegeneration with brain iron accumulation (NBIA) [33, 34]. Excessive brain iron accumulation is caused by mutations that target proteins directly implicated in iron metabolism, as for example ceruloplasmin in aceruloplasminaemia, resulting in impaired cellular iron efflux and thus iron accumulation [33]. As these studies show that iron can cause neurodegeneration, it is very plausible that iron accumulation in neurodegenerative diseases also contributes to disease progression.

In AD, the most frequent cause of dementia, the pathological hallmarks amyloid beta ($A\beta$) and hyperphosphorylated tau are still considered key mediators in AD pathogenesis [35]. However, changes in iron metabolism might also play a role and are shown to be increased in cortical, subcortical and white matter regions affected by the diseases [36, 37]. Also, high concentrations of iron are found to be present in $A\beta$ plaques, neurofibrillary tangles and the surrounding glial cells [38–40]. Evidence from in vitro studies suggests that increased iron concentrations accelerate $A\beta$ and hyperphosphorylated tau formation and increases the toxicity of these proteins [41]. Further supportive evidence comes from a longitudinal clinical study showing that levels of ferritin in the cerebrospinal fluid of AD patients are negatively associated with cognitive performance and predict the conversion from mild cognitive impairment to AD [42].

Increases in iron levels in disease-specific regions are also reported in diseases with a clear monogenetic origin. In HD, a rare autosomal dominant inherited progressive neurodegenerative disorder [43], the largest increase of iron is reported in the basal ganglia and more specifically in the striatum [44–46]. Interestingly, iron levels were found to be already increased in asymptomatic gene-carriers and are subsequently shown to correlate with the severity of the disease [47, 48].

In addition, we know that the pathogenesis of many neurodegenerative diseases is not restricted to the neuronal compartment, but includes strong interactions with immunological mechanisms in the brain. This pathomechanism, also known as neuroinflammation, is evident in both AD and HD brains through the observation of increased numbers of activated microglia in disease-specific regions [49–51]. Whereas in AD clusters of activated microglia are found in the vicinity of $A\beta$ plaques in the cortex [51, 52], microglia and astrocytes are found in regions with neuronal loss in HD brains [53, 54]. Genome-wide analysis suggests that several genes encoding for immune receptors are associated with AD and increase the risk of sporadic AD [55–57]. Also in HD, microglia activation is already evident in gene-carriers without any symptoms suggesting that neuroinflammation has probably not only a passive role but might be a key player early in the pathogenesis [54]. Also systemic inflammation, both acute and chronic, is suggested to interfere with immunological processes of the brain, thereby inducing or contributing to disease progression [58].

Microglia often accumulate iron, as has been shown in both AD and HD [39, 59].

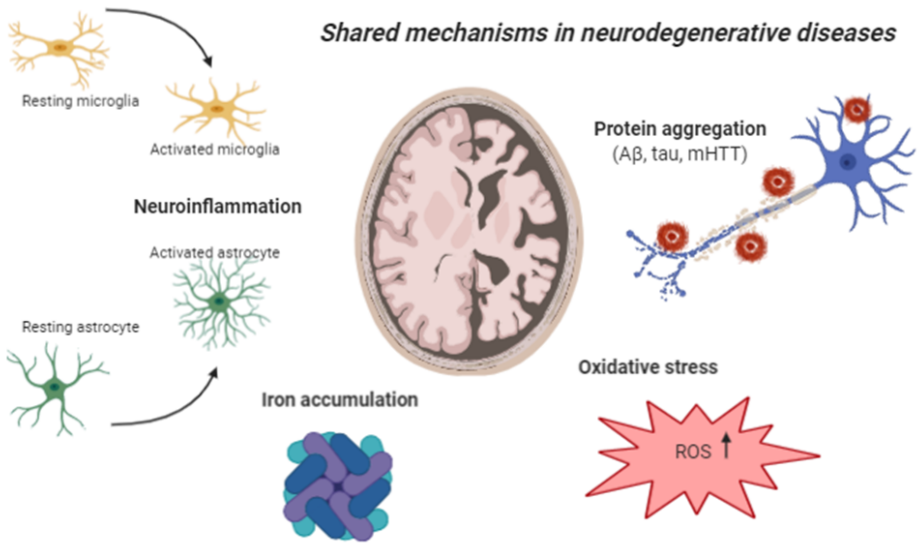

Figure 1.2: **Overview of the shared mechanisms in neurodegenerative diseases as Alzheimer's and Huntington's disease.**

The exact mechanisms of iron accumulation in microglia are not known, but microglia have been reported to have DMT1, transferrin receptors and ferroportin, and are able to store large amounts of iron in ferritin [60, 61]. The increased release of pro-inflammatory cytokines like TNF-alpha and IL-6 by microglia induce the synthesis of DMT1 and promote iron accumulation in microglia [62]. Also, astrocytes that have been activated by pro-inflammatory cytokines show increase iron uptake due to up-regulated expression of DMT1 [63]. As such, iron accumulation is closely linked with both microglia and astrocyte activation.

Despite all these correlative findings, it is currently unknown whether iron accumulation is a cause or consequence of neurodegeneration, and what the role of iron accumulation is with respect to the many other shared mechanistic links between neurodegenerative diseases (Fig. 1.2). The fact that iron accumulation is not a diffuse effect but found in brain regions specifically affected by the disease, stresses the interaction between iron accumulation, protein aggregation and neurodegeneration. This suggests that iron can be used as marker for neurodegeneration, but the question remains how this is related to other disease-specific mechanisms and mechanisms such as neuroinflammation.

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1.3. T_2^*-WEIGHTED MRI AS A PROXY FOR IRON CONCENTRATION

The advent of high-field MRI is of great relevance to study brain iron accumulation non-invasively, creating the possibility to use iron as a potential imaging biomarker in neurodegenerative diseases and to further investigate the questions raised above.

Susceptibility-based MRI techniques make use of the strong paramagnetic properties of iron, as the presence of iron causes intravoxel dephasing of the MR signal and subsequently accelerated T_2^* decay on gradient echo (GRE) images [64]. These iron-induced susceptibility changes can be detected as hypointense regions on T_2^*-weighted images. Measuring the signal decay of subsequent echos from a multi-echo GRE scan allows the calculation of $R_2^* = 1/T_2^*$, which is frequently used for quantification of the observed contrast changes [64]. As variations in tissue susceptibility also cause changes in the phase image, phase data has also been used as a qualitative measure for susceptibility changes. However, intrinsic limitations of the phase data are the non-local nature and the high dependency of the orientation of the object with respect to the main magnetic field [65, 66]. Susceptibility-weighted imaging (SWI) combines the magnitude and phase information to enhance the susceptibility contrast on T_2^*-weighted images [67]. Although clinically used to image vascular structures and microhaemorrhages, SWI does not provide a quantitative measure for tissue susceptibility changes [68]. Quantitative susceptibility mapping (QSM) overcomes the non-local nature of the magnetic field distribution and might therefore be a more reliable technique to quantify brain iron. Apart from being highly sensitive to iron as shown by multiple post-mortem studies, QSM also makes it possible to distinguish between paramagnetic and diamagnetic materials such as calcifications and myelin [69–71].

Currently, non-invasive biomarkers for detection of neurodegeneration at an early disease stage are lacking. Such a biomarker would not only increase our understanding of the pathophysiological process, but would also allow diagnosis at an early disease stage which is currently not possible in disease like AD, as the definitive diagnosis can only be made post-mortem. Therefore, all above mentioned iron-sensitive MRI techniques have been used in the last decades to investigate and quantify brain iron accumulation in several neurodegenerative diseases. For example, increased R_2^* values, phase shifts and susceptibility values have been reported in the cortex of AD patients and striatum of HD patients (Fig. 1.3) [26, 46, 48, 68, 72, 73]. Even more promising, some studies showed the potential of iron-sensitive MRI as a marker for disease progression by identifying iron accumulation in early disease stages in both these diseases [48, 74].

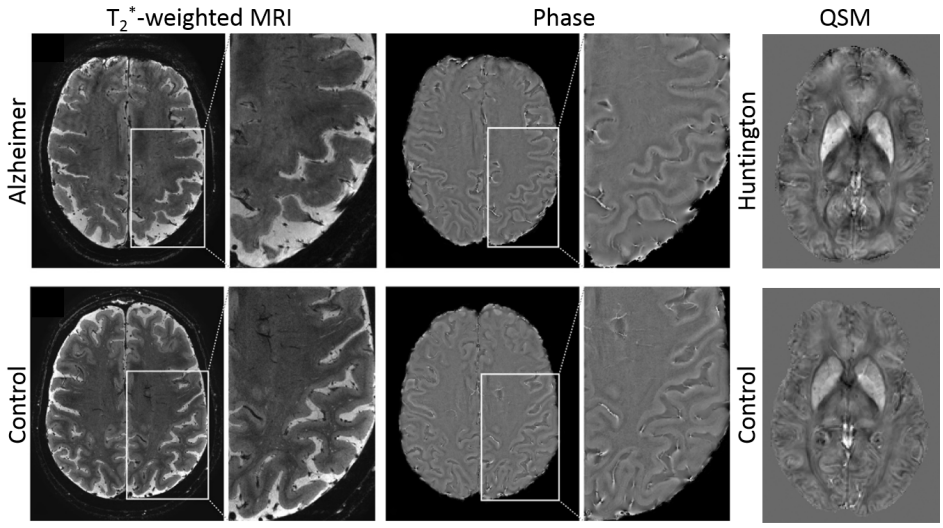

Figure 1.3: **Susceptibility-weighted MRI in patients with Alzheimer's and Huntington's disease.** Phase images show enhanced contrast between cortical gray and white matter in AD patients compared to control subjects, indicating a larger cortical phase shift. QSM shows significant susceptibility differences within the striatum of HD patients compared to control subjects. Figure adapted from van Rooden, et al., 2014 [26] and van Bergen et al., 2016 [48]

1.4. THE MISSING LINK

Although the potential of iron-sensitive MRI has been shown and magnetic susceptibility techniques are now sufficiently developed to allow quantification, the interpretation of the data in the biological context remains challenging. This is particularly difficult when there are clearly defined differences between brains from healthy controls and patients with neurodegenerative diseases, yet the interpretation of data is mostly restricted to a correlative level. We clearly need a better understanding of the nature of the changes on T_2^*-weighted MRI and comparing them with *in vivo* biomarkers is required to allow greater biological insights. Validation and interpretation of *in vivo* MRI findings using post-mortem MRI and histology on post-mortem brain tissue will provide some answers to these questions as they provide unique information on the underlying pathological substrate of MRI contrast.

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1.5. AIM OF THIS THESIS

In this thesis I aim to gain more insight into the role of iron in neurodegenerative diseases using high-field MRI. It is my goal to unravel what iron imaging reflects on the pathological level and to determine how iron accumulation is associated with disease progression both ex vivo and in vivo. I focus on two main neurodegenerative diseases: Alzheimer's disease and Huntington's disease. Additionally, I explored the use of iron as a potential biomarker for neuroinflammation, independent from neurodegeneration, in systemic lupus erythematosus (SLE), an autoimmune disease not marked by neurodegeneration. The thesis is further organized as follows:

In *chapter 2* I first aim to validate MRI-based detection of iron in post-mortem cortical brain tissue of both AD patients and non-demented controls using a unique pipeline that allows a direct comparison between full spatial maps of iron detected by T_2^*-weighted MRI, histochemistry, and a gold standard for iron detection, laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS). In addition, I will measure iron levels in AD patients and controls with LA-ICP-MS and all three quantitative MRI methods.

In *chapter 3* I further investigate the histopathological correlates of previously observed T_2^*-weighted MRI contrast changes to determine the exact pattern of iron accumulation and the colocalization with specific cells in the frontal cortex of AD patients. I will also investigate the differences in iron accumulation in two subtypes of AD, namely early-onset and late-onset AD.

In *chapter 4* I extend the research in *chapter 3* to investigate the spatial distribution pattern of the observed MRI contrast changes over the entire cortex, using tissue blocks from different cortical regions selected based on the distribution pattern of tau pathology. By applying the same pipeline as used in *chapter 3*, I correlate the MRI contrast changes with the severity of AD pathology in each region. Finally, I investigate the differences in distribution patterns of AD pathology and MRI contrast changes between subtypes.

In *chapter 5* I move to Huntington's disease to gain insight into the histopathological correlates of the well-known T_2^*-weighted contrast changes in HD. Also here, I perform ultra-high field *ex vivo* MRI and histopathological examination on post-mortem tissue of the striatum of HD patients to determine the exact pattern of iron accumulation and the colocalization with specific cells.

Based on the results in *chapter 5*, I describe the rationale and design for an in vivo 7T MRI study in *chapter 6*. This study focusses on the correlation of brain iron levels obtained from 7T MRI of HD patients in different disease stages, to specific and well-known clinical cerebrospinal fluid markers for iron accumulation, neurodegeneration and neuroinflammation.

In *chapter 7* I use QSM to investigate iron accumulation in the basal ganglia of SLE patients with neuropsychiatric complaints to gain more insight into the link between iron accumulation and neuroinflammation. Histological examination of post-mortem brain tissue including the putamen and globus pallidus is done to further support the in vivo findings.

In *chapter 8* I summarize the main findings of this thesis, followed by a general discussion. The thesis ends with suggestions for future research.

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