METHODS FOR BRAIN IRON EVALUATION IN NORMAL AGING:
T2 AND PHASE MEASUREMENTS AT 3 TESLA AND 7 TESLA

DISSERTATION

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ABSTRACT

During the last few years, magnetic resonance imaging (MRI) systems operating at high (≥3 Tesla) and ultra-high (≥7 Tesla) magnetic field strength, have been extensively developed and used. However, there are still open questions about what determines the improved contrast among tissues in the brain, visible in susceptibility weighted, T2, T2*, and phase images.

Brain iron deposition is age and region specific, with a pattern that is disturbed in neurological diseases (Alzheimer, Parkinson). Understanding the effect of tissue iron in the transverse relaxation and contrast mechanism in susceptibility weighted T2* images may be useful to non-invasively detect the distribution of iron in the brain. Studies of normal subjects and age related changes are needed to provide baseline data.

Iron is capable of affecting the MRI signal by influencing the phase of the diffusing spins. This causes an increased transverse relaxation rate, visible in a T2 or a T2* weighted image, and a strong dephasing of spins, which effects are seen in a gradient echo T2* weighted image with a long echo time.

Our experiments, conducted at both 3 Tesla and 7 Tesla, looked into the relationship between measured T2 using the commercially available Dual
Echo and GRASE sequences, and the published estimated regional brain iron content, in the first study. The second study was dedicated to the correlation between phase spin quantifications, using phase images obtained from a gradient echo T2* acquisition and the same estimated brain iron content.

We found that T2 correlated with brain tissue iron concentrations at both 3T and 7T. However, accurate relaxation measurements using the vendor supplied sequences are difficult to obtain, especially at 7 Tesla. RF inhomogeneities probably caused the failure to detect significant correlation between T2 values and normal brain iron distribution using GRASE. The correlation between brain iron and phase shift proved to be dependent on the region of interest. Moreover, the high visual resemblance between the phase images at the midbrain level and the same region on postmortem India ink stained microvasculature suggests that tissue iron may not be the sole influence in the contrast mechanism at ultra-high field strength.
Dedicated to

Licsandra & Marin Serban, Cosmin Mihai
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INTRODUCTION

Magnetic Resonance Imaging (MRI) is a non-invasive technique able to provide images with high resolution and a high degree of contrast between soft tissues. Imaging at high ($\geq 3$ Tesla) and ultra-high ($\geq 7$ Tesla) magnetic field strength improved greatly the signal to noise ratio (SNR) and resolution of acquired images, which may allow for better diagnosis of many diseases.

Recently, the need to understand the contrast mechanism at higher magnetic field increased as the number of high field systems used for diagnosis went up over the last few years. Even if the ultra-high field systems are only used for research purposes, they illustrate improvement in image quality that goes beyond the increase in resolution and SNR. For example, Susceptibility Weighted Imaging, more precisely phase images reconstructed from T2* gradient echo with a long echo time acquisition shows greater white matter–gray matter differentiation, improved vasculature depiction, and white matter fiber bundle orientation. While all of these are new and exciting, the contrast mechanism in both magnitude and phase images is not completely understood at ultra-high field. At lower field strengths the phase contrast in brain images was attributed to
normal iron tissue deposition, which also affects the transverse relaxation through spin dephasing.

Brain tissue iron deposition that is age and region dependent is a feature of normal brain aging. This dissertation is dedicated to checking the relationship between the estimated regional brain iron and transverse relaxation time, T2 and phase shifts in phase images, respectively. Moreover, the effects of brain iron on the contrast mechanism at high field imaging are investigated. Results obtained at ultra-high field (7 T) are compared to similar acquisition sequences results obtained at high field (3 T).

In summary, the specific aims of the studies presented in this dissertation include:

1. Evaluate the correlation between brain tissue iron estimates in normal aging and transverse relaxation rates T2 obtained using the commercially available, non-altered Dual Echo and GRASE sequences.

2. Investigate the relationship between phase shifts in phase images obtained using gradient echo T2* susceptibility weighted acquisitions and normal brain iron tissue estimates.

3. Develop an understanding about the effect of iron deposition on the improved contrast seen in Susceptibility Weighted Imaging at ultra-high field strength.

4. Evaluate and compare the 7 T and 3 T results.
CHAPTER 1

INTRODUCTION TO NORMAL BRAIN AGING. RELATION TO NEURODEGENERATION

During normal aging, the brain undergoes morphological and functional modifications, underlined by biochemical and molecular changes. These changes affect synapses, neurotransmitters, brain circulation and metabolism, motor and sensory systems, and ultimately result in an increased vulnerability to neurodegenerative disorders.

1.1. STRUCTURAL BRAIN CHANGES

1.1.1. Gross anatomy

Examination of structural brain alterations may help to understand age related functional changes and the role they play in neurodegenerative processes. The most striking structural change of the brain with aging is the overall brain tissue shrinkage. Brain volume in healthy individuals beyond the age of 50 years decreases by about 5% per decade, with region-specific brain atrophy shown in both in vitro studies and in vivo imaging (Troller, 2001).
In aging, heterogeneous brain atrophy is gender specific, with an overall increased brain shrinkage in males compared to females (Xu, 1999). Both white and gray matter volumes are affected by age, but show a different pattern. Gray matter (GM) is affected first and seems to show a linear decline with age, while the white matter (WM) begins shrinking later in life (Jernigan, 2001; Resnick, 2003; Ge, 2002). For very old individuals WM shows an overall greater loss than GM. According to Salat et al. (Salat, 2004), the thinning of the cerebral cortex in normal brain aging is not limited to just the prefrontal cortex, but widely affects the primary motor and visual areas as well. Also, both longitudinal and cross sectional studies have shown agreement regarding an increase in ventricular CSF space with age, which is not gender-specific and is accelerated with advancing age (Jernigan, 2001; Resnick, 2003).

Initially, shrinkage of brain tissue with age was attributed to neuronal loss, but more recent reports consistently show that the loss of cortical neurons with aging is minimal (Morrison, 1997), indicating that other age related changes of the brain may be responsible for the overall brain shrinkage, such as the ones described below.

1.1.2. Cytology

Myelin transformation and nerve fiber loss are two age related brain tissue changes that may be correlated with the cognitive decline seen in normal aging (Peters, 2002). Electron microscopy preparations of rhesus monkey
brain showed shearing defects, splitting of the myelin lamellae due to the continued formation of myelin with age, and focal volume increase of the myelin sheaths. These myelin volume increases range in size from 1 to 10 microns, and the larger ones may be fluid-filled, resulting in a potential increase in the T2 signal in MRI measurements (Bartzokis, 2004). This age-related myelin breakdown seems to first affect the fibers having smaller diameters and the brain regions that have been myelinated later during brain development: the prefrontal, temporal and parietal lobes (Bartzokis, 2004a). This damage to the myelin sheets correlates well with the cognitive decline observed in primates, which is attributed to the decrease in conduction velocity along nerve fibers (Peters, 2002), and the increase in the refractory period of the axon (Bartzokis, 2004a).

Overall, the destruction of axonal fibers seems to be related to the combined effect of demyelination of the axonal tracts and cerebrovascular diseases affecting the penetrating arteries of the brain (Troller, 2001). There is also evidence showing that the loss of nerve fibers in the WM, related with these changes is severe, while the number of nerve fibers affected in cerebral cortex is much lower. The nerve fiber loss adds to the conduction dysfunction brought up by myelin defects, and increases cortical disconnections that have been associated with cognitive dysfunction in the elderly (Peters, 2002; O'Sullivan 2001).
1.2. VASCULAR AND HEMODYNAMIC BRAIN CHANGES

In vitro and in vivo studies also suggest that there is an important relationship between aging and central nervous system (CNS) vascular changes that affects the cognitive process. The rarefaction of cerebral arterioles and significant reduction of the capillary density in the brain, especially in the hippocampus and cerebral cortex (Riddle 2003), are accompanied by a decrease in cerebral blood flow (CBF) and decreased metabolic rates of glucose and oxygen (Farkas, 2001).

Changes in capillary density in cortical and hippocampal regions of the brain, as well as alterations in the normal structure of the capillary walls, were observed in both human and experimental animal aging models. Animal studies have shown that microvascular fibrosis and basement membrane thickening are typical vascular abnormalities in CNS aging (Farkas, 2001).

It was also demonstrated that the decline seen in blood perfusion and CBF is caused by both an impaired vasodilatory response and a domination of vasoconstriction mechanisms that limits the increase in vascular caliber in healthy aging brain (Farkas, 2001). However, SPECT, MRI and PET studies in humans failed to completely agree about the effects of gender, brain location or age-specificity on the CBF decrease (Meltzer, 2000; Van Laere 2001) possibly due to the spatial resolution limitations in PET and SPECT compared to MRI. The reduction in CBF affects the transport of metabolites to tissues, which is further worsened by age-altered transport across capillaries. There is an
increased leakage through the blood brain barrier and reduced passage of amino acids and glucose, due to these vascular aging-related abnormalities (Riddle, 2003). The lower CBF seems to trigger the decrease in cerebral metabolic activity as well as the decline in oxygen and glucose utilization in normal brain with aging (Farkas, 2001).

It is known that the CNS microvasculature is actively modified through angiogenesis to match the level of neuronal activity and the metabolic and neuronal support for that activity, a process known as microvascular plasticity (Riddle, 2003). In aged brain tissue, this process shows a reduced response to the increase in neuronal activity and angiogenesis factors. The decrease in microvascular plasticity, correlated with the reduced CBF with advanced age, seems to contribute to and also explain the cognitive and functional decline seen in the elderly (Farkas, 2001; Riddle, 2003).
1.3. MOLECULAR BRAIN CHANGES

Aside of the structural and vascular changes there are also other neurobiological transformations that affect the aging brain at the cellular level. Among the most important of these are the formations of neurofibrillary tangles, neuritic and amyloid plaques, and granulovacuolar degeneration. Alzheimer’s disease pathology changes are also seen in the normal brain aging to same extent.

Neurofibrillary tangles, which consist of contorted and thickened neurofibrils, appear in neuronal cytoplasm and seem to represent a nonspecific neuronal response to injury (Cummings, 1992). They are found in the brains of people as young as 30 years of age and are localized at the level of entorhinal cortex and hippocampus, showing an exponential increase with age (Price, 2003). Granulovacuolar degeneration, represented by clusters of intracytoplasmic vacuoles of approximately 5 microns in diameter with a central granule of up to 1.5 microns, appears in hippocampi of normal brain even before people turn 60 years of age. Also present in the normal brain are neuritic plaques, corresponding to tissue degeneration with granular deposits and other remnants of neuronal processes. Their formation impairs the normal intercellular communication and affects the synaptic role in learning, memory, and cognition (Cummings, 1992, Golob, 2001). Amyloid plaques, which represent an extracellular aggregate of 39 to 42 residue peptides containing the beta amyloid peptide (Aβ), have a low and nonspecific distribution in the normal aging cerebral cortex. Recently, the amyloid rich plaques in normal or diseased brain
were seen to be directly correlated to the presence of microhemorrhages found adjacent or around small vessels, suggesting that the microvascular breakdown at the site of senile plaques is a precursor of dementia (Cullen, 2006).

All of these microscopic changes are normal for the aged brain in small number, but when they show up in great number and follow a specific regional distribution they become hallmarks for neurodegenerative diseases (ND), such as Alzheimer’s disease (AD) (Cummings, 1992), and Parkinson’s disease. For example, AD, which may also have a genetic predisposition given by the presence of apoE4 gene and others, shows pathological changes that affect associatival areas of the parietal, temporal, and frontal lobes and hippocampus (Cummings, 1992). It is histologically characterized by significant deposits of Aβ amyloid plaques and intracellular neurofibrillary tangles containing filaments of abnormally phosphorylated tau proteins. The presence of these deposits determines a loss of synaptic densities and neuronal death that translates in a profound cognitive and functional decline (Cummings, 1992; Peinardo, 1998), which ultimately results in the death of the affected individual.

In conclusion, increased age accompanied by normal macroscopic and microscopic transformation of the brain tissue, genetic in nature or otherwise, represents a major risk factor to neurodegeneration (Morris, 2003).
CHAPTER 2

INTRODUCTION TO BRAIN IRON DEPOSITION

Iron is one of the most essential trace elements in the body and plays a crucial role in normal brain functioning. Deficiency or excess of iron in the brain have neurological consequences, and as a result iron uptake and storage inside the brain is highly regulated. Genetic or environmental disruption of iron metabolism is believed to be a primary cause of neurodegenerative disorders (Ke, 2003).

As part of many enzymes and oxygen carrier proteins, iron has an important role in cell metabolism. One of its major functions is the involvement in the electron transport chain of respiration in mitochondrial oxidation reactions. During this process, through the breakdown of sugars and fats in the presence of oxygen, chemical energy in the form of adenosine triphosphate production (ATP) is generated. Deoxynucleic acid (DNA) and neuronal transmitters synthesis as well as myelogenesis and myelination in the brain are also iron dependent. As a consequence, iron availability to the brain has to satisfy regional and cellular needs in addition to age-dependent requirements. Due to the high-energy consumption in the brain, oxygen utilization and metabolic processes
involving iron take place at a high rate. This emphasizes the need for immediate iron availability in order to sustain such high activity (Connor, 2001).

2.1. PARENCHYMAL BRAIN IRON

The two forms of iron in the body, heme and non-heme iron may exist in two oxidation states: ferrous (Fe$^{2+}$) and ferric (Fe$^{3+}$) iron. Heme iron, mostly uniformly distributed inside the blood pool, is part of the oxygen transport metalloprotein hemoglobin found in red blood cells of the blood. In humans, hemoglobin contains more than half of the total iron in the body. Non-heme iron is mostly contained in the iron transport and storage proteins, such as transferrin and ferritin, respectively.

2.1.1. Regional brain iron distribution

It is well known from the work of Hallgren and Sourander (1958) that iron distribution inside the brain is heterogeneous, with a nonuniform deposition even in the same brain structure. In their work, Hallgren and Sourander showed, by doing hystochemical iron determination for 17 brain regions using 81 age-variant normal brains, that regional iron is also age-dependent. For example, the highest iron value is found in the globus pallidus with an average of 21.30 mg iron per 100g of fresh weight tissue, followed by the red nucleus with 19.48 mg, and the substantia nigra with 18.46 mg. The medulla oblongata has the lowest concentration of 1.02 mg iron per 100g fresh weight tissue.
In different regions of the brain, the age dependence of iron varies. This is seen in the structures such as the globus pallidus, where the iron concentration increases rapidly from birth until 20 years of age, with no increase after 30 years of age, while in the putamen and caudate nucleus the increase in iron’s concentrations values is slower and does not end until 50-60 years of age. The same paper (Hallgreen, 1958) provides both means of iron concentrations for subjects that are older than 30 years of age (Table 2.1), and convenient equations that allow approximate regional iron determination based on the age of the subject. The regression lines that relate iron concentration (y) in mg/100g wet tissue and the age of the subject (x) in years is calculated in the paper by the method of least squares to provide equations with the general form:

\[ y = a \{1 - \exp(-bx)\} + c \]  

2.1

The coefficients a, b, and c for the specific brain regions are summarized in Table 2.1.

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>Age&gt;30 years Fe (mg/100g wet tissue)</th>
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<tr>
<td>Frontal WM</td>
<td>3.95</td>
<td>0.1</td>
<td>0.31</td>
<td>4.24±0.88</td>
</tr>
<tr>
<td>Prefrontal GM</td>
<td>2.43</td>
<td>0.07</td>
<td>0.58</td>
<td>2.92±0.41</td>
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<tr>
<td>Motor GM</td>
<td>4.79</td>
<td>0.05</td>
<td>0.40</td>
<td>5.03±0.88</td>
</tr>
<tr>
<td>Globus Pallidus</td>
<td>21.41</td>
<td>0.09</td>
<td>0.37</td>
<td>21.3±3.49</td>
</tr>
<tr>
<td>Caudate Nucleus</td>
<td>9.66</td>
<td>0.05</td>
<td>0.33</td>
<td>9.28±2.14</td>
</tr>
<tr>
<td>Thalamus</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>4.76±1.16</td>
</tr>
<tr>
<td>Putamen</td>
<td>14.62</td>
<td>0.04</td>
<td>0.46</td>
<td>13.32±3.43</td>
</tr>
<tr>
<td>Parietal GM</td>
<td>3.31</td>
<td>0.06</td>
<td>0.6</td>
<td>3.81±0.67</td>
</tr>
<tr>
<td>Sensory GM</td>
<td>3.97</td>
<td>0.07</td>
<td>0.49</td>
<td>4.32±0.58</td>
</tr>
<tr>
<td>Temporal GM</td>
<td>2.70</td>
<td>0.07</td>
<td>0.58</td>
<td>3.13±0.57</td>
</tr>
<tr>
<td>Cerebral GM</td>
<td>2.70</td>
<td>0.085</td>
<td>0.68</td>
<td>3.35±0.87</td>
</tr>
<tr>
<td>Occipital GM</td>
<td>4.03</td>
<td>0.06</td>
<td>0.72</td>
<td>4.55±0.67</td>
</tr>
</tbody>
</table>

Table 2.1. Coefficients that relate iron concentration with age, and the mean iron concentrations for subjects between 30 and 100 years of age, as they were listed in Hallgren and Sourander paper (Hallgren, 1958). Note: GM =gray matter, WM=white matter, NA=non-applicable
These normal age iron depositions are perturbed in neurological disorders such as Alzheimer’s, Parkinson’s and Huntington’s diseases, as well as Hallervorden-Spatz syndrome, resulting in neuronal death through the increased activity of oxidative stress mechanisms (Ke, 2003).

### 2.1.2 Cellular Distribution of Iron

The staining of the normal brain tissue through Perl’s reaction showed that oligodendrocytes are the neural cells that contain the most iron, which is deposited in the cytoplasm and processes (Levine 1991, Connor 1995). Even if they can be found everywhere, including iron-rich brain nuclei, oligodendrocytes are mostly present in white matter tracts. Quantitative studies (Rajan, 1976) showed that white matter has more iron than corresponding gray matter in some regions. The iron-rich oligodendrocytes are not uniformly distributed in the white matter tracts, however. The only exceptions are the oligodendrocytes within the U fibers at the border of cerebral gray matter with white matter, which stain strongly and uniformly for iron (Connor, 1995). The deep brain nuclei, including the dentate nucleus, globus pallidus and substantia nigra normally show high iron concentration (Koeppen, 2001). In general, areas associated with motor function, especially the motor cortex have elevated iron concentration compared with the rest of the cerebral cortex (Connor, 1991).

In brain parenchyma, the iron that is not immediately utilized by the cell is stored as stable Fe\(^{3+}\). The storage is carried out in ferritin proteins (Hallgreen, 1958; Vymazal 1996), with small deposits found also in
hemosiderin, which is a pathological form of ferritin (Quitana, 2004), as well as in lipofuscin and in neuromelanin proteins (Zecca, 2001). Ferritin molecules, present in cellular cytoplasm and near to myelinic structures (Quitana, 2004), consist of a protein shell with an outer diameter of approximately 12nm and an inner diameter of 8nm with 24 subunits of heavy (H-ferritin) and light (L-ferritin) isoforms, which have an iron storage capacity of up to 4500 iron atoms per ferritin molecule (Gossuin, 2004). According to Chasteen et al. (1999), the H-ferritin contains ferroxidase centers that rapidly convert divalent iron to trivalent iron, which is subsequently stored in the ferritin molecule. Conversely, L-ferritin lacks the ferroxidase center and is slower in sequestering iron into ferritin, but seems to be involved in mineralization and storage of iron. Neurons contain mostly H-ferritin, microglia has ferritin that is predominantly L-rich, while oligodendrocytes, which have the highest iron concentration in the brain, contain similar amounts of the two ferritin isoforms (Connor, 1994). Connor et al. (1995) showed that in the normal brain the predominant form of isoferitin is H-ferritin, and that its concentration increases with age. H-ferritin presumably has a protective role against continuous iron uptake with age and is a readily available source for iron utilization, as required by the high-energy consumption of the brain. The L-ferritin is also age dependent, and its role seems to be that of a long-term safe storage for iron atoms inside the cell. The same authors (Connor, 1995) demonstrated a region-specific distribution of H/L forms of isoferitin, with age dependent ratios, that are disturbed in Alzheimer (AD) or Parkinson disease (PD) patients. According to Friedman et al. (2006) the lower levels of L-
ferritin chains in some brain regions, such as the hippocampus may suggest a limited potential to safely store the iron available to that region of the brain. This limitation is worsened by the inability of the H-ferritin chain to take up more iron, so that it can safely detoxify the tissue of free iron. Phenomena such as this suggest that some brain regions are predisposed to neurodegeneration (Friedeman, 2006), and may provide evidence that, besides iron deregulation, and abnormal iron deposition in diseased states increases the potential for oxidative damage and neuronal death associated with neurodegenerative diseases (Ke, 2003).

2.1.3. Brain Iron Transport

The major route for the plasma iron to reach the brain is transport across the blood brain barrier (BBB). The iron is first complexed to transferrin (Tf) in the blood, and then the Tf-iron complex binds to a Tf receptor (TfR) expressed in endothelial cells of the brain microvasculature. Another proposed mechanism for iron uptake into the brain or release from the brain is the ventricular system, through choroids plexus, in which epithelial cells contain transferrin, iron and ferritin. Connor et al., (Connor, 2001) proposed that the transferrin receptors of the brain microvasculature are responsible for regional regulation of iron, while choroids plexus epithelial cells transferrin receptors are involved in a more homogenous iron uptake throughout the brain. There is also evidence that the iron transport to the brain tissue is related to the iron status of the endothelial cells of the microvasculature. According to Burdo et al. (2003), the
ratio of the non-transferrin-bound iron to transferrin-bound iron transported across endothelial cells is dependent upon their iron status, more exactly the Fe3+ stored in epithelial ferritin molecules of the brain vasculature.

The way iron gets inside the brain cells is not completely elucidated (Rouault, 2006). However, a more complete understanding of genetic and non-genetic factors involved in iron-brain regulation could help treat or prevent neurodegenerative disorders in which iron deregulation has a major influence in neuronal death (Ke, 2003; Qian, 2000).

2.2. BRAIN IRON AND FREE RADICAL MITOCHONDRIAL DECAY THEORY OF AGING

The free radical theory of aging is one of the most plausible of many aging theories meant to explain aging and age related diseases (Wickens, 2001; Schipper, 2004). This theory states that aging is the sum of oxidative damage to the cell, as well as to the tissues of the body, due to an increase in oxidative stress with age that is not accompanied by an increase in antioxidant defense mechanisms (Barja, 2004; Droge, 2007).

The most abundant source of free radicals in the cell is the mitochondria, which uses 90% of the oxygen in the cell for metabolic water production, by sequential reduction of $O_2$ (Wickens, 2001). This process generates radical oxygen species (ROS), such as highly reactive and toxic superoxide ($O_2^-$), hydroxyl radical ($^*OH$), and hydrogen peroxide ($H_2O_2$). The unstable Fe2+ iron form, which is involved in ATP production in
mitochondria, catalyzes these reactions: and via Fenton (equation 2.1) and Haber-Weiss reactions (equations 2.2 and 2.3), transforms superoxide \( O_2^- \) and hydrogen peroxide into the aggressive hydroxyl radical (Wickens, 2001, Schipper, 2004), as shown below:

\[
\begin{align*}
Fe^{2+} + H_2O_2 & \rightarrow Fe^{3+} + OH^- + OH^- \\
H_2O_2 + OH^- & \rightarrow H_2O + O_2^- + H^+ \\
H_2O_2 + O_2^- & \rightarrow O_2 + OH^- + OH^- \tag{2.3}
\end{align*}
\]

In small quantities, ROS are beneficial in cell homeostasis since they are involved in cell signaling and gene regulation. However, these functions are compromised when their production is increased inside the cell. The damaging action of ROS is associated with hallmarks of aging such as lipid peroxidation of membranes, cross-linkage of protein, DNA damage and mitochondrial function decline (Polla, 2003). Mitochondrial DNA is more prone to damage from oxidative stress than nuclear DNA, due to both generation of ROS in mitochondria and the close proximity of mitochondrial DNA. In the brain, damage from ROS is more aggressive than in many other cells of the body. The largely post-mitotic nature of the neurons does not allow replacement of lost and damaged neurons by mitosis of healthy ones, and the significant availability of iron in the cell and mitochondria potentiates the toxic effect of ROS (Barja, 2004).

This interdependence between brain iron, oxidative stress and affected normal mitochondrial function form the basis of free radical-
mitochondrial theory of aging. A disruption of any of these factors, therefore, could create a cascading effect on the others (Schipper, 2004). In any case it is clear that iron plays an important role in brain aging and neuropathology, and a complete understanding of its impact is necessary (Droge, 2007).

There is evidence that increased deposition of iron in the aging brain, or iron deregulation associated with neurodegeneration, correlates with increased oxidative stress and mitochondrial dysfunction. For example, the role of iron deposition on amyloid-β (Aβ) plaques that have binding sites for cooper and iron in AD, is believed to be that of an enhancer of the oxidative processes. While all transferrin, melanotransferrin and ferritin have been associates with pathology and oxidative stress in AD, the iron regulatory protein 2 is held responsible for the disturbance in brain iron homeostasis and the overall decompartmentalization of iron that results in ROS production (Hossein Sadrzadeh, 2004). In PD, iron has been suggested to be the main cause for nigrostriatal dopamine neurons degeneration due to its ability to mediate toxic ROS production that cause lipid peroxidation (Hossein Sadrzadeh, 2004). The neuromelanin pigment in the substantia nigra in PD might be involved in iron storage, while overexpressing of lactoferrin receptors on neurons and microvessels from PD affected brain tissue suggested a relationship with iron overload in affected brain regions (Hossein Sadrzadeh, 2004). All of these mechanisms suggest that disturbances in iron uptake, storage, intracellular metabolism, release and transcriptional control provide a perfect environment for free iron/ROS to cause permanent cellular damage in AD and PD.
CHAPTER 3

ASSESSMENT OF BRAIN TISSUE IRON USING T2 MEASUREMENTS
DUAL ECHO AND GRASE RESULTS AT 7T AND 3T

3.1. ESTIMATION OF TRANSVERSE RELAXATION TIME (T2)

The contrast in MRI magnitude images is determined by intrinsic parameters, such as tissue proton density and the T1 and T2/T2* relaxation times. Relaxation times represent a measure of the mechanisms that take place when a tissue sample experiences relaxation back to equilibrium and loss of phase coherence following energy absorption at resonance. The relaxation mechanisms can be grouped into those that transfer energy from the spins to the lattice, named spin-lattice interactions, and those that redistribute it within the spin system, known as spin-spin interactions (Abraham, 1961; Slichter, 1963; Callaghan, 1991). The first mechanism contributes significantly to the spin-lattice relaxation time (T1). The latter mechanism contributes exclusively to the decay of the transverse component of the bulk magnetization by causing the spins to lose phase coherence. This is quantified in the transverse relaxation time T2 and/or T2*. The reader is referred to one of the classical MRI books (Slichter 1963;
Haacke, 1999) for more details on relaxation mechanisms and further explanations on how the spin excitation and relaxation occurs.

T1, also called the spin lattice or longitudinal relaxation time, is a time constant that characterizes the rate of recovery of longitudinal magnetization back to equilibrium. This recovery is caused by fluctuating magnetic fields generated by the motion of molecules in close proximity to the magnetic moments. In essence, T1 gives information about the mobility of water molecules and hence their binding to macromolecules. Since the focus of this dissertation is not T1 measurements, this will not be discussed further, and the reader is referred to classical books of MRI for more details.

3.1.1. Introduction to transverse relaxation time

Following the RF excitation the total magnetization of the sample has both a longitudinal (Mz) and a transversal component (Mxy) (Figure 3.1).
Figure 3.1. The vector components of the magnetic moment in z and x-y plane of the three dimensional Cartesian coordinate axis system.

The signal in MRI is generated by the transversal component of the magnetization. Initially, the transversal component is large because the nuclear magnetic moments lying in the transverse plan point in the same direction and have phase coherence. The phase coherence of the spins is lost in time as spins experience the randomly fluctuating background magnetic field generated by the sample itself. The most important source of this background field is the dipole-dipole interaction. Each of the nuclear 1/2 spins of $^1H$ has associated with it a dipole field. As two nuclei came in close proximity with each other they experience a small disturbance to the main magnetic field $B_0$, due to the spin-spin interaction. Because the corresponding molecules undergo random Brownian motion in solution, so the background field varies randomly. This random variation of the field experienced by spins generates the loss of phase coherence in the transverse plan.
For a homogenous sample, placed in a perfect magnetic field $B_0$, the loss of phase coherence of the precessing spins results in a signal decay characterized by $T_2$ relaxation. The loss of phase coherence can be accelerated by the presence of imperfect magnetic field $B_0$ and external magnetic field inhomogeneities, such as ferromagnetic and paramagnetic materials (see Chapter 4). The signal loss in this case is determined by the relaxation time $T_2^*$, which is shorter than $T_2$. While both $T_2$ and $T_2^*$ characterize the loss of phase coherence of the spins that results in signal decay, the relation between $T_2$ and $T_2^*$ is shown graphically in Figure 3.2.

One way to describe the time constant $T_2^*$ is to look at a Free Induction Decay (FID) curve (Figure 3.2), which shows the decay of the acquired signal as spins lose their phase coherence due to the different magnetic fields they experience.

Figure 3.2. A FID sequence that shows the relation between $T_2$ and $T_2^*$ relaxation times.
In a very simplified form, the dephasing of the spins is described by the decay constant $T2^*$:

$$S(t)=S_0 \exp[-t/T2^*]$$

Where $S(t)$ is the acquired signal at time $t$, $S_0$ is the maximum signal, and the $T2^*$ is the exponential decay constant of the FID. Another way of expressing $T2^*$ is by the use of relation:

$$1/T2^* = 1/T2' + 1/T2 \quad \text{or} \quad R2^* = R2' + R2$$

Where $R2'=1/T2'$ represents reversible-dephasing mechanisms, which may be corrected by successive refocusing of the signal, and $R2=1/T2$ shows the irreversible dephasing measurements due to water diffusion and intrinsic dipole-dipole interaction.

One way of measuring $T2^*$ in MRI is by use of a gradient echo sequence, which is in essence a FID technique that uses gradients to form an echo during the signal sampling. Since $T2^*$ is not a topic of this dissertation, $T2^*$ determinations methods will not be discussed further.

### 3.1.2. Methods to estimate transverse relaxation time, $T2$

**Hahn spin echo**

The spin-spin or transverse relaxation time ($T2$), which is also the irreversible part of the $T2^*$ decay, is estimated by using spin echo based techniques. A very basic schematic of a spin echo sequence, which is known as a Hahn spin echo (Hahn, 1950) is shown in Figure 3.3.
The spin echo pulse sequence starts with a $90^\circ$ RF pulse and produces an FID that decays according to $T_2^*$ relaxation. After a delay time equal to $TE/2$, a $180^\circ$ RF pulse inverts the spins, in order to regain phase coherence and produce an echo at time $TE$. However, some spins became irreversible dephased because of their random (thermal) motion leading them into a region of the sample with a different main magnetic field to that which they were prior to the application of the $180^\circ$ RF pulse. This is how the choice of the echo time, more precisely the allowed time for spins diffusion influence the $T_2$ estimate (see Section 3.1.4). As a result the Hahn spin echo is very sensitive to diffusion mechanisms.

It is possible to measure $T_2$ of a tissue sample by repeatedly acquiring a spin echo Hahn sequence with different echo times and plotting
the signal intensity amplitude as a function of echo times. One of the fitting parameters of the signal decay envelope is tissue T2. However, due to the very long scan time necessary for T2 measurements that may result in subject motion between scans, the Hahn spin echo is not the best choice for T2 estimation in vivo.

**CP spin echo**

Carr and Purcell (1954) minimized diffusion effects of the Hahn spin echo sequence by adding multiple refocusing 180° pulses in the same direction as the 90° RF pulse, which allows acquisition of a train of echoes separated by an interecho time $2 \tau$ equal. The number of acquired images matches the echo train length. A schematic of the Carr-Purcell (CP) spin echo is shown below (Figure 3.4).

![Carr-Purcell spin echo diagram](image)

**Figure 3.4.** Carr-Purcell spin echo showing a train of three echoes separated by a time $2 \tau$. The signal decay of the echoes is determined by the T2 relaxation time.
While this method requires shorter acquisition time than Hahn spin echo, the errors in RF refocusing pulses are cumulative and successive pulses will generate errors leading to underestimated T2 measurements (see Section 3.1.3).

**CPMG spin echo**

The problem of errors in the RF refocusing pulses was solved by Meiboom and Gill (1958) with a simple modification of the CP spin echo sequence. By applying the 180° RF pulse in quadrature with the initial 90° RF pulse, the loss of spin coherence due to the imperfect 180° RF pulse is corrected after the second echo, and for every other echo following it. Thus, by using only the even echoes, a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence gives an accurate T2 estimate. On the other hand, the odd echoes are plagued by errors caused by the imperfect pulse profile (see Section 3.1.3). However, overall, by using both even and odd echoes the estimated T2 is more accurate than a T2 measured by using a CP sequence.

For spin echo sequences, the signal dependence on an inhomogeneous magnetic field and diffusion is expressed by the relation (Carr, 1954):

\[ S = \exp\left(- \tau / T_2\right) \exp\left[- \gamma^2 G^2 D \tau^3 / 12n^2 \right] \]  

3.3

Where \( \tau \) is the interecho time, \( D \) is the diffusion coefficient of the sample, \( n \) is the echo number (\( n=1 \) for Hahn spine echo), and \( G \) is the linear
field gradient a sample may encounter. In other words, the signal loss in a spin echo sequence depends, not only on the dipole-dipole relaxation (T2), but it is also dependent on the linear gradient in an imperfect magnetic field and spin diffusion ($\gamma^2 G^2 D$). If a monoexponential fitting is used for T2 determination, a combined effect of gradient susceptibility, diffusion and dipole-dipole interaction is reflected in the measured T2.

Both CP and CPMG pulse sequences are less sensitive to diffusion effects than the Hahn spin echo. Successive refocusing of the signal reduces the effect of spins diffusion on the loss of transverse relaxation by not allowing the accumulation of susceptibility and gradient effects. The short $\tau$ usually used in a CPMG sequence is typically too short for the accumulation of phase differences. Conversely, Hahn spin echoes allow spins to experience susceptibility and gradient effects over a longer dephasing period (longer TE) without multiple refocusing pulses. As a consequence, the Hahn spin echo is more sensitive to susceptibilities effects of iron deposited in the brain.

While each of the above discussed spin echo sequences are classic pulse sequences used to determine T2, there are a few other sequences that are also used to calculate transverse relaxation times (see section 3.1.3). Among these there are the gradient echo sampling of free induction decay and echo (GESFIDE), gradient echo sampling of the spin echo (GESSE), and gradient-spin-echo (GRASE).
GESFIDE

This sequence was introduced by Ma et al. (Ma, 1996), and is a modified spin echo sequence that is able to provide $R_2^*$, $R_2$ and $R_2'$ maps in a single acquisition. An oscillating read gradient produces a train of gradient echoes between the $90^\circ$ and the $180^\circ$ RF pulses, and a second set of echoes after the $180^\circ$ RF pulse. The schematic of this sequence is provided in Figure 3.5. One of the important properties of this sequence is that can be performed with two RF pulses that have an arbitrary flip angle, as long as strong refocusing crusher gradients are applied around the second pulse. As a consequence this $T_2$ measuring method is relatively insensitive to nonuniform RF pulses.

![Figure 3.5](image)

**Figure 3.5.** The GESFIDE pulse sequence. The $R_2$ and $R_2'$ can be computed from the sum and the difference of $R_2^*$ and $R_2'$. Adapted from Song et al. (2007).
**GESSE**

The gradient echo sampling of the spin echo (GESSE) sequence is a close relative of GESFIDE sequence (Ma, 1996), and was proposed by Yablonskiy and Haacke in 1997 (Yablonskiy, 1997). This sequence incorporates gradient echoes in a standard spin echo sequence to sample only the rephrasing and dephasing parts of the spin echo signal (see Figure 3.6).

![Figure 3.6. The GESSE sequence schematic. Observe the symmetric sampling of the signal on both sides of the refocusing pulse.](image)

The symmetric train of gradient echoes collected after the echo time of a single spin echo sequence reverse the spin dephasing that occurs within the voxel from macroscopic static field inhomogeneities. As a consequence this sequence is insensitive to the slice profile imperfection effects.
**GRASE**

This sequence, which combines gradient echoes with spin echoes, was first introduced by Oshio and Feinberg (1991). A schematic pulse sequence is shown below in Figure 3.7. A train of spin echoes is generated using a CPMG sequence (N_{SE}). Centered about each of the spin echoes a number of gradient echoes (N_{GE}) are produced by switching the polarity of the readout gradient. Compared to a Hahn spin echo imaging sequence a GRASE sequence has a speed advantage, whose factor is proportional to the total number of echoes per 90° RF pulse excitation and equals N_{SE}xN_{GE}. Moreover, due to the successive use of 180° RF pulses, GRASE nulls some of the field inhomogeneity errors seen in a Hahn spin echo sequence. Also, the GRASE sequence uses a discontinuous phase encode order during the echo train to eliminate the phase errors and chemical shifts acquired during each group of N_{GE} echoes. In essence, a GRASE acquisition is faster and has the contrast of a fast spin echo technique.
3.1. 3. Sources of errors in T2 measurements

The principle behind T2 measurement is simple, exponential fitting of the signal decay curve as a function of echo time. However, there are a lot of factors that may influence the accuracy of transverse relaxation measurements. These are briefly discussed below.

**RF Inhomogeneity**

RF inhomogeneity, resulting from imperfections in the transmit/receive field of the RF coil, may result in certain regions of the imaging volume receiving more or less than the required RF power (Simmons, 1994).
This MR instrumentation flaw at low fields (<3 T) is actually a feature of the ultra high field imaging (≥7 T). These RF inhomogeneities have the potential to create phase artifacts and stimulated echoes that ultimately result in erroneous T2 measurements. Specifically, non 90°/180° flip angles lead to the generation of additional transverse and longitudinal components of magnetization. These additional components cause T1 contamination of the transverse relaxation time in a long echo train. This problem is exacerbated when a fast spin echo sequence (FSE), with an effective TE greater than T2 and less than T1, is used for T2 estimation (Williams, 1996). However, some of the stimulated echoes can be eliminated by the application of crusher gradients on either side of the 180° RF pulse as can be seen in Figure 3.8. (Majumdar, 1985).

**Figure 3.8.** A CPMG pulse sequence with crusher gradients on the slice direction to correct for simulated echoes, as was proposed by Majumdar et al. (1985)
**Slice profile**

When multi-slice experiments are performed, slice selective RF pulses have to be applied to the sample. Unfortunately, the slice profile of the 180° RF pulse does not generate a rectangular slice profile, because the actual flip angle value decays when approaching the edge of the slice. This problem is eliminated if a single-slice multiple-echo CPMG sequence is employed, as the 180° RF pulse then does not have to be slice-selective.

Another way of avoiding imperfect slice excitation is to use an 180° refocusing pulse with a wider bandwidth than the 90° excitation pulse. This will avoid erroneous T2 estimates only if the slices are widely separated, so that cross talk between slices is minimized.

**Partial volumes**

Erroneous T2 estimates may also be associated with slice thickness, and partial volume effects. A thin slice is desired to avoid introduction of multi-exponential decay into the relaxation curve, especially in brain T2 measurements, where tissues with different T2s are in close proximity.

**3.1.4. Influence of number of echoes and interecho times in T2 estimates**

Absolute T2s are difficult to measure, as the estimated transverse relaxation time for a certain tissue is affected by pulse sequence types, such as single spin echo and multi-echo sequences, and imaging parameters such the
number of echoes and inter-echo time, as it is explicitly shown by the equation 3.3, and its discussion on section 3.1.2.

**Numbers of echoes**

The number of echoes is very important for an accurate and reproducible T2 determination. For example, consider the in vivo T2 relaxation of the human brain. There is no clear method or a specific number of echoes that will guarantee data reproducibility. However, it is possible to obtain a T2 estimate even if just a dual-echo acquisition method is employed, because an exponential decay can be computed with just two data points. Unfortunately, simulations and measurements (Whittall, 1999) concluded that mono-exponential fits to two echoes give a poor estimate for T2 calculations. The same study of Whittall et al. (1999) suggested that the best choice for T2 measurements must use a multi-echo sequence and a multi-exponential analysis to account for the fundamental differences in microscopic structure of the white and gray matter, as well as partial volume averaging. The iron presence in the brain complicates things further. The same authors (Whittall, 1999) suggested as a gold standard a 32-echo CPMG sequence, with a 10ms inter-echo time, and multiexponential analysis for T2 determination in the brain.

**Interecho time**

The other parameter that widely affects the T2 estimates, especially in tissue with iron deposition, is the time between echoes in a multi-echo
sequence. There is a clear dependence between the acquired signal decay over time and the echo spacing. This is related with the influence of the diffusion time of water protons, in the range of milliseconds, in the magnetic field. According to Vymazal et al (1995) this longer diffusion time is explained in vivo either by the decreased diffusion coefficient of water in tissue compared with free water, or in both in vivo and in vitro by the increase in size of the magnetic inhomogeneities (Vymazal, 1995). For example, in the brain the apparent diffusion coefficient decreases in comparison with water or blood, and due to the clustering of ferritin in the organells the size of the magnetic inhomogeneity increases. Both these effects have their influence on the choice of the echo time TE, and T2 calculated values. The same study of Vymazal et al. (1995) showed that for both blood and the globus pallidus the transverse relaxation times were highly dependent on the choice of interecho times, which were inversely proportional to the measured T2.

Moreover, increased efforts were made to reduce the variability in T2 estimates by concentrating on both number of echoes and echo spacing. A systematic examination using simulations of echo spacing and number of echoes needed for T2 measurements, done by Song et al. (2007), demonstrated that the optimal total data acquisition time (interecho time $\tau \times$ number of echoes N) for echo trains in single exponential decay spin echo sequences has to be twice the transverse relaxation time. This is true as long as the bandwidth is kept constant during the acquisition. For GESFIDE sequence the optimum acquisition time for each echo train was shown to be approximately equal to T2*.  


All of these considerations suggest that an absolute value for T2 is hard to determine, but optimization and comparison of the T2 estimates are possible if the sources of variations are known and taken into consideration.

Most likely the simple mono-exponential decay used for T2 determination is incomplete and does not account for diffusion effects in the magnetic inhomogeneity field, such as the one induced by iron in the brain. Rather, when brain tissue iron is involved, a multi-exponential analysis for T2 measurement is appropriate as the iron deposits will induce gradients effects from susceptibility and diffusion of spins. For example, the term $\gamma^2 G^2 D$ from equation 3.3 most likely assesses the influence of iron in the T2 measurement.

Shown later in this chapter, are our T2 estimates, which were used for a comparison of a dual echo sequence and an eight-echo GRASE acquisition. The comparison was aimed at bringing out the differences in T2 values, mostly to assess the brain iron deposition. By choosing the long inter-echo time $\tau$ for the dual echo, compared with shorter $\tau$ for GRASE sequences, we expected dual echo sequence to be more sensitive to iron than GRASE eight echoes were. We were limited in our choice of long $\tau$ with many echoes by the fact that at ultrahigh field (7T) the effective echo time $TE >> T2$, and as a consequence the signal is lost in the noise level after the first few echoes.
3.2. BRAIN IRON AND TRANSVERSE RELAXATION T2

Magnetic species such as iron have the ability to influence tissue MRI contrast by affecting T1 and T2 relaxation times. This effect is dependent on iron concentration and the physical-chemical environment in which iron is located. The non-heme iron with sufficient concentration to generate endogenous MR contrast in human tissue is ferritin and hemosiderin (Schenck, 2003). As it was shown in Chapter 2 of this dissertation, brain-iron, which is mostly in the form of ferritin, is region and age dependent (Halgreen, 1958). Brain tissue iron in ferritin/hemosiderin deposits is shielded from the water by the protein shell, and affects the MR contrast through the outer sphere mechanism by dephasing the spins of the protons that diffuse in their vicinity. The dephasing of the spins shortens the brain T2, which shows up as darker areas in T2 weighted images, the relative intensities depending on iron-tissue concentration. Conversely, small magnetic ions in solutions, such as contrast agents, affect MR contrast through the inner sphere mechanism. This process involves a direct contact with the water molecules, resulting in T1 shortening and the presence of hyperintense areas in T1 weighted images (Schenck, 2003). Due to the outer sphere mechanism, the iron in brain tissue affects mostly T2 relaxation. This effect is increased at higher magnetic fields because the magnetization of iron increases and affects more diffusing spins. The difference between the behaviors of the ferritin molecules homogenously distributed in solution and the ferritin clusters most likely found in brain tissue, is reflected in different T2 values.
Since heterogeneously distributed brain iron was recognized as having an influence on T2 relaxation (Drayer, 1986), there has been an intense interest focused on T2 shortening and the quantitatively assessment of brain iron in vivo. As already mentioned (section 3.1.3) the heterogeneous loss of brain signal in T2 weighted images with a long echo time is attributed to water molecule diffusion in the vicinity of the microscopic field inhomogeneities created by iron deposition. Many MR studies have looked at the relation between this contrast mechanism and brain iron localization in an attempt to better understand brain pathology that involves iron abnormalities. However, the complexity of modeling tissue iron distribution as well as the impossibility of accounting for all the other factors influence on transverse relaxation, have made a precise quantitative tissue iron calculation from a measured T2 value difficult to achieve (Schenck, 2003). Nevertheless, a multitude of studies show a direct relation between estimated human brain iron concentration and T2 measurements.

One of the first methods that found a good correlation between T2-weighted MRI images and age-based estimates of brain iron concentrations was the method proposed by Bartzokis et al. (1993). According to this method the difference in transverse relaxation rates with magnetic field strength, termed the Field Dependent R2 Increase (FDRI), is a measure of tissue iron stores, especially of ferritin. R2 data obtained with a dual spin echo sequence (TR=2500ms, TE=20, 90 ms) at high field (1.5T), was subtracted from the R2 data obtained with the same sequence but a lower field (0.7T). The regional brain FDRI behavior matched the iron postmortem distribution pattern.
(Hallgren, 1958) and indicated that this method is effective at eliminating the field independent contribution to R2, such as the dipole-dipole interaction, water content and intrinsic T2. Their study also demonstrated, through the ferritin phantom study, that ferritin is the primary factor in T2 relaxation.

A later study of the same group (Bartzokis, 1997), which involved subjects of ages 21-77, focused on FDRI effects at the level of frontal white matter, globus pallidus, caudate and putamen. This study confirmed the initial FDRI data (Bartzokis, 1993) and extended its validity to age correlation (Hallgren, 1958), indicating it as a good method for ferritin iron quantification. The latest study involving the FDRI method (Bartzokis, 2007) illustrates age and gender related differences in estimated iron distribution that may be related with increased risk of neurodegeneration.

Another MR study, which demonstrated the interdependence between ferritin iron and both T1 and T2 relaxation time was that of Vymazal et al. (1995a). The authors of this work started from the hypothesis that 1/T1 and 1/T2 in normal gray matter have "baseline" values determined by non-iron effects, and they have to increase from these baseline values in proportion to iron concentration and magnetic field strengths, respectively. In this study the authors retrospectively analyzed T1 and T2 weighted images of 153 subjects of different ages acquired at 0.5 T and 1.5T. This work showed a linear increase in relaxation rates with field strength and age estimated iron concentrations (Hallgren, 1958), from the baseline value. The observed difference between their
results and relaxation in ferritin solutions was attributed to slower water diffusion and larger clusters of ferritin in tissue (Vymazal 1995; Gossuin, 2004).

Another study (Vymazal, 1996) used gray matter samples and iron determination techniques to correlate the relaxation times (T1 and T2) and tissue iron concentration at different magnetic field strengths. Their results showed a T1-shorthening that falls off at higher fields, a T2 shortening that is field independent and a contribution to 1/T2 that increases linearly with the field strength and becomes more important at higher fields. Moreover, all of the above effects display a good linear correlation with iron concentration. Also, this study showed an increase in 1/T2 with interecho time that levels off at interecho time $\tau=25\text{ms}$, and is a good indication of ferritin/ferritin cluster presence.

It was always clear that iron from brain tissue is not the only factor influencing T2 relaxation time. As a consequence, implementations of different pulse sequences with different sensitivities to iron presence were attempted, in order to separate the effects of iron from non-iron contributions to T2 relaxation. Ordidge et al. (1994) implemented a multi-echo sequence, which enabled measurements of both T2 and T2*, so that a more accurate T2' broadening component was determined. Ordidge claimed that his study, especially the T2', was able to provide a more specific measure of tissue iron content, compared with previous T2 measurement protocols.

Gelman et al. (1999) used a GESFIDE pulse sequence that allows multi-section maps of transverse relaxation rates R2*, R2 and R2' in a single acquisition at 3T field strength. This study showed a linear relationship
between relaxation rates $R_2$ and $R_2'$, and the regional iron concentration in gray matter as a function of age estimated from Hallgren and Sourander (1958). $R_2'$ showed more sensitivity to iron than $R_2$ estimates. Their conclusion came as no surprise, because another accepted hypothesis is that the local magnetic inhomogeneity caused by iron deposition is reflected in $R_2'$ rather than $R_2$ measurements.

The study of Hikita et al. (2005) is the most recent to look into the issue whether the local field inhomogeneity induced by iron deposits is affecting only $R_2'$ or both $R_2$ and $R_2'$. By using a GESFIDE sequence and a multiple spin echo sequence (MSE), the authors found that $R_2$ from both sequences shows correlation with estimated brain iron as a function of subject’s age (Hallgren, 1958). Moreover, it was seen that $R_2'$ could not detect differences in iron deposition between basal ganglia and cortex and was easily affected by susceptibility artifacts. Hikita et al. concluded that in the issue of sensitivity to iron deposition $R_2$ from GESFIDE is the most robust between the three sequences (higher slope of the linear regression line). On the issue of measurement precision, the most precise results were provided by $R_2$ from MSE (highest correlation coefficient). They also concluded that a method as simple as calculating $R_2$ is enough to estimate the amount of iron in the basal ganglia of a healthy population.

Ye et al. (1996; 1996a) proposed a CPMG sequence with different interecho times, which was based on the model of water diffusion in the field gradients produced by heterogeneous distribution of cellular susceptibility
caused by ferritin. The hypothesis of this study was that glial accumulation of iron gives rise to local, highly nonuniform paramagnetic susceptibility, which produce field gradients over spatial length of order of tens of microns. Diffusing water molecules sample such magnetic field heterogeneity in a time that is typical for interecho times of CPMG pulse sequences. In consequence, the apparent relaxation rate becomes function of interecho time. Their study (Ye, 1996a) concluded that T2 measurements with different $\tau$ may prove efficient for disease progression evaluation in neurodegenerative diseases that show iron content increase in gray matter.

A lot of effort was also concentrated in analytical and quantitative determination of signal behavior in the presence of iron, especially in the brain gray matter (Jensen, 2000; 2000a; 2001). In one of these studies (Jensen, 2000), the iron is represented as microscopic spatial inhomogeneities in the static magnetic field. Their theory applies when the magnetic inhomogeneities are week in magnitude and the nuclear spins diffuse a significant distance in comparison with a length scale of the inhomogeneity. The theory was also used to fit experimental data for the dependence of the relaxation rate on the interecho time for a CPMG sequence, from an experiment that used samples of human brain gray matter. Another study (Jensen, 2001) proposed a quantitative model for computing the dependence on the interecho time of the relaxation rate in iron rich gray matter obtained with a CPMG sequence. The model represents iron bearing oligodendrocytes as identical magnetic spheres arranged in a spatially random pattern, and approximates water diffusion as isotropic and
unrestricted. Numerical predictions of this model were calculated using Monte Carlo simulations. Their model seemed to provide a good fit to experimental measurements of in vitro samples of monkey brain at 1.0 T and 1.5 T, and claimed to have a potential use in neurodegenerative diseases.

Nevertheless prolonged controversy exists about what exactly creates T2 relaxation in the brain. It is acknowledged that the variations in T2 relaxation of various regions are based on the differences in non-heme iron concentration, water content, diffusivity and cytoarchitecture (Georgiadis, 2001, Bartzoskis, 2004a), without a clear measure of their individual influence on transverse relaxation. It has also been argued that iron from deoxyhemoglobin plays a significant role in the brain tissue relaxation (Schenck, 2004), and this effect must be stronger at higher magnetic fields (Yablonskiy, 1994; Hoogenraad, 2001). Despite this controversy, the regional shortening of T2 that correlates with iron stores and age increase, the transverse relaxation behavior at higher fields, and the good correlation with imaging of pathological brain iron deposition support a major contribution of brain iron stores to the T2 relaxation (Schenck, 2004). As it was already mentioned the T2 decreases with increasing magnetic field, and as such a higher sensitivity to the iron effects and their action on the transverse relaxation time is expected at ultra-high magnetic field.
3.3. DUAL ECHO AND GRASE T2 ESTIMATES AT 7T AND 3T

3.3.1. Introduction

Based on the effects of paramagnetic iron on $T_2$ and $T_2^*$ relaxation, it has been known for over 20 years that MRI is a sensitive indicator for brain iron content (Drayer, 1986). Consequently, there is a large body of work aimed at finding the quantitative relationship between brain iron and $T_2$ and $T_2^*/T_2'$ measurements. One line of research includes correlation of in vivo $T_2$ with measured tissue iron in an animal brain such as the Rhesus monkey (Vymazal, 1996; Hardy, 2005), or postmortem normal and diseased human brains (Vymazal, 1996; House 2007). Another area of inquiry focused on the correlation of brain iron, estimated from subject’s age based on published histochemical data (Hallgren, 1958), to $T_2$ or $T_2'$ measurements (Vymazal, 1995 and 1999; Gelman, 1999; Hikita, 2005; Wang, 2006).

Despite this ongoing and significant effort, there are limitations and controversies. A clear linear dependence between $T_2$ and estimated iron was shown for the basal ganglia (which includes the caudate, putamen, and globus pallidus), at all studied field strengths (Vymazal, 1999; Gelman, 1999; Hikita, 2005). On the other hand, less agreement was achieved for the cortical white and gray matter, which seems to switch contrast and vary depending on position. For example, Zhou et al. (2001) reported gray/white matter contrast flip in the occipital lobe at 1.5T, which was contradicted by the study of Stefanovich et al. (2003) at the same field strength.
Moreover, significant efforts were also focused on the modeling and analytical characterization of water relaxation behavior in ferritin’s susceptibility fields (Ye, 1996; Jensen, 2000; 2001). While a significant correlation between transverse relaxation and iron deposition is undeniable, a series of factors make the estimated T2s vary widely across studies.

Despite controversies and limitations, the regional shortening of T2 correlates with iron stores and increased age, in both the normal and diseased brain. These suggest that a major contribution to the transverse relation is given by tissue iron deposition (Schenck, 2004).

Availability of a sensitive tool for brain iron measurement is of significant interest, because brain iron is known to change in a number of neurological disorders. Some of these include Multiples Sclerosis (MS) (Bakshi, 2001; Whittall, 2002), restless leg syndrome (Earley, 2006; Allen, 2001), and Parkinson’s (Bartzokis, 1999), Alzheimer’s (Bartzokis, 2000) and Huntington’s (Bartzokis, 2007) diseases. A sensitive and non-invasive tool for brain iron measurement would allow for serial studies following disease progression. This would help elucidate the role of iron in disease processes by evaluating if iron change is a cause or consequence of a specific disease. It could also monitor disease progression in treatment protocols.

Lastly, the availability of T2 and T2* values for different brain regions at 7T will be highly valuable for contrast optimization in a wide range of pulse sequences for ultra-high-field MRI. Presently, there exists only limited availability of such data (Whitaker, 2004; Peters, 2007).
The goal of this work was to evaluate standard commercially available sequences for T2 measurement at 7T in different regions of the brain in healthy volunteers of different ages. To the best of our knowledge, data comparing 7T T2 versus age is not yet available. In addition to providing 7T T2-values for contrast optimization, this work can help to assess shortcomings with commercial sequences for T2 measurements, and identify techniques to further improve accuracy and sensitivity of T2-based brain iron assessment. For comparison, T2 measurements were also done at 3T using almost identical sequences.

3.3.2. Materials and Methods

Subjects:

For the MR study, we imaged 22 subjects of ages 18-58 years (mean 37 years, median 34.5 years) at 7T, and 17 subjects of age 18-70 years (mean 40.7 years, median 40 years) at 3T. A total of 14 subjects were scanned at both field strengths, and 9 of them were over 30 years of age. The study was approved by the OSU Institutional Review Board, and consent and HIPPA forms were signed by all participants prior to the MRI study.

Acquisition:

Our study was conducted using Philips Achieva 7T and 3T MRI systems with vendor supplied transmit-receive head coils. The imaging protocol included two T2 acquisition sequences, Dual Spin Echo and GRASE, which
were run with different times between refocusing pulses, in order to obtain three acquisitions at each of the investigated field strengths. A total of 10 slices of 3mm thickness with a 9mm gap to avoid cross talk were acquired with a TR=2000ms, FOV=230x172mm$^2$ and NSA=1. Independent of the acquired matrix size of the imaging voxel, all MR images were reconstructed by zero-filling k-space to a 512x512 matrix size, resulting in a reconstructed 0.45x0.45x3mm$^3$ image voxel size. Details of the acquisition sequences are as follows:

1) Dual Echo sequence, with TE=10/50ms at 7T and TE=10/60ms at 3T, an acquired voxel size of 0.9x1.2x3mm$^3$ in a 9:48 min scan time at 7T, and 6:30 min at 3T. This acquisition will be referred as DUAL-long $\tau$ in the rest of the paper.

2) Eight-echo GRASE sequence with TE from 21ms to 168ms, a time $\tau$=21ms between the $120^0$ refocusing pulses, three gradient-echo acquisitions per spin-echo and an acquired voxel of 0.9x1.2x3mm$^3$ in a 5:06 min scan time at 7T and a 2:16 min at 3T. This sequence will be referred to as GRASE- $\tau$21 in the following paragraphs.

3) Eight-echo GRASE sequence with TE from 9ms to 72ms, a time $\tau$= 9ms between the $120^0$ refocusing pulses, three gradient-echo acquisitions per spin echo, and an acquired voxel of 1.8x2.4x3mm$^3$ in a 2:16 min scan time at 7T and a 1:12 min at 3T. This sequence will be referred to as GRASE-$\tau$9 below.

In all cases, the longer scan time at 7T compared to 3T is due to the specific absorption rate (SAR) limitations. The scanner software and
hardware settings force multiple acquisition passes to limit RF power deposition at 7T.

It is well understood that the pulse sequences used in this work are sub-optimal for T2-measurements. They were merely chosen because of their availability as standard sequences on both 3T and 7T scanners, and because they allowed for imaging with different times ($\tau$) between refocusing pulses. This is important because of different sensitivities to diffusion between the Hahn spin echo sequence and CPMG sequences. As such, spin echo sequences with different interecho spacing give different T2-values when single exponential fitting is applied.

**Limitations/Justification for DUAL echo:**

Using a dual-spin-echo sequence instead of multiple spin Hahn echo sequences is a compromise that shortens scan time and avoids problems with subject motion between longer individual scans. The time between refocusing pulses was selected to be slightly longer than T2 to maximize effects from spin diffusion in the susceptibility field from paramagnetic iron. However, having only two data points restricts the freedom of T2 fitting, thus the resultant T2 becomes dependant on the choice of the two TE’s. Furthermore, T2 measurements are dependent on flip angle variations due to RF field inhomogeneity at 7T, as well as imperfect profiles for the slice selective refocusing pulses in the multi-slice scans (Poon, 1992; Sled, 2001). The last problem was somewhat reduced by using slice spacing which was thrice the slice thickness (3mm slice with 9mm gap, respectively).
**Limitations/Justification for GRASE:**

A GRASE sequence with eight refocusing pulses and three (epi-factor) gradient echoes for filling k-space was used instead of a standard multi-echo sequence. This introduces some susceptibility artifacts and blurring, but speeds up the acquisition and minimizes SAR limitations at 7T. For example, a multi-echo (non-GRASE) acquisition needs an approximately twenty minute acquisition time to acquire a data set similar to a GRASE-$\tau_{21}$. This is because the scanner forces multiple acquisition passes as the result of SAR increase at higher field strength.

Also, SAR limits dictated the use of the 120 degree refocusing pulses instead of 180 degree ones. This may contaminate T2-measurement with T1 effects introduced by stimulated echoes, but non-180 degree refocusing pulses were mandated by 7T SAR limits. As with the dual-spin-echo sequence, B1 inhomogeneity and slice profile imperfections further contaminate T2 measurements. Finally, the resolution of the GraSE-$\tau_{9}$ scan was lowered to compromise between getting short interecho spacing and maintaining sufficient resolution to differentiate the brain regions to be analyzed.

**T2 Analysis:**

The T2 images were reconstructed off-line from unprocessed image data (Philips, ParRec format) using IDL software (Interactive Data Language, ITT, Boulder, Co). Regions of interest (ROIs) were manually traced in each hemisphere of the brain to include 7 brain regions: frontal and motor white
and gray matter, caudate, putamen and globus pallidus. For the DUAL-long τ, T2 values were obtained using the equation $T_2 = \frac{(TE_2 - TE_1)}{(\log SE_1 - \log SE_2)}$, where SE1 and SE2 are the mean pixel intensity values at the short and long echo, respectively. Standard deviation estimates were calculated from error propagation equations. For GRASE acquisitions, T2 estimates were obtained from the nonlinear least-squares-fit of the mean pixel values for the multiple echo measurements at each echo time. Two $\{A \exp(-T_2/\text{TR})\}$ and three parameters $\{A \exp (T_2/\text{TR}) + K\}$ fits were tested, but due to the use of only eight points corresponding to the eight echoes, two parameters fitting was used throughout. Additionally, exclusion of the first echo, (which sometimes had lower signal than the second echo) was explored. Because of the resulting non-significant alteration of the T2 estimates, we included all eight acquired echoes. Finally, the T2 estimates for each individual brain region were obtained as a mean of the left and right hemisphere estimates.

3.3.3. Results

In the ROIs measured for T2 analysis at 3T, SNR ranged from 137 to 110 in the first echo (DUAL–long τ and GRASE-τ9), from 50 to 35 in the second DUAL-long τ, and from 10 to 6 in the last (TE=168ms) GRASE-τ21 echo. The DUAL-long τ at 3T showed good image contrast between different tissue types, especially in the second echo, even if the signal intensity compared to the first echo seems to decrease noticeably. The GRASE-τ9 image contrast evolves from a proton density (PD) weighted image in the first echo to a T2 weighted
image at the last echo (CSF being the brightest brain region). While the last echo signal intensity is still well above noise level, the low acquired image resolution contributed to the blurred appearance of the brain regions. This made it a difficult task to distinguish different brain regions, as well as differentiating gray and white matter. GRASE-<i>τ</i>21 allows better differentiation between tissue types, due to the higher acquired image resolution. Its contrast is more similar to the DUAL–long <i>τ</i> contrast.

For 7T, SNR ranged from 450 to 150 in the first echo (DUAL-long <i>τ</i> and GraSE-<i>τ</i>9), from 55 to 40 in the 2<sup>nd</sup> DUAL-long <i>τ</i>, and from 15 to 5 in the last (TE=168ms) GRASE-<i>τ</i>21 echo. The DUAL–long <i>τ</i> at 7T, while having higher SNR than the 3T DUAL–long <i>τ</i>, is contaminated by B1 inhomogeneities that result in regional loss of the signal. These losses exhibit either laterally or in the middle of the image, depending on the shape of the head. This is more obvious in the second echo (TE=50ms). GraSE-<i>τ</i>9 has less contrast than DUAL–Long <i>τ</i> 7T images, but more than the contrast seen at 3T GRASE-<i>τ</i>9, and the 8<sup>th</sup> echo signal is well above noise level. The loss of the signal due to the B1 inhomogeneities is less obvious than on the second echo of the DUAL–long <i>τ</i> sequence. Conversely, GRASE-<i>τ</i>21 shows better image contrast than GRASE-<i>τ</i>9 at 7T, but the eight echo also approaches noise level, making it difficult to distinguish between different tissue types. GRASE images also showed mild susceptibility artifacts near the air-tissue interfaces. The artifacts were more severe at 7T than 3T.
Table 3.1 lists average T2 values for the subjects over 30 years of age for different brain regions and measurement methods at both 3T and 7T. We decided to list the mean estimates of T2 for subjects older than 30 years of age, due to the fact that Halgren and Sourander (Hallgren, 1958) found in their study that iron estimates for individuals after age 30 years change very slowly with increasing age for most brain regions. Overall, T2 values are shorter at 7T than at 3T. T2 estimates increase with decreasing $\tau$ such that GraSE-$\tau$9 values are nearly twice as long as DUAL-long $\tau$ T2 values, both at 3T and 7T. Also, note that in the T2 for frontal GM is longer than T2 for adjacent WM; but in the motor area the WM and GM T2 estimates are inverted. Specifically, motor WM T2 values are about 15% above the motor cortex T2 at both 7T and 3T.

Figure 3.9 shows the resultant contrast inversion in T2-weighted images at 3T and 7T Dual-long $\tau$, (2$^{nd}$ echo) for a 52 year old subject. It is clearly seen that in the frontal area GM signal is higher than WM signal, but in the motor area GM signal is lower than WM signal. This pattern is more obvious in older than in younger subjects and more obvious at 7T than at 3T. This observation and T2 estimated values (Table 3.1) are consistent with the published iron content in frontal GM, frontal WM, and motor GM of 2.92±0.41, 4.24±0.88, and 5.03±0.88 mg/100g wet for subjects over 30 years of age (Hallgren, 1958).

Figure 3.10 and 3.11 shows graphs of R2 (DUAL-long $\tau$ sequence) versus iron content, computed from the subjects’ age using published data (Hallgren, 1958) for six individual tissues. Figure 3.12 illustrates graphs of R2 versus iron for all tissues combined, and all three interecho time intervals $\tau$. 
<table>
<thead>
<tr>
<th>Field Strength</th>
<th>7T</th>
<th>3T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>$\tau$ (ms)</td>
<td>40</td>
<td>21</td>
</tr>
<tr>
<td>FGM</td>
<td>34.9±2.5</td>
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<tr>
<td>FWM</td>
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<td>57.7±10.5</td>
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<tr>
<td>MGM</td>
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<td>63.9±26.0</td>
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<tr>
<td>MWM</td>
<td>32.1±4.5</td>
<td>79.0±27.0</td>
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<tr>
<td>Caudate</td>
<td>28.4±2.3</td>
<td>56.8±10.6</td>
</tr>
<tr>
<td>Putamen</td>
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<td>51.0±0.7.0</td>
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<tr>
<td>Pallidus</td>
<td>19.9±2.2</td>
<td>38.2±2.4</td>
</tr>
</tbody>
</table>

Table 3.1. Average T2 for subjects over 30 years of age for 7T and 3T, with different acquisition sequences.
Figure 3.9. Second echo images of a 52 years old subject from DUAL-long τ acquisitions at 7T (upper row) and 3T (lower row). Observe the inverted contrast between white and gray matter in frontal (left) and motor (right) areas (white arrows).
Figure 3.10. DUAL-long $\tau R_2$ versus mean iron as estimated from subjects' age (Hallgren, 1958) at 7T (left) and 3T (right) for frontal gray matter (FGM), frontal white matter (FWM), and motor gray matter (MGM). The curves illustrate the 95% prediction interval of the regression and the straight lines represent the regression lines. Data points are illustrated as mean values with standard deviation error bars for R2 estimates. Statistically significant correlation is found for all three listed regions at 3T, but just for only MGM at 7T.
Figure 3.11. DUAL-long $\tau$ R2 versus mean iron as estimated from subject's age (Hallgren, 1958) at 7T (left) and 3T (right) for caudate nucleus (CAU), putamen (PUT), and globus pallidus (GP). The curves illustrate the 95% prediction interval of the regression and the straight line represents the regression line. Data points are illustrated as mean values with standard deviation error bars for R2 estimates. At both 7T and 3T there is statistically significant correlation between R2 and estimated iron content for the three listed regions.
Figure 3.12. R2 versus mean iron for DUAL-long $\tau$ (upper row), GRASE- $\tau_{21}$, and GRASE- $\tau_{9}$ at 7T (left) and 3T (right) for all combined six brain regions. All graphs show statistically significant correlation between R2 and estimated iron content.
Regression analysis statistics for all sequences are listed in Table 3.2. Except for the frontal GM and WM at 7T, all DUAL-Long $\tau$ measurements for the individual tissues show significant correlation between R2 and iron. Likewise, all sequences show strong correlation for all tissues combined. Conversely, correlation between R2 and iron for the GRASE sequences was only observed at 3T for the high iron content tissues (caudate, putamen, and globus pallidus). No correlation was observed with GRASE at 7T, except for the putamen in the GRASE-$\tau$9 sequence. In Table 3.2, also note that the slope of the correlation increases with increasing $\tau$. This indicates that the Dual-long $\tau$ sequence is more sensitive to tissue iron. Finally, for the combined data for all tissues (Figure 3.9 and Table 3.2), the R2 versus iron slope is larger for 7T than for 3T indicating increased sensitivity at 7T.
<table>
<thead>
<tr>
<th>Brain Region</th>
<th>7T Dual</th>
<th>GraSE (8echoes)</th>
<th>3T Dual</th>
<th>GraSE (8echoes)</th>
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</thead>
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<tr>
<td>FGM</td>
<td>τ (ms)</td>
<td>p</td>
<td>Rsqr</td>
<td>slope</td>
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<td>40</td>
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<td>9</td>
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<td></td>
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<td>Globus</td>
<td>p</td>
<td>0.025</td>
<td>0.006</td>
<td>0.85</td>
</tr>
<tr>
<td>Pallidus</td>
<td>Rsqr</td>
<td>0.225</td>
<td>0.942</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>slope</td>
<td>1.4</td>
<td>-3.2</td>
<td>-0.14</td>
</tr>
<tr>
<td>All Brain Regions</td>
<td>p</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Rsqr</td>
<td>0.676</td>
<td>0.562</td>
<td>0.764</td>
</tr>
<tr>
<td></td>
<td>slope</td>
<td>0.96</td>
<td>0.59</td>
<td>0.73</td>
</tr>
<tr>
<td>Subjects</td>
<td>21</td>
<td>4</td>
<td>13</td>
<td>17</td>
</tr>
</tbody>
</table>

Table 3.2. Summary of the statistical regression analysis correlating R2 and estimated regional brain iron content. The statistically significant correlations between R2 and iron are represented by gray shading.
The second echo of the DUAL-long $\tau$ sequence showed the contrast inversion between white and gray matter in the occipital cortex, and helped clarify the controversy (Zhou, 2001; Stefanovich, 2003) regarding the change in contrast between white and gray matter in the occipital lobe compared to frontal lobe. The white-gray matter contrast inversion is better visualized at 7T than at 3T (Figure 3.13).

**Figure 3.13.** 7T and 3T DUAL-long $\tau$ magnified images of occipital region that show the same brain location in a 52 year old subject. Observe the inverted gray-white matter contrast (gray matter signal lower than white matter signal) compared to the frontal region that is visible more accurately at 7T than at 3T.
3.3.4. Conclusion & Discussion

In summary, the measured T2 values for all the investigated brain regions illustrate the known behavior of T2 with increasing field, increasing iron concentration and inter-echo time variability. Specifically, the T2 values for each of the brain regions at 7T were lower than T2 values at 3T. The increase in the estimated iron content resulted in a shortening of measured T2 values at both 3T and 7T. Also, the increase in the inter-echo time resulted in a decrease in the T2 value.

Our estimated R2 values from the DUAL-long $\tau$ showed significant correlation with estimated brain iron for all six analyzed brain regions at 3T, and only for four regions at 7T. At 7T, the frontal white and gray matter R2 measurements failed to show any correlation with estimated iron. The R2 values from GRASE sequences failed even more at 7T, as only the putamen for GRASE-$\tau$9, and the combined analysis of all brain regions showed significant correlation between T2 measurements and estimated iron. Once again 3T behaved more appropriately, as GRASE sequences showed significant correlation between measured T2 and iron for three out of six investigated regions.

Both sequences reflect tissue water mobility and effects from iron, such as water diffusion in the susceptibility field gradient due to inhomogeneously distributed iron. DUAL-long $\tau$ is more sensitive to iron than GRASE-$\tau$9, and this is expected from the models and is experimentally seen in our 3T data. Since both water mobility and iron affect all sequences, albeit
with different sensitivity, one might expect that GRASE would show correlation only for high iron tissue. For low iron tissue, the weak contribution from iron to the total measured T2 is no longer observed, as can be observed from 3T results.

The failing of dual echo sequence to show correlation between T2 and iron in the frontal area at 7T is probably due to the B1 inhomogeneities, which are more prominent at higher field. Moreover, the same is true for GRASE sequences, which are even more sensitive to the magnetic field inhomogeneities. It is known that flip angle deviations may lead to erroneous T2 measurements. This has been extensively evaluated for imperfect refocusing pulses (Poon, 1992; Sled, 2001), and as such, the 120 instead of 180 degree refocusing pulses for GRASE would introduce errors in T2 estimates. However, the present work gives fundamentally the same results as single slice multi-echo at 8T using 180 degree refocusing pulses (Whitaker, 2004), and Laser sequence (Bartha, 2002). The effects of non-90 degree excitation pulses due the B1 inhomogeneity at 7T have not yet been evaluated in detail either theoretically or through phantom studies.

In conclusion, while this still has to be proven through phantom studies, we speculate that B1 inhomogeneity is the cause for the significant stray in T2 values at 7T, especially for GRASE, and thus the lack of significant correlation between R2 and estimated iron.

Our study demonstrated that T2 measurements, using the commercially available sequences at 7T, are not ideal to obtain an accurate T2 estimate for brain tissue. Corrections for B1 inhomogeneity have to be
implemented in order to gain more precise information on the transverse relaxation of the brain and its relation with estimated brain iron.
4.1. INTRODUCTION TO MAGNETIC SUSCEPTIBILITY

4.1.1. Introduction/Types of susceptibility

Magnetic susceptibility is a measure of the degree of magnetization of a material in response to an applied magnetic field. In other words, magnetic susceptibility describes the change in magnetic field when an object is placed in that field, as shown by equation 4.1.

\[ B = B_0 (1 + \chi) \]  

Where \( B \) is the magnetic field through the object, \( B_0 \) is the main magnetic field, and \( \chi \) is the magnetic susceptibility.

The main magnetic field can be either enhanced by the presence of the object (\( \chi > 1 \)), which means its molecules align with the field and so the material is called paramagnetic, or it can be weakened (\( \chi < 1 \)) as the molecules tend to slightly oppose the field, in which case the material is a diamagnet. The perturbation created in the magnetic field by an object tends to be larger at
higher magnetic fields, as the magnetic susceptibility effects are stronger at higher magnetic fields.

Almost all tissue types are diamagnetic due to the high water content. Sources of paramagnetic susceptibility in brain are both intracellular deposited iron (non-heme iron), as well as iron from vascular blood (heme iron). While overall the brain tissue is diamagnetic, the paramagnetic effect of iron particles manifests locally and has an influence on phase and T2*/T2 relaxation of the local spins. This is due to the diffusion of spins in the intravoxel magnetic susceptibility fields created by iron, which affects the phase of the spins, as well as shortening the transverse relaxation times.

Magnetic susceptibility effects in MRI may be divided, based on the spread of the susceptibility effects into macroscopic, mesoscopic, and microscopic susceptibility effects.

**Macroscopic susceptibility effects**

Their action, which manifests over several voxels, is due to the neighboring of materials with large magnetic susceptibility differences, such as paramagnetic materials adjacent to diamagnetic materials. The tissue-air interface is one of the clearest examples for this phenomenon. When such materials of opposite susceptibility are subject to MR imaging, the resulting image shows loss of acquired signal at the tissue interface or misplacement of the signal that may be mistaken for pathology. Water protons will precess at slightly different frequencies due to the different magnetic fields they
experience. As a result, the reconstructed MR image shows distortions, regional signal voids, and/or signal enhancements depending on the pulse sequence used for MR imaging. Gradient echo images illustrate mostly signal voids and distortions, while spin echo images, due to the 180° refocusing pulse that corrects for some spins dephasing, display only distortions, not signal loss.

**Mesoscopic susceptibility effects**

These effects are generated by intravoxel dephasing mechanisms, resulting from the presence of small magnetic perturbers in both endogenous (i.e. deoxygenated blood in vessels, iron/iron clustering in tissue) and exogenous (i.e. iron based contrast agents) forms.

Mesoscopic susceptibility is characterized by the signal behavior in magnetically inhomogeneous tissues, which is modeled and characterized based on the magnetic, geometric and dynamic properties of the system (Hardy, 1991; Kennan, 1994). According to Kennan et al., when diffusion is fast with respect to the intravoxel magnetic field variations, then the system is in a motionally narrowed regime, and the signal loss is similar in spin echo and gradient echo sequences. If the dephasing of the spins and the loss of signal occur faster than the diffusion phenomena manage to average out the phases of different nuclei, then the regime is known as a static dephasing regime. In this regime, the magnetic field inhomogeneities are the main influence in the loss of signal.
This is more obvious in a gradient echo sequence, as theory suggested, and simulations and experiments showed (Yablonsky 1994; Weisskoff, 1994; Kennen, 1994).

**Microscopic susceptibility effects**

Microscopic susceptibility effects are known as outer and inner sphere mechanisms, and they are caused by molecular interactions. These effects also influence phase and relaxation mechanisms. The outer sphere mechanism manifests when magnetic particles shielded from the water by a protein shell (e.g. ferittin and hemosiderin) dephase the magnetic spins of the protons diffusing in their immediate vicinity without direct contact. The influence of the outer sphere mechanism is seen in spins dephasing and shortening of T2 relaxation time. Conversely, small iron particles that have direct contact to water molecules, such as iron based contrast agents, bond to the diffusing spins through a transfer of magnetization. This is the inner sphere mechanism, and its effect is mostly illustrated through a shortening of the longitudinal relaxation time (T1) of the tissue (Schenck, 2003).
4.1.2. BOLD MRI

Since Ogawa et al. (1990) discovered that the MRI signal in tissue space around veins decreases or increases as a function of the oxygen content of inspired air, the Blood Oxygenation Level Dependent (BOLD) effect has been mainly used in fMRI experiments performed to map brain activity related to motor, sensory, or cognitive functions. The BOLD effect is based on one biophysical and one physiological phenomenon. Deoxyhemoglobin generates magnetic field gradients through and around blood vessels that decrease the MRI signal. During brain activation there is an increase in cerebral blood flow (CBF) with a local reduction in oxygen extraction fraction (OEF), resulting in a decreased concentration of deoxyhemoglobin in blood. A drop in deoxyhemoglobin level because of increased neuronal signaling, which generates the above described mechanisms, results in an elevation of the MRI signal (Buxton, 2002).

The change in MRI signal due to brain activation is attributed to both the change in T2 relaxation of the blood (an intravascular effect), and the phase change difference between blood and surrounding tissue (extravascular effect). The individual contributions of these to the overall BOLD effect are difficult to assess and separate. However, the initial approaches to this problem were the analytical models (Kennen, 1994) and the Monte Carlo (MC) simulations (Weisskoff, 1994; Boxerman, 1995). These have also taken into consideration the degree of oxygenation, size of the blood vessels, rate of water diffusion, pulse sequence, echo time and field strength, in an attempt to
understand and take advantage of the BOLD effect. It is now clearly established that all of the above factors have their specific influences on BOLD MRI. Gradient echo (GE) acquisition has been shown higher sensitivity to the BOLD effect compared to spin echo (SE) acquisition, suggesting that the primary effect of the reduction of blood deoxyhemoglobin is an increase in the local T2* value (Buxton, 2002).

Hoogenraad et al. (2001) combined analytical models with three GE experiments followed by volume fraction analysis. This was done in an attempt to clarify the influence of extravascular and intravascular effects on an in vivo BOLD experiment. By using inversion recovery sequences (IR) in two of their multiecho experiments, the authors were suppressing the signal for either gray matter (GM) or cerebrospinal fluid (CSF), discriminating between the intravascular and extravascular BOLD models. The conclusion of their study was that the major influence on the signal change in a GE experiment comes from the extravascular signal dephasing around single veins in GM and CSF. The extravascular dephasing effect in GM around capillaries, phase dephasing of blood, and blood T2 changes due to oxygenation proved to be minor influences in BOLD signal. This conclusion suggests the mechanism involved in BOLD fMRI is macroscopic rather than microscopic susceptibility.
4.1.3. Susceptibility Weighted Imaging

Due to the complex nature of the acquired MRI signal, it is always possible, to generate a magnitude and a phase image with a single MR acquisition. For example, the complex signal in a 2D acquisition can be expressed at each pixel location by the relation:

\[ S(x, y) = S_0(x, y)(\cos \varphi + i \sin \varphi) = \text{Re}S(x, y) + i \text{Im}S(x, y) \]  \hspace{1cm} 4.2.

Where \( S_0(x, y) \) is the signal magnitude, \( \varphi \) is the accumulated phase of the spins and \( \text{Re}S(x, y) \) and \( \text{Im}S(x, y) \) represent the real and the imaginary component of the signal.

However, the phase image or phase map is obtained from:

\[ \varphi (x, y) = \tan^{-1}\{\text{Im}S(x, y) / \text{Re}S(x, y) \} \]  \hspace{1cm} 4.3.

The inverse tangent function is periodic over the \((-\pi, \pi)\) interval, making the phase image limited to this interval; although the true phase value may take any real value. Any phase values outside of \((-\pi, \pi)\) spread will be wrapped back into the allowed interval by adding or subtracting \(2\pi\) from the real phase value. As a result, spins with phase values differing by multiples of \(2\pi\) would have the same intensity (Haacke, 1999) in the phase intensity map. Figure 4.1 shows an example of magnitude (A) and unwrapped (B) phase image of the brain at 7T.
Figure 4.1. Magnitude (A), aliased phase image (B), and high pass filtered phase image (C) of a 18 years old subject at 7T.
Phase aliasing, as well as the multiple sources of phase in MRI, make the extraction of phase information a difficult process. This may explain why phase images are many times ignored, rather than used in clinical MRI.

Sources of phase variation may include strong magnetic field inhomogeneities, such as imperfect shim or tissue-air interface susceptibility differences, as well as less obvious susceptibility differences between structures, such as deoxygenated blood and surrounding tissue or parenchymal iron induced susceptibility differences. Fortunately, field variations from imperfect shim or large tissue-air cavities have low spatial frequencies. In other words, the changes they generate in the phase shifts of spins are rather slow in comparison to the phase changes generated by subvoxel susceptibility effects that give rise to high spatial frequencies. These frequency characteristics help in separating susceptibility effects. By applying a high-pass filter to the phase images both aliasing of phase and enhancement of the small anatomical structures are obtained, making the phase images important sources of unique anatomical information. Figure 4.1.C shows an example of a phase image obtained after application of a high pass filter which removed the wrapping of the phase and enhanced small brain tissue structures.

The spin echo sequence, due to the $180^\circ$ rephasing pulse, corrects for some of the acquired phase of the spin especially due to the local inhomogeneities in the static regime. Conversely, the gradient echo sequence which does not have a refocusing pulse is more sensitive to intravoxel phase accumulation, while at the same time being plagued by all other susceptibility
sources. The use of a T2* weighted gradient echo sequence with a long echo time (TE), which allows for sufficient spin dephasing due to local susceptibility differences, forms the basis of the Susceptibility Weighted Imaging (SWI) method (Haacke, 2004). It is referred to as a SW image, a magnitude and/or a phase image, or a combination (sum or product) of magnitude and phase images.

In general, SWI is seen as a BOLD-sensitive method capable of visualization of small vessels in high details by exploiting the magnetic susceptibility properties of blood (Rauscher, 2005). SWI is also able to enhance WM and GM differences in the brain (Abduljalil, 2003), especially at high field where, due to the increase in T1 and decrease in T2 of the brain tissue, the white-gray matter contrast in magnitude images is diminished. Another application in which SWI showed the capacity to separate tissue susceptibility effects is the comparison of iron loaded tissues to the background tissue (Haacke, 2005).

In conclusion, SWI is able to pick up any susceptibility difference between different tissues that otherwise could be missed with other MRI methods.

4.1.4. Phase images and their relation to brain iron

As was already mentioned before, the contrast in magnitude image is a function of the proton density (PD) and the intrinsic properties of the tissue, such as T1, T2, and/or T2*. In a phase image the contrast is determined by the differences in local precession frequencies, due to bulk magnetic
susceptibility differences of tissues or different levels of blood oxygenation. By getting both magnitude and phase images one is able to obtain two images that have independent, nonredundant information. A phase image can reveal anatomical information that is many times invisible in magnitude images or in a combined display of magnitude and phase images (e.g. sum, product).

The acquired phase of the spins develops as a function of the echo time according to the formula:

$$\varphi = -\gamma \Delta B^*TE$$  \hspace{1cm} (4.4)

where $\gamma$ is the gyromagnetic ratio of protons, $\Delta B$ is the change in the magnet field induced by inhomogeneity, and TE is the echo time at which the data are acquired (Haacke, 1999). The phase shifts became a function of the main magnetic field or the field difference, since the magnetic susceptibility is changed with the magnetic field. We are expecting to see either a higher phase effect when the field strength of the MRI system is increased and the TE is kept constant, or a similar phase accumulation if the $\Delta B^*TE$ is kept constant at different field strengths, i.e. shorter TEs are used.

Iron, either from venous blood (heme-iron) or deposited in the brain parenchyma (non-heme iron) is one of the main sources of phase variation, which is emphasized in a high pass filtered phase image.
When brain iron particles are part of deoxyhemoglobin molecules confined to blood vessels, the phase shift of the spins neighboring the iron is also influenced by the sizes and orientations of vessels, as well as their density, as is shown by BOLD venography (Reichenbach, 1997; 2001). However, iron deposited in the brain tissue induces phase shifts of the spins which are function of its parenchymal concentration (Haacke, 2005).

So far, brain tissue susceptibility differences in T2* weighted images with long echo time have indicated that phase quantification in phase images is a valid approach to detect iron deposited in the brain tissue (Ogg, 1999). The study of Ogg et al., conducted at 1.5T, did quantitative phase measurements in frontal GM and WM, motor GM, globus pallidus, caudate and putamen of 45 normal and diseased human subjects varying in age from 0.8 to 45 years old. Phase shifts were correlated with estimated regional brain iron depositions (Halgreen, 1958). A statistically significant correlation between estimated regional iron deposition and phase shift with age was found in all investigated brain regions, except frontal white matter. The study Ogg et al (1999) was the first and so far the only one that linearly related non-heme iron and phase shifts in the aging brain.

While both heme and non-heme iron are obviously sources of inhomogeneity in the brain, there is still a prolonged controversy about what exactly influences the T2* relaxation and the contrast to noise ratio (CNR) at high magnetic field. This is of interest due to the fact that at ultrahigh magnetic field, the MR images generated from a gradient echo acquisition with a long echo time (TE~T2*) show an improved contrast between brain tissues and
microvasculature, GM and WM, as well as between known iron loaded tissues and background brain tissue, which is more evident in phase images. This phenomenon suggests that phase images may hold the key to understanding the contrast mechanism in susceptibility weighted images at ultrahigh magnetic field.
4.2. REGIONAL PHASE QUANTIFICATION AND RELATION TO NORMAL IRON DISTRIBUTION IN THE BRAIN. 3T AND 7T RESULTS

The contrast in T2* weighted images is influenced by the subtle susceptibility differences between different tissues with different levels of blood oxygenation and/or different parenchymal iron concentrations. The controversy regarding which of these two factors, heme iron (ferritin) or non-heme iron (deoxygenated blood iron), is the main influence in contrast generation is still under heavy debate. While the study of Ogg et al. (1999) reported statistically significant correlation between phase measurements and iron concentration for almost all studied brain regions, the preliminary study of Xu et al. (2007) found a clear statistically significant correlation between phase shift measurements and parenchymal iron concentration only for the putamen and red nuclei. The latest result is closer to our findings. However, the details of our study conducted at 3T and 7T, as well as the emerging conclusions are presented below.

The primary objective of our study was to measure phase shifts at both 3T and 7T in different regions of the brains of normal individuals of different ages and to correlate these findings with calculated regional iron as a function of age using Hallgren’s equations (1958). The secondary goal of our study was to evaluate some of the factors that affect phase measurements and their variability in the brain. First, we have checked the influence of high pass filter strength on quantitative phase shift measurements and its influence on the correlation between phase and parenchymal iron concentration. Second, the relation between phase standard deviation (STD) and signal to noise ratio was
also verified in an attempt to understand the high variability of the phase shifts inside the same brain region. It is known (Hackee, 1999) that phase measurements are more accurate when the SNR of the image is high, and inaccuracy of phase shifts measurements is influenced by an image with low SNR.

4.2.1. Materials and Methods

Experimental design:

The experimental design of our study followed that of Ogg et al. (Ogg, 1999), but adjusted to the longer T1 and shorter T2 at higher fields. Ogg study done at 1.5 T used a 2D gradient echo sequence with TR/TE/flip angle= 1165/60ms/65°, FOV=230x173mm² and an acquisition matrix of 256x192 with a 5mm slice thickness.

Subjects:

We scanned 16 normal human subjects of ages 18-70 years (mean 41.1 years, median 40 year) at 3T and 15 normal subjects of age 18-56 years (mean 39.1 years, median 40 year) at 7T. A total of 14 subjects were scanned at both field strengths, and 10 of them were over 30 years of age. The study was approved by the OSU Institutional Review Board, and HIPPA forms were signed by all participants prior to the MRI acquisition.
**Acquisition:**

Our study on normal volunteers was conducted using Philips Achieva 3T and 7T MRI systems with a vendor supplied transmit-receive (T/R) head coil. The MRI protocol included a 2D gradient echo sequence (T2W_FFE) with moderately long echo time (TE~T2*), and full echoes. A total of 40 slices and 20 slices respectively with no gap between them were acquired using TR/TE/Flip angle=1300/25ms/60° at 3T, parameters that were adjusted to the larger T1 and shorter T2 at 7T, TR/TE/Flip angle=1600/12ms/50°. The field of view (FOV) was 230x172.5 mm², while two matrix sizes (256x192, 512x384) and two slice thicknesses (5mm and 2.5mm), to generate a low and a high image resolution, were used for image acquisition. As a result, the two image data sets were acquired at both fields with 0.9x0.9x5mm³ and 0.45x0.45x2.5mm³ resolutions.

**Raw Data Processing:**

Prior to the offline image reconstruction, the k-space was zero filled to a matrix size of 1024x768 for both acquired resolutions. Magnitude and phase images were reconstructed offline from the time domain data using IDL software (Interactive Data Language, ITT). For phase image reconstruction, a high pass filter operation was used to remove effects from slowly varying field inhomogeneities due to main field imperfection and air/tissue susceptibility effects (Abduljalil, 2003). To obtain a phase image of the low spatial frequency components, we used a two dimensional Gaussian filter of the form
\[ \exp\left(-2\pi^2k^2\zeta^2\right) \], where \( k = -a_x..1-a_x \), \(-b_y..1-b_y\) is normalized to 1 over the matrix size, \( N_x/N_y \), \( a_x/b_y \) are the echo centers in k-space in the frequency and phase directions respectively, and \( \zeta \) is related to the full weight high maximum (FWHM) of the Gaussian function such that \( FWHM = \sqrt{2\ln(2)/\pi\zeta} \). The phase of the complex low pass filtered image is complex subtracted from the original wrapped phase image to produce the high pass filtered phase image (Figure 4.2.), which preserves the phase generated by the subvoxel susceptibility differences. The strength of the Gaussian filter is controlled by the scaling parameter \( \zeta \) which determines the range of spatial frequencies to be filtered out.

\[ \_ = ABC \]

\[ \_ = \_ = ABC \]

**Figure 4.2.** Steps involved in the generation of the high pass filtered phase map. Subtraction of the low pass Gaussian filtered image (B) from the unwrapped phase map (A), results in a high pass filtered phase image (C).
Prior to the phase map generation the k-space time domain data were centered to avoid any addition to the phase induced by the Fourier transform of the shifted time domain data in k-space, as shown by the relation:

\[ f(t - t_0) \overset{FFT}{\leftrightarrow} e^{-it_0\omega} F(\omega) \]  

Where \( f(t - t_0) \) represents the time domain k-space data shifted from the center of the k-space with \( t_0 \), \( e^{-it_0\omega} \) contains the phase addition due to the shift \( t_0 \), and \( F(\omega) \) is the frequency data corresponding to the Fourier transformed time domain data \( f(t) \). An example of the high pass filtered phase map generated from an uncentered, shifted k-space data in y direction is shown in figure 4.3.

![Figure 4.3](image)

**Figure 4.3.** Examples of (A) wrapped phase map that was not k-space centered prior to phase generation, (B) Gaussian low pass filtered non centered phase map, and (C) a high pass filtered non centered phase map. Observe the phase variation across phase map that are not removed by the application of the Gaussian filter and subtraction of image B from image A.
For our study, different Gaussian filters strengths were tested. A filter strength of $\zeta = 6$ sufficiently removed slowly varying field inhomogeneities at both fields, and was used for phase map generation and phase measurements. In addition, the high resolution data sets of all subjects at 7T were used to assess the influence of filter strength on phase quantification and its dependence on brain iron. For these three additional phase images data sets were generated using $\zeta = 12, 18$ and 25.

**Image analysis:**

Data analysis was performed using both magnitude and phase images. Selected brain regions were chosen for quantitative measurements of the phase shift, magnitude signal to noise ratio (SNR) and standard deviation of the phase (STD), including frontal and motor white and gray matter, globus pallidus, caudate, putamen, thalamus and the ventricles (see Figures 4.4, 4.5, 4.6). Except for ventricles, and motor white matter that were selected just to assess the influence of magnitude image SNR on STD of the phase, all the other chosen brain regions were in addition used to correlate regional iron to phase shifts. Regional iron as a function of age was determined according to regression equations listed in Table 2.1, Chapter 2 as they were generated by the study of Hallgren and Sourander (1958).

Regions of Interest (ROIs) were manually traced on magnitude and/or phase images using another IDL program (see Figures 4.4, 4.5, and 4.6). This program allowed the display and the alternate switching between
magnitude and phase images, as well as the ROI selection on either one of them. After each tracing the mean phase value, STD of the phase and mean signal intensity were recorded for further data processing. Additionally, for each slice in which a brain region was selected, a ROI placed outside of the brain, in the air filled space, was also traced and STD of the noise was recorded. The regional SNR for each selected brain region was calculated as the mean value of the signal intensity divided by the STD of the noise.

For three regions of the brain, the globus pallidus, caudate nucleus and putamen, three sets of ROIs were traced. The first one included the whole anatomical region displayed in a particular slice, the second one included only the darkest region from the phase image and a last one included a small, homogenous region seen on both magnitude and phase images. An example of the three different ROI selections is provided in Figure 4.6. Care was taken to avoid inclusion of the visible vasculature inside the selected region, by checking both magnitude and phase images prior and after tracing. Exceptions were made for ROIs delineating an entire anatomical region.
Figure 4.4. High resolution magnitude (left and right top row) and histogram equalized phase (right, bottom row) images of a 56 years old subject at 7T, showing examples of ROIs depicting frontal white matter (blue, phase image), and frontal gray matter (red, magnitude image). These ROIs drawings were typical for all subjects.
Figure 4.5. High resolution magnitude (left) and histogram equalized phase (right) images of a 56 years old subject at 7T, showing examples of ROIs depicting motor gray matter (red), and motor white matter (blue).
Figure 4.6. Magnitude (left) and histogram equalized phase (right) high resolution images of a 56 years old subject at 7T, showing the ROIs depicting globus pallidus (red), caudate (yellow) and putamen (blue) for the whole anatomical region (larger area) and for the darkest region of the phase (smaller area). The filled green objects placed inside the brain regions represent typical choices for homogenous ROIs.
**Statistical data processing:**

In summary, the steps involved in statistical data analysis to address the listed objectives of our studies were:

1. Linear regression analysis with phase$= a + \text{slope} \cdot [\text{Fe}]$ to determine the correlation between phase shifts from quantitative phase measurements with estimated iron concentration ($[\text{Fe}]$). The iron concentration was determined for each subject, for all 6 brain regions (caudate nucleus, putamen, globus pallidus, frontal white and gray matter, as well as motor cortex), at each field strength (3T and 7T) and each resolution (low and high). A Gaussian filter of strength 6 was used.

   (A). Each region was independently evaluated with regression analysis by including data from all subjects at a certain field strength and resolution (caudate, putamen and globus pallidus were separately evaluated for the whole, dark and homogenous ROI selected).

   (B). All brain regions were evaluated together for a given field strength and resolution (three cases were generated for each field strength and resolution, corresponding to the three ROI types selected for the caudate, putamen and globus pallidus).

   (C). For each subject over 30 years of age (10 scanned at both 3T and 7T), from the high resolution images at each field strength, the measured phase shifts for 7 analyzed brain regions (thalamus was also included) were displayed as a function of the average regional brain iron, as listed in Hallgreen and Sourander (1958) for subjects over 30 years of age (Table 2.1, Chapter 2).
Regression analysis was performed to correlate phase shifts and iron concentration in an attempt to avoid intersubject variability of calculated regional iron that Halgreen and Sourender’s equations may not account for. It is expected that even if the exact iron concentration cannot be known/or determined in an alive subject, the high to low iron concentrations in the brain should follow the order: globus pallidus, putamen, caudate, thalamus, motor cortex, frontal white and gray matter. The statistical analysis was done just for the ROIs that included the whole anatomical structure and the darkest region of the phase image.

2. Regression analysis in a similar fashion as in 1.(A) for the high resolution 7T data, for all subjects, obtained using different Gaussian $\zeta$ values (6, 12, 18 and 25). The purpose of this was to evaluate the influence of filter strength on the correlation between phase shifts and age related regional iron concentrations. The analysis was performed just for the ROIs that included the whole anatomical structure and the darkest region of the phase image. We did not check the influence of filter strengths on phase shifts obtained from the homogenous regions as no correlation between iron and phase shifts were observed after the first filter ($\zeta=6$) was checked.

3. Regression analysis using the first order inverse equation, STD phase $= a + \text{slope}/\text{SNR}$, to check if STD phase is related with magnitude SNR through the known relation (Haacke, 1999) or STD phase is an indicator of subvoxel variability. For this, five subjects’ STD phase versus 1/SNR data were evaluated independently for each image resolution (low and high), as well as with combined data (low and high together) at both field strengths.
4.2.2 Results

*Image Quality*

As it was expected, overall, the image quality at 7T on both magnitude and phase images is superior to 3T, even if the magnitude images at 7T show contamination of $B_0$ and RF inhomogeneity. Microvasculature is depicted at both field strengths but due to higher image SNR at 7T compared to 3T, depiction of microvasculature and anatomical structural detail is improved at 7T (Figures 4.7 and 4.8). Magnitude images have mixed proton density/T2* contrast. As is typical of proton density images, white matter is darker than gray matter and CSF is bright. T2* effects seem evident in brain structures with high iron content such as the caudate, putamen, globus pallidus, substantia nigra and red nucleus, and in veins, where high levels of deoxyhemoglobin lead to a very short T2*, seen as a regional signal decrease in magnitude images and negative phase shifts in phase images, respectively (Figure 4.7).
Figure 4.7. High resolution magnitude (left) and histogram equalized phase (right) images of a 22 years old subject at 3T (A, top row) and 7T (B, bottom row), showing deep gray matter structures (e.g. caudate, putamen, globus pallidus and thalamus). The higher SNR of the 7T images results in better visualization of structures and microvasculature in both magnitude and phase images (image resolution 0.45X0.45X2.5mm³).
Figure 4.8. High resolution magnitude (left) and histogram equalized phase (right) images of a 22 years old subject at 3T (A, top row) and 7T (B, bottom row) showing the upper brain (a few slices above the slices depicted in Figure 4.7). The higher SNR of the 7T images results in better visualization of structures and microvasculature in both magnitude and phase images (image resolution 0.45X0.45X2.5mm$^3$).
Phase shifts versus iron estimates

Phase shifts measurements as a function of brain iron estimations ([Fe]) using Hallgren’s equations (1958), at 3T and 7T, for both resolutions are presented for the six analyzed brain regions in figures 4.9 to 4.17. The numerical values for the phase shift measurements for 3T and 7T are similar due to the adjustment of the TE at 7T (TE=12ms) relative to 3T (TE=25ms). When comparing the high to the low resolution phase measurements the results are similar in terms of phase spread. More importantly they are consistent in showing or not showing statistically significant correlation between phase shifts and iron concentrations estimated as a function of age. When a statistically significance correlation is found for a specific brain region, the significance holds true at both resolutions and both field strengths. Figures 4.18 and 4.19 present the phase shifts vs. iron concentration for the all brain regions, by including the data from the six analyzed brain regions. Significant correlation and fairly significant negative slopes (phase shifts decrease with increasing estimated brain iron concentration), are observed only when including the darkest regions. Slightly significant correlation but a near zero slope, is also observed for the 7T date encompassing the entire caudate, putamen and globus pallidus. For the curves showing the homogeneous regions, there appears to be a small but non-significant upwards slope.
Figure 4.9. Phase versus iron concentration for the caudate, measured from the low resolution data set at 3T (left) and 7T (right) for the whole anatomical region (top row, A), the darkest region from the phase image (middle row, B) and a small homogenous region of the caudate (bottom row, C). The black line represents the regression analysis line, and the blue and red lines show the 95% confidence interval, and 95% prediction interval of the regression, respectively. Marked with * are the cases where a statistically significant correlation (p<0.05) between phase shifts and iron concentrations was shown by the regression analysis. The details of the regression analysis are presented in Table 4.1.
**Figure 4.10.** Phase versus iron concentration for the caudate, measured from the high resolution data set at 3T (left) and 7T (right) for the whole anatomical region (top row, A), the darkest region from the phase image (middle row, B) and a small homogenous region of the caudate (bottom row, C). The black line represents the regression analysis line, and the blue and red lines show the 95% confidence interval, and 95% prediction interval of the regression, respectively. Marked with * are the cases where a statistically significant correlation (p<0.05) between phase shifts and iron concentrations was shown by the regression analysis. The details of the regression analysis are presented in Table 4.1.
Figure 4.11. Phase versus iron concentration for the putamen measured from the low resolution data set at 3T (left) and 7T (right), for the whole anatomical region (top row, A), the darkest region from the phase image (middle row, B) and a small homogenous region of the putamen (bottom row, C). The black line represents the regression analysis line, and the blue and red lines show the 95% confidence interval, and 95% prediction interval of the regression, respectively. Marked with * are the cases where a statistically significant correlation (p<0.05) between phase shifts and iron concentrations was shown by the regression analysis. The details of the regression analysis are presented in Table 4.1.
Figure 4.12. Phase versus iron concentration for the putamen measured from the high resolution data set at 3T (left) and 7T (right) for the whole anatomical region (top row, A), the darkest region from the phase image (middle row, B) and a small homogenous region of the putamen (bottom row, C). The black line represents the regression analysis line, and the blue and red lines show the 95% confidence interval, respectively 95% prediction interval of the regression. Marked with * are the cases where a statistically significant correlation (p<0.05) between phase shifts and iron concentrations was shown by the regression analysis. The details of the regression analysis are presented in Table 4.1.
**Figure 4.13.** Phase versus iron concentration for the globus pallidus, measured from the low resolution data set at 3T (left) and 7T (right) for the whole anatomical region (top row, A), the darkest region from the phase image (middle row, B) and a small homogenous region of the globus pallidus (bottom row, C). The black line represents the regression analysis line, and the blue and red lines show the 95% confidence interval, and 95% prediction interval of the regression, respectively. No statistical significance correlation between phase shifts and globus pallidus iron concentrations was found no matter the field strength (3T or 7T) or ROI selection (whole, dark or homogenous). The details of the regression analysis are presented in Table 4.1.
Figure 4.14. Phase versus iron concentration for the globus pallidus measured from the high resolution data set at 3T (left) and 7T (right) for the whole anatomical region (top row, A), the darkest region from the phase image (middle row, B) and a small homogenous region of the globus pallidus (bottom row, C). The black line represents the regression analysis line, and the blue and red lines show the 95% confidence interval, and 95% prediction interval of the regression, respectively. No statistical significance correlation between phase shifts and globus pallidus’s iron concentrations was found no matter the field strength (3T or 7T) or ROI selections (whole, dark or homogenous). The details of the regression analysis are presented in Table 4.1.
Figure 4.15. Phase versus iron concentration for the frontal white matter (FWM) at 3T (left) and 7T (right) for the low resolution data set (low, top row), and the high resolution data set (high, bottom row). The black line represents the regression analysis line, and the blue and red lines show the 95% confidence interval, and 95% prediction interval of the regression, respectively. No statistical significant correlation between phase shifts and iron concentration was found for the FWM independent of magnetic field strength or resolution. The details of the regression analysis are presented in Table 4.1.
Figure 4.16. Phase versus iron concentration for the frontal gray matter (FGM) at 3T (left) and 7T (right) for the low resolution (low, top row), and the high resolution (high, bottom row). The black line represents the regression analysis line, and the blue and red lines show the 95% confidence interval, and 95% prediction interval of the regression, respectively. No statistical significant correlation between phase shifts and iron concentration was found for the FGM independent of magnetic field strength or image resolution. The details of the regression analysis are presented in Table 4.1.
Figure 4.17. Phase versus iron concentration for the motor gray matter (MGM) at 3T (left) and 7T (right), for the low resolution (low, top row), and the high resolution (high, bottom row) images. The black line represents the regression analysis line, and the blue and red lines show the 95% confidence interval, and 95% prediction interval of the regression, respectively. Marked with * are the cases where a statistically significant correlation (p<0.05) between phase shifts and iron concentrations was shown by the regression analysis. The details of the regression analysis are presented in Table 4.1.
Figure 4.18. Phase versus iron concentration for all brain regions at 3T (left) and 7T (right) for the low resolution data set. The measurements were done for the whole anatomical regions (top row, A), the darkest region from the phase image (middle row, B) and a small homogenous region (bottom row, C). The black line represents the regression analysis line, and the blue and red lines show the 95% confidence interval, and 95% prediction interval of the regression, respectively. Marked with * are the cases where a statistically significant correlation (p<0.05) between phase shifts and iron concentrations was shown by the regression analysis. The details of the regression analysis are presented in Table 4.1.
Figure 4.19. Phase vs. Iron concentration for all brain regions at 3T (left) and 7T (right) for the high resolution data set. The measurements were done for the whole anatomical regions (top row, A), the darkest region from the phase image (middle row, B) and a small homogenous region (bottom row, C). The black line represents the regression analysis line, while the blue and the red lines represent the 95% confidence interval, respectively 95% prediction interval of the regression. Marked with * are the cases where a statistically significant correlation (p<0.05) between phase shifts and iron concentrations was shown by the regression analysis. The details of the regression analysis are presented in Table 4.1.
Table 4.1 summarizes the regression analysis results for both field strengths (3T and 7T), both resolutions (low and high) as well as for the three ROI selections (whole anatomical region, darkest region of the phase image, and homogenous region).

The comparison between the results of Ogg’s study conducted at 1.5T (1999), the study of Xu et al. at 1.5T (2007) and our low resolution data at both 3T and 7T are listed in Table 4.2. Our low resolution study and Ogg’s study were investigating the same brain regions and used the same acquisition methods. The preliminary study of Xu et al. (2007) used a 3D acquisition with the same inplane resolution as our low resolution acquisition, but with a 2mm slice thickness. The only brain regions investigated by Xu that overlapped with our study were: frontal white matter, caudate, putamen and globus pallidus. It is easy to observe the similarity of the regression analysis results for the caudate, putamen, globus pallidus and frontal white matter between our 3T and/or 7T results and Xu’s results. Our results just partially match, in terms of significance, with Ogg's results, more specifically they show statistical significance correlation between phase and iron only for putamen, motor gray matter and all brain region.
Table 4.1. Summary of the regression analysis results for both field strengths (3T and 7T), both image resolutions, as well as for the three ROI selections (whole anatomical region, darkest region of the phase images, and homogenous region on both magnitude and phase image). The statistically significant correlation ($p<0.05$) between phase shifts and iron concentrations as a function of subjects’ age is illustrated using bold style and gray shading. Observe the consistency of results across the field strengths (exception made by All Brain Regions, high, whole) as well as across slice thicknesses.
<table>
<thead>
<tr>
<th>Field Strength</th>
<th>Caudate</th>
<th>Putamen</th>
<th>Pallidus</th>
<th>FWM</th>
<th>FGM</th>
<th>MGM</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5T (Ogg, 1999)</td>
<td>R 0.72 0.76</td>
<td>0.59 0.05</td>
<td>0.19 0.68</td>
<td>0.73</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p &lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>slope -2.1</td>
<td>-1.7</td>
<td>-1.2</td>
<td>0.3</td>
<td>-1.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5T (Xu, 2007)</td>
<td>R 0.447</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p NS</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>NA NA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>slope -0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3T</td>
<td>R &lt;0.001</td>
<td>0.529</td>
<td>0.074</td>
<td>0.026</td>
<td>0.007</td>
<td>0.446</td>
<td>0.5</td>
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<tr>
<td>p 0.726</td>
<td>0.001</td>
<td>0.3</td>
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<td>0.757</td>
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<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>slope 0.22</td>
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<td>-1.03</td>
<td>-0.54</td>
<td>-2.4</td>
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<tr>
<td>7T</td>
<td>R 0.059</td>
<td>0.557</td>
<td>0.004</td>
<td>0.226</td>
<td>0.211</td>
<td>0.329</td>
<td>0.621</td>
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<tr>
<td>p 0.321</td>
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<td>0.811</td>
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<td>-2.5</td>
<td>-3.3</td>
<td>-2.5</td>
<td>-0.8</td>
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Table 4.2. Summary of the comparison of regression analysis results between our studies and the studies of Ogg et al. (1999) and Xu et al. (2007), for the overlapping studied brain regions. The statistically significant correlation (p<0.05) between phase shifts and iron concentrations as a function of subjects’ age is illustrated using bold style and gray shading. Note: NS=non significant correlation, NA=not applicable.

**Phase shifts versus mean iron for subjects over 30 years of age**

Figures 4.20 to 4.22 show the phase shift versus iron concentration for 3 out of the 10 subjects with ages over 30 years. The whole region, as well as the darkest region from the phase images of caudate, putamen and globus pallidus are considered. Observe that when statistical significance is found for one subject, it is always for the case in which phase shifts from the dark region are considered. Figure 4.23 shows the phase shift versus mean iron for all subjects and all investigate brain regions, respectively. Significant correlation and fairly negative slope are found for dark region cases when all 10 subjects are considered together.
Figure 4.20. Phase versus mean iron concentration for a 54 years old subjects at 3T (top row) and 7T (bottom row) for the whole region (right column) and the dark region (left column). The black line represents the regression analysis line, and the blue and red lines show the 95% confidence interval, and 95% prediction interval of the regression, respectively. Marked with * are the cases where a statistically significant correlation (p<0.05) between phase shifts and iron concentrations was shown by the regression analysis. The details of the regression analysis are presented in Table 4.3.
Figure 4.21. Phase versus mean iron concentration for a 34 years old subject at 3T (top row) and 7T (bottom row) for the whole region (right column) and the dark region (left column). The black line represents the regression analysis line, and the blue and red lines show the 95% confidence interval, and 95% prediction interval of the regression, respectively. Marked with * are the cases where a statistically significant correlation (p<0.05) between phase shifts and iron concentrations was shown by the regression analysis. The details of the regression analysis are presented in Table 4.3.
Figure 4.22. Phase versus mean iron concentration for a 42 years old subjects at 3T (top row) and 7T (bottom row) for the whole region (right column) and the dark region (left column). The black line represents the regression analysis line, and the blue and red lines show the 95% confidence interval, and 95% prediction interval of the regression, respectively. Marked with * are the cases where a statistically significant correlation (p<0.05) between phase shifts and iron concentrations was shown by the regression analysis. The details of the regression analysis are presented in Table 4.3.
Figure 4.23. Phase versus mean iron concentration for all 10 subjects at 3T (top row) and 7T (bottom row) for the whole region (right column) and the dark region (left column). From left to right inserted green objects delineate the data points for frontal gray matter (FGM), frontal white matter (FWM), thalamus (T), motor gray matter (MGM), caudate (Cau), putamen (Put) and globus pallidus (GP), in order of increasing mean iron concentrations. The black line represents the regression analysis line, and the blue and red lines show the 95% confidence interval, and 95% prediction interval of the regression, respectively. Marked with * are the cases where a statistically significant correlation (p<0.05) between phase shifts and iron concentrations was shown by the regression analysis.
Tables 4.3 and 4.4 summarize the results of phase iron correlation from regression analysis for each of the 10 subjects over 30 years of age. The phase shifts measurements from the high resolution data (2.5mm slice thickness) for seven brain regions (frontal white and gray matter, motor gray matter, caudate, putamen, globus pallidus and thalamus) versus mean regional iron listed in Hallgreen’s paper (1999) were analyzed. For the phase measurements including the entire caudate, putamen and globus pallidus there was no statistically significant correlation in any subject at 3T or at 7T. When including only phase data for the darkest region statistically significant correlation was observed for 3 of 10 subjects at 3T and 7T, for 7 subjects at 3T and only for 6 subjects at 7T.
<table>
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<tr>
<th>Subject</th>
<th>ROI</th>
<th>Analysis</th>
<th>7T</th>
<th>3T</th>
<th>Subject</th>
<th>ROI</th>
<th>Analysis</th>
<th>7T</th>
<th>3T</th>
</tr>
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<td></td>
<td>R²</td>
<td>0.541</td>
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<td></td>
<td>R²</td>
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<td>0.587</td>
<td>6</td>
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<tr>
<td>54 years</td>
<td>R²</td>
<td>0.276</td>
<td>0.752</td>
<td>40 years</td>
<td>R²</td>
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<td>0.489</td>
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<td>dark</td>
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<tr>
<td>42 years</td>
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<td>R²</td>
<td>0.469</td>
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<tr>
<td>dark</td>
<td>p</td>
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<tr>
<td>56 years</td>
<td>R²</td>
<td>0.643</td>
<td>0.335</td>
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<td>R²</td>
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<tr>
<td>dark</td>
<td>p</td>
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<td>R²</td>
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<td>0.023</td>
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<tr>
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<td>-0.06</td>
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<td></td>
<td>slope</td>
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<td>-0.65</td>
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</tr>
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<tr>
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<td>&lt;0.0001</td>
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<tr>
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Table 4.3: Regression analyses results for each subject over 30 years of age, when phase measurements for the high resolution data obtained with a filter strength $\zeta = 6$ were correlated with mean iron for each of the seven brain region (caudate, putamen, globus pallidus, frontal white matter, frontal and motor cortex, and thalamus) as listed in Hallgreen’s paper (1958). The statistically significant correlation between phase shifts and mean iron is not consistent at both fields, or for the both analyzed cases (whole anatomical region and the darkest region from the phase image).
Table 4.4. Summary of the table 4.3 showing the number of subjects over 30 years of age scanned at both 3T and 7T that showed statistically significant correlation between phase shifts and the mean regional iron concentrations as listed in Hallgren and Sourander (Hallgren, 1958).

<table>
<thead>
<tr>
<th>Number of Subjects</th>
<th>ROI</th>
<th>Both 3T &amp; 7T</th>
<th>Neither 3T &amp; 7T</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 whole</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>10 dark</td>
<td>7</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>

**Gaussian filter strengths and their influence on phase iron correlation**

Figures 4.24 and 4.25 show examples of linearly scaled and histogram equalized phase images of a high resolution data set at 7T obtained by high pass filtering the time domain data using Gaussian filters of strength $\zeta = 6, 12, 18$ and 25. As $\zeta$ increases the images show progressive degradation, due to susceptibility effects, in the form of alternating bright/dark bands that are more obvious around air tissue interfaces (see Figure 4.24), and extend throughout the brain when the filter strength is 25. The phase shifts versus iron concentration for each of the six studied brain regions are shown for all the Gaussian filters strengths in Figures 4.26 to 4.29. The details of the regression analysis are summarized in Table 4.5. The graphs and the statistical analysis show that in all cases where significant correlation was observed (i.e. for the dark area of the putamen and motor cortex), this correlation was maintained for higher filter values. The slope for the linear regression increased with increasing filter strength, however the p-value also increased. This demonstrates that the filter strength has no effect on the overall outcome, nor does it change the phase/iron correlation, increasing the filter value merely spreads out the phase values, without providing additional information.
Figure 4.24. Histogram equalized phase images of a 22 years old subject at 7T obtained using different filter strengths: 6 (A), 12 (B), 18 (C), 25 (D). Observe the failure of the filters to completely unwrap the phase.
Figure 4.25. Phase images of a subject at 7T obtained using different filter strengths: 6 (A), 12 (B), 18 (C), 25 (D). Observe the failure of the 25 filter strength to completely unwrap the phase.
Figure 4.26. Phase versus iron concentration for caudate (high resolution data set) when different Gaussian filter strengths are used (ζ = 6, 12, 18 and 25) for the whole anatomical region (top graph) and the darkest region from the phase images (bottom graph). Black lines represent the regression analysis lines, while * illustrates the statistical significant correlation between phase and iron concentration for caudate when measurements of the whole anatomical region are employed. More details about the regression analysis results are found in Table 4.5.
Figure 4.27. Phase versus iron concentration for putamen (7T, high resolution data set) when different Gaussian filter strengths are used ($\zeta = 6, 12, 18$ and 25) for the whole anatomical region (top graph) and the darkest region from the phase images (bottom graph). Black lines represent the regression analysis lines, while * illustrates the statistical significant correlation between phase shifts and iron concentration. More details about the regression analysis results are found in Table 4.5.
Figure 4.28. Phase versus iron concentration for globus pallidus (7T, high resolution data set) when different Gaussian filter strengths are used ($\zeta =$ 6, 12, 18 and 25) for the whole anatomical region (top graph) and the darkest region from the phase images (bottom graph). Black lines represent the regression analysis lines. More details about the regression analysis results are found in Table 4.5.
**Figure 4.29.** Phase versus iron concentration for frontal white and gray matter (top and middle graphs) and motor gray matter (bottom graph) when different Gaussian filter strengths are used ($\zeta = 6, 12, 18$ and $25$). Black lines represent the regression analysis lines, while * illustrates the statistical significant correlation between phase shifts and iron concentration. More details about the regression analysis results are found in Table 4.5.
<table>
<thead>
<tr>
<th>Brain Region</th>
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<th>Regression Analysis</th>
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<tr>
<td></td>
<td></td>
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<td></td>
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<tr>
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</tr>
<tr>
<td>Motor GM</td>
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</tr>
<tr>
<td></td>
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</tr>
</tbody>
</table>

**Table 4.5.** Regression analysis results (7T, high resolution data set) for Gaussian filter strengths of 6, 12, 18, and 25. The statistical significant correlation (p<0.05) of phase shifts with iron concentration is shown by bold style and gray shading. Observe the consistency of regression analysis results (one exception is the putamen, whole anatomical region) for filter strengths of 6, 12, and 18. The results obtained from measurements from phase images generated with filter strength 25 are not similar to the other filter strengths and are consistent with the visual appearance of the phase images that show unwrapping failure throughout the entire brain.
**STD versus SNR**

A summary of the regression analysis on the correlation between phase STD and magnitude images inverse SNR is summarized for all 5 analyzed subjects in Table. 4.6. A statistical significant correlation of the phase STD with magnitude inverse SNR was found, for each of the low and high image resolutions, in 3 subjects at 3T and just 1 subject at 7T. When the low and high resolution data were combined, the results clearly followed the expected behavior STD ~1/SNR (Haacke, 1999) and showed a statistically significant correlation in all evaluated subjects at 3T and just 3 out of 5 subjects at 7T. As an example figure 4.30 shows the phase STD versus 1/SNR with regression curves for one subject at both 3T and 7T. Note that the magnitude SNR at 3T varies from 15 to 145, while at 7T ranges from 40 to 350. The phase STD at 3T goes approximately between 4 and 14, while at 7T ranges between 2 and 8.

These results may suggest that for the very high SNR achieved in this study especially at 7T, magnitude SNR may not be the only factor involved in the variability of the phase signal. It is possible that variability of the phase between neighboring voxels, even for the very small ROIs may reflect true anatomic variability generated, for examples, by deoxyhemoglobin in microvessels and or ferritin/ferittin clusters. Further, local phase differences within each voxel may exceed $2\pi$ or more thus causing overall signal cancellation and reduced effective $T2^*$. 
Figure 4.30. Standard deviation (STD) of phase versus 1/SNR for a 53 years old subject at 3T (top) and at 7T (bottom). The black line represents the regression curve and the blue and red curves show the 95% confidence band and the 95% prediction band, respectively. Each graph shows data points for 15 brain regions (3 caudate, 3 putamen, 3 globus pallidus, 1 frontal white matter, 1 frontal gray matter, 1 motor cortex, 1 motor white matter, 1 ventricle, and 1 thalamus) obtained from measurements on the low resolution image and 15 brain regions obtained from measurements on the high resolution magnitude image. The statistical significant correlation between STD Phase and 1/SNR is found at both 3T and 7T (p<0.001), which is a typical result for many of the other subjects.
Table 4.6. Regression analysis results of standard deviation (STD) of phase versus inverse SNR for each of the investigated 5 subjects. The statistically significant correlation (p<0.05) of phase shifts with iron concentration is shown by bold style and gray shading. Observe the differences between 3T and 7T correlation results.
4.2.3 Discussion & Conclusion

In summary, our study illustrated that PD/T2* weighted magnitude and phase images, obtained at 7T were superior in terms of tissue contrast and vasculature depictions to the ones obtained using similar parameters at lower field strength, namely 3T. Regression analysis showed that phase measurements do not clearly correlate with regional iron estimates as a function of age or mean iron values as provided by Hallgren and Sourander's histochemical brain iron determination (1958). However, the results of phase measurements and their correlation with regional parenchymal iron were very similar in our study for both image resolutions, as well as for the 3T and 7T field strengths.

Despite the improved tissue contrast due to the imaging at higher magnetic fields, the findings of our study regarding phase measurements-regional iron correlations are in contradiction with the results of earlier work by Ogg et al. (1999), conducted at 1.5T. While the study of Ogg showed a clear linear correlation between phase measurements and regional estimated iron concentrations for 5 out of 6 investigated brain regions, our study only clear finding is that phase measurements and their correlation with iron are highly dependent on the choice of ROI within each brain region. However, the regional variation of phase measurements within the same brain region was not evaluated in the earlier study by Ogg et al. (1999) or in a recent preliminary report by Xu. et al (2007). However, the study of Krishnamurthy et al. (2006) showed variability of
phase shifts measurements in the putamen, which is similar to our finding.

The different conclusion of our study compared to Ogg’s study is not due to the use of a different MRI technique or acquisition. For instance, we all used a 2D T2* gradient echo acquisition with a long TE to emphasis susceptibility differences between brain tissues. In addition, part of our study and Ogg’s study were very similar in terms of acquisition parameters (TR~T1 gray matter, flip angle<Ernst flip angle for gray matter, contrast between white and gray matter) and image resolution (0.89x0.89x5mm³). In spite of these, the results were very different, although some of the differences may be related to the age and number of involved human subjects as well as with their brain iron normality. Due to the IRB limitations we registered for our study only normal subjects over 18 years old, while Ogg et al. examined subjects from 0.4 to 45 years old, with some subjects having brain tumors or sickle cell disease, which most likely had a very different brain iron concentration than normal subjects. More than these, we employed different phase generation techniques, i.e. different filter strengths, with similar outcomes.

However, our technique for SWI acquisition and phase images generation can be further improved. The use of the 2D-SWI technique was inspired by the study of Ogg et al. (1999), but a 3D SWI technique with multiple receiver array seems more appropriate due to the possibility to achieve high SNR and contrast between white and gray matter for voxel volumes of about 0.24x0.24x1mm³ (Duyn, 2007). Furthermore, in our study, we only tested Gaussian low-pass filters for generating the phase images.
Different filters shapes, and/or different methods to optimize the removal of the low spatial frequency from the phase images, e.g. using polynomial fitting, should be checked for sensitivity to intravoxel susceptibility, and may prove to be appropriate for optimal phase images generation.

As was mentioned above one of the clear findings of the present work is that phase quantification correlates with iron estimates as a function of age (Hallgren, 1958) in a highly ROI selection-dependent manner within a given brain structure, i.e. when encapsulating the entire globus pallidus or just the darkest region of the caudate or globus pallidus there is no statistical significant correlation between phase shifts and iron estimates. However, it is known that non-heme iron is highly inhomogenous distributed inside a single brain region.

While the regional brain iron values used in this work were based on the regression equations of Hallgreen and Sourander (1958), we are aware of that these age based estimates are very different than the real brain iron concentrations for many subjects. According to Hallgreen and Sourander their chemical parenchymal iron determination was based on non-heme iron extraction from “homogenized tissue samples”. Thus it seems conceptually flawed to select only the “darkest” region for the MRI analysis for correlation with Hallgren’s iron content estimates. Furthermore, it is highly unlikely that Ogg et al. (Ogg, 1999) or our study’s phase measurements included the exact tissue sample from a specific brain region looked at by Hallgreen and Sourander.

However, even though the estimated values are probably not true for any specific subject, the values for iron after 30 years of age in different
brain regions are probably within the range of the mean values listed in Halgreen and Sourander (1958). Our attempt to correlate phase measurements and mean regional iron from subjects over 30 years of age was again not showing a clear statistical significant dependence, suggesting that the relationship between phase shifts and estimated non-heme iron is more complex than a linear, direct correlation.

A different representation of tissue iron distribution is given by Morris et al. (1992). Based on Fe$^{3+}$ Perl staining, with and without diaminobenzidine (DAB) in optimally prepared samples, the paper describes details of iron distribution in different brain regions. The globus pallidus stained most intensely with large amounts of iron found in glial cells, which were filled with iron granules. In the globus pallidus, iron was also found around blood vessels, and was described as amorphous concretions or encrustation up to 500$\mu$m in size. Pale staining was also observed in the neuropil, fibrous astrocytes and even neurons which did not stain in other brain regions. In the putamen and caudate, staining was associated with myelinated fibers originating from the globus pallidus; abundant iron filled glia were observed as well.

Overall iron staining was much less in white matter, but distinct staining was observed along fiber bands with a distinct beaded appearance. Though these descriptions cannot explain the presence or lack of correlation between high field MRI phase images and total iron content, they point out the complexity of the connection between MRI signal and brain tissue iron, which is present in variable sized (10-25$\mu$m) and variably loaded spherical glia and
along myelinated fibers of variable length and orientation to the magnetic field. Increased iron along vessels was observed in some but not all regions. These complex iron distribution patterns make modeling of MRI magnitude and phase a complex task, but might explain the significant variability seen in the phase images (see Figures 4.7 and 4.8), and exemplified by the measured very large standard deviation of the average phase shifts despite the high overall SNR.

Alternatively, another source of contrast in MRI phase images is the heme iron, respectively iron from deoxygenated blood. Furthermore, vasculature depiction is one of the features of the high field susceptibility weighted imaging (Reichenback, 1997). The difference in vasculature concentration between white and gray matter may explain the phase difference between them, while the high microvascular density in gray matter seems to correlate to the low cortical signal seen in phase images. Comparison of the 7T phase images with India ink stained brain microvasculature show a high visual resemblance (Figure 4.31) suggesting a relation between phase and microvasculature. Moreover, direct correspondence between phase (Figure 4.31 B) and India ink sections (Figure 4.31 C) is observed in the red nucleus and substantia nigra. It can be seen that high vessel density in the magnocellular part and lower vessel density in the parvocellular part in the India ink image of the red nucleus match the signal pattern in the phase image, whereas contrast is reversed on the magnitude image (Figure 4.31 A). On iron stains Morris et al. observed about twice the iron reactivity in the medially placed pars dorsalis and oralis of the red nucleus than in the pars caudalis (Morris, 1997). Likewise medial of the substantia
nigra, the pars compacta (SNc), is dark on phase and India ink images, with a central bright region on both phase and India ink but not on magnitude images. Linear striations extend though the lateral pars reticulate of the substantia nigra (SNr) into the cerebral peduncle (cp). Conversely, findings in the irons stains show the opposite pattern where the pars reticulate (SNr) shows about twice the iron reactivity as the past compacta (SNc) (Figure 4.31 D (Morris, 1997)). This observation strongly suggests a vascular basis for the phase images.
Figure 4.31. Midbrain structures (red nuclei and substantia nigra) depicted in (A) magnitude image, (B) histogram equalized phase image, (C) India ink stained microvasculature (Duvernoy, book) and (D) iron stained brain tissue (Morris, 1992).
In short our findings regarding the correlation between phase shifts and age related iron concentrations, and the comparison between MRI phase, India ink and iron stained images suggest that SWI is not primarily influenced by non-heme iron concentration as was concluded by Haacke et al. (Haacke, 2006). This phenomenon is probably due to a combination of heme and nonheme iron effects plus more or less myelination and geometrical effects as was suggested by Duyn et al. (Duyn, 2007).

Better understanding of contrast mechanisms in phase images may entail a combination of careful histological analysis of the human cadaver brain, and animal in vivo and in vitro work, i.e. experiments aimed at altering blood flow and blood signal as well as staining for tissue iron and vasculature assessment. Animal studies will probably play a crucial role in finding the exact contribution of each of the factors involved in phase manipulation. An overview of the possible studies involving in vivo and in vitro animal work will be presented in some details in Chapter 5.

A complete understanding of the contrast mechanism that makes high field phase images of such high interest is highly desired, due to the potential of SWI to add to the diagnostic and monitoring value in cerebrovascular diseases, tumor angiogenesis (Christoforidis, 2004), multiple sclerosis, trauma and neurodegenerative diseases such as Alzheimer’s, Parkinson’s and Huntington’s disease.
CHAPTER 5

INTRODUCTION TO THE CANINE AS A MODEL OF HUMAN BRAIN AGING AND IRON DEPOSITION

This chapter is concerned with briefly introducing the canine model for human brain aging and iron deposition, as well as outlining possible future work to separate heme and non-heme brain iron contribution to phase measurements.

5.1. CANINE AS A MODEL FOR NORMAL HUMAN BRAIN

Dogs are an easily available source of research material, and they are in many respects a suitable model for normal human brain aging. Depending on the breed, they have a moderate life span between 12 to 20 years of age, and exhibit with age a series of behavioral changes, labeled as normal aging and/or “senile” (Cummings, 1996). These changes seem to be very similar with the ones observed in aged humans. This is also true when brain pathologies of human and dog brains are compared.

A series of in vivo and in vitro dog studies were concerned with depicting the changes in the brain that are closely related with cognitive and
behavioral changes seen in old specimens. Most of these studies were reviewed by Cummings et al. (1996), in addition to presenting their own results on the behavioral and cognitive aspects of normal dog aging, and their relation to structural, functional and cytological transformation of the brain.

Briefly, the aging dog brain shows extended β-amyloid accumulation, which initially is built in and around the neurons and seems to precede the β-amyloid plaque formation. Amyloid angiopathy is also present, as are cerebral microhemorrhages and some neuronal loss. Iron deposition in the brain tissue and lipofuscin accumulation in neurons is also part of the aging and neurodegeneration process in the aged brain dog. Furthermore, it is acknowledged that there is a genetic contribution to some of the pathological changes in the aged canines, similar with the ones seen in the human brain. One of the few differences between a dog’s brain and a normal human brain aging is the absence of the neurofibrillary tangles and as so, of the neuritic plaques. This makes the canine model ideal to study the influence of the β-amyloid plaque, by itself, to neurodegeneration and cognition.

Similar structural changes as the ones encountered in the aged human brain are also present in the canine, such as cerebral vascular changes, atrophy of the brain with up to 22% loss of brain volume, dilatation of the ventricular spaces and thickening of the meninges. All of these structural transformations are detectable using MRI, as two of the most recent studies showed (Su, 1998; Kimotsuki, 2005).
The MRI study of Su et al. (1998) was mainly focused on determining the vascular blood volume using dynamic contrast MRI (DC-MRI) and permeability of the blood brain barrier (BBB) in the dog brain, which is expected to be affected by amyloid angiopathy as a result of aging. This study proved that there is a correlation between each of the cerebral volume, the cerebral tissue volume, the volume of the lateral ventricle, the cerebellar volume and aging. However, DC-MRI did not find any correlation between vascular volume and aging, perhaps since it was applied only to non atrophied brain regions, but it showed a slight correlation between permeability of the BBB and aging. One dog, which performed poorly on the behavior and cognition tests, and showed significant brain atrophy and unexpectedly high leakage of BBB as determined by the use of the pharmacokinetic model in DC-MRI, showed upon brain histochemistry significant β-amyloid plaque deposition. This result suggested that early diagnosis of dog brain pathology associated with extensive plaque formation may be assessed by the use of DC-MRI techniques.

The study of Kimotsuki et al. (2005) is more interesting from our point of view because it also looked at the relation between hypo and hyperintensity of the signal in different beagle brain regions as a function of age. As with the human brain aging, hyperintensity was found in the white matter of the dog brain, and was associated with myelin transformation and fluid increase. Signal hypointensity ratios showed a significant correlation with aging for structures such as substantia nigra and globus pallidus. These are regions that are also known in the human brain to have an age increase in non-heme
iron, and as such seem to be the cause of signal decrease in these brain regions. The sacrifice of a 16-year old beagle, and subsequent Perl's staining of the brain regions that showed increased hypointensity of the signal, illustrated that indeed the non-heme iron accumulation with age was responsible for the signal change as a function of age. This clearly indicated that non-heme iron presence affects the contrast in T2 weighted images of the brain dog as is the case for humans.

All of the above discussed studies demonstrate that the canine brain, due to the high structural, biochemical and pathological similarities to human brain, may be a realistic model for evaluating the relation between phase and non-heme iron. This may help in our attempt to separate influences of heme-, non-heme iron/deoxyhemoglobin and microvascular density on human brain image contrast in high field SWI.

5.2. POTENTIAL FUTURE STUDIES FOR SEPARATING BRAIN HEME AND NON-HEME IRON CONTRIBUTIONS TO PHASE MEASUREMENTS

Our human in vivo experiments presented in Chapter 4 of this dissertation showed an unclear correlation between estimated brain iron as a function of subject age and phase measurements in a few brain regions. Moreover, the phase images through the midbrain illustrated that for the substantia nigra and red nuclei there is a high visual resemblance between dark phase and microvascular density, as shown by India Ink stained vasculature of the same brain region of different subjects (Duvernoy, 1998).
According to our previously discussed studies, the contrast mechanism in a SWI phase image seems to be affected mainly by three factors: non-heme iron, heme iron/deoxyhemoglobin presence, and microvasculature density. The latest two factors affect the phase and contrast in SWI in a combined fashion through the BOLD effect. However, we demonstrated that the correlation between estimated iron and phase quantification is both region and ROI selection dependent. Moreover, microvasculature density seems to relate to negative phase quantification with a high visual resemblance to phase images. In conclusion, a series of in vivo and in vitro canine studies are required to find and separate the influence of each of the above mentioned factors to phase accumulation in SWI.

5.2.1. In vitro studies for non-heme iron and microvascular density determination

To understand the contrast mechanism in SWI at high field, a direct correlation between MR measures such as hypointensity in magnitude images and phase decrease in phase images, and the amount of non-heme iron and microvasculature density is necessary.

Non-heme iron visualization and concentration determination

The use of chemical reactions (hystochemical determination) on prepared brain tissue samples allows for tissue iron visualization. Perl's staining is one of the most commonly used techniques to assess the spatial
distribution of either one of the ferric (Fe$^{3+}$) and ferrous (Fe$^{2+}$) iron, depending on what it is of interest. More details and references are listed in Whitacker (2004).

Since phase shifts of the spins diffusing around iron deposits are directly influenced by the amount of non-heme iron in the tissue, the exact amount of iron in a specific tissue or fragment of a brain tissue is required for a direct correlation with MR measures. One of the methods that are discussed and referenced in Whitaker (2004) is inductively coupled plasma (ICP) mass spectroscopy, which seems to be highly sensitive to the measurement of total elemental content in a tissue sample.

The exact details on the above techniques and the ones listed below are not discussed here, as the present chapter is concerned with outlining possible future work. This will require an extensive literature search to evaluate the utility of these techniques in more detail.

**Microvasculature visualization**

There are different techniques for spatial determination of microvasculature distribution. One of them is India ink stain (Duvernoy, 1998), which involves staining fresh post-mortem brain vasculature by flushing it with India ink. This method already seemed to allow a direct comparison between phase images and tissue images.

Another method involves intravascular injections using low viscosity resign to outline the lumen of the vessels after the resin is allowed to polymerize. Moreover, a three dimensional distribution of
vasculature, which may be sectioned and studied with scanning electron microscopy, may be obtained by allowing the digestion of the surrounding tissue with alkali (Logothetis, 2004).

However, it is clear that MR images and/or phase quantification has to be directly spatially matched with the region/slice stained for iron and microvasculature and/or total iron measurement. This will involve extremely high caution and clear spatial brain landmarks, which are visible in both MR images and brain anatomy.

5.2.2. Influence of heme-iron/deoxyhemoglobin to phase quantification

Heme-iron, predominantly iron from deoxygenated blood influences the signal and spin phase in a T2* weighted image through the BOLD effects, which was briefly discussed in Chapter 4. Finding ways to manipulate blood signal when susceptibility weighted magnitude and phase images are acquired may prove useful to determine how much the blood pool contributes to the phase alteration in the brain.

There are three different approaches to change the signal from vasculature, and to affect the phases of the surrounding spins. One of them involves the use of contrast agents, mostly paramagnetic or superparamagnetic agents, which will increase blood susceptibility. Another one uses drugs that will alter the flow by vasoconstriction (e.g. caffeine, theophylline) or vasodilatation (e.g. azetazolamine) and by such the BOLD effect, and the third one is through direct manipulation of the level of oxygen in the blood.
The contrast agent method may be very useful for determination of microvasculature density (Wu, 2004; Jensen, 2000; Jensen, 2006). It also offers the possibility to compare the obtained results with histology in animal studies. A similar outcome between calculated and histology obtained microvasculature density may prove useful for reducing the number of in vitro studies necessary for microvasculature visualization. The drug based method may not help too much in an animal study as the exact effect of the vasoconstrictor or vasodilatator dosage must be carefully checked prior to the MRI phase study.

The increase in cerebral blood flow (CBF) that is not accompanied by a similar increase in oxygen extraction fraction (OEF) is the basis for the BOLD effect, which can be manipulated in a series of experiments with inhaled oxygen mixes as MR contrast agents. Tumor MRI characterization and treatment response (Taylor, 2001; Howe, 1999), or the visualization of small cerebral veins in high detail (Rauscher, 2005) are just a few of the studies that have exploited the BOLD effect. Increasing oxygen hemoglobin saturation by breathing air with a high oxygen concentration results in an increase in the tissue signal in a T2* weighted image. Inhalation of 100% O2 was associated with increased vessel vasoconstriction that results in decreased CBF (Watson, 2000). The decreased CBF partially counteracted the increase in hemoglobin saturation given by the breathing of the high O2 concentration. However, adding carbon dioxide (5%) eliminates the vasoconstrictor effect of pure oxygen, and results in an increased MR signal compared with air or 100% oxygen breathing. For example, in brain
gray matter the MR signal increases by 8%, due to both the increase of oxygen in capillaries and increased flow with 5% CO2 (Taylor, 2001).

In conclusion, based on the MRI BOLD effect, the signal increase in brain gray matter with carbogen breathing (95% O2 and 5% CO2) suggests the decrease of deoxyhemoglobin iron effect on transverse relaxation and phase shifts measurements, respectively. This is a method that seems easy to implement in a canine experiment, as the blood oxygenation level can be directly and constantly checked through arterial and blood samples collected during the MRI study.

5.3. PROPOSED IN VIVO AND IN VITRO CANINE EXPERIMENT

A possible sequence of MRI experiments followed by tissue iron and microvasculature determination may include the following steps:

1. Image the brain of the normal air breathing dog using a series of SWI sequences with different echo times (TEs), and multiple echoe T2 weighted sequences. They’ll be used to determine T2*, absolute phase and T2 values. Samples of arterial and venous blood must be collected during MR scanning to determine the level of oxygen/deoxyhemoglobin in the blood.

2. Image the brain of carbogen breathing (95% oxygen, 5% carbogen) dog using the same as above MRI sequences. Again, samples of arterial and venous blood must be collected during scanning to determine the level of oxygen/deoxyhemoglobin in the blood.
3. Sacrifice the dog by increasing the anesthesia level while still in the magnet. Flush out the blood from the body (if it is possible) and substitute it with saline solution. Another MRI acquisition with the same sequences as above will be acquired afterwards.

4. The dog brain must be removed from the skull. It could be either India ink stained for microvasculature visualization or Perl's stained for non-heme iron localization. Comparison between phase images and India ink microvasculature will be done. Another possibility is to section the dog brain in thin slices that spatially correlate with acquired MRI images. Samples of different brain tissues (that will match the location and volume of the ROI used for phase quantification) will be used with mass spectroscopy for iron quantification.

5. The in vivo and in vitro MRI images will be used to look at the relation between phase and level of deoxyhemoglobin in the microvasculature. After ROI drawings and phase quantifications, an analytical expression that will correlate phase shift and heme-iron/deoxyhemoglobin level will be obtained. The effect of heme-iron on phase shifts will be determined.

6. The phase images and phase shift quantifications will be visually and/or analytically correlated with microvascular stains from the matching locations. This will involve the same brain locations as in step 5.

7. The tissue iron concentration from the exact locations as before will be determined.
8. All the data will be analytically combined, and based on the level of involvement in phase shift measurements, a conclusion, as well as a relation between the three mentioned factors, will be drawn.

The above summarized experiment is one of the possible variations of the in vivo combined with in vitro canine experiments, which may help find a clear answer to the question of contrast generation in SWI in high field imaging.
CONCLUSION

MRI studies conducted at low magnetic field strength (~1.5T) showed that transverse relaxation measurements correlate with the age-dependent increases in iron concentration in many brain regions. Moreover, phase shifts seen in the phase images were also highly correlated with brain iron deposition. Therefore, we hypothesized that at high (3T) and ultra-high magnetic field (7T) the correlation between brain tissue iron concentration with both transverse relaxation and phase shifts has to be stronger than at 1.5T.

Our investigation of the correlation between iron and transverse relaxation values obtained using Dual Echo and GRASE sequences illustrated that the Dual Echo sequence is more sensitive to iron than the GRASE sequence. GRASE showed correlation between transverse relaxation time and iron concentration only for the high iron content brain areas. RF inhomogeneities introduced errors in the transverse relaxation measurements at the ultra-high field that affected the correlation with brain tissue iron.

Phase shift measurements showed significant correlation with regional brain iron estimates only for the motor gray matter. Phase quantification of each of the brain regions with high iron content (putamen, caudate and globus pallidus) illustrated correlation with iron only in a region-selection dependent
manner. However, the results of phase measurements and their correlation with regional parenchymal iron were very similar in our study for both image resolutions, all investigated high pass filters, as well as for the 3T and 7T field strengths. Comparison of the 7T phase images with India ink stained brain microvasculature at the midbrain level showed a high visual resemblance, suggesting a relation between phase and both microvasculature and deoxygenated blood iron.

**Future work**

The work from this dissertation can be further extended to investigate both transverse relaxation measurements and phase shift dependence on brain tissue iron.

Corrections for the magnetic field inhomogeneities have to be implemented before accurate transverse relaxation estimates will be acquired. Furthermore, a different method for transverse relaxation data fitting, such as using an equation with multiple exponentials (equation 3.3), may be more sensitive to brain iron content than the mono-exponential fitting used in our work. This aspect may be further investigated after RF corrections are implemented at ultra-high field.

Better understanding of the contrast mechanisms in phase images may entail a combination of postmortem or animal tissue sample comparison work. These experiments should be aimed at altering the local blood flow or the blood signal, as well as theoretical modeling of the susceptibility
mechanism with respect to the phase signal. Studies comparing MRI findings with direct tissue measures may include postmortem or animal studies of MRI followed by vessel assessment using India ink, silicon vessel casts, or other methods like iron quantification using histochemistry or laser ablation inductively coupled plasma mass spectroscopy (La-ICP-MS). Alternatively, phase shift effects from vascular deoxyhemoglobin or non-heme parenchymal iron may be distinguished by modifying blood flow through vasoconstrictors (e.g. caffeine, theophylline) or vasodilators (e.g. azetazolamine), or respiratory manipulations (breath holding, oxygen or carbogen breathing) as it was described in Chapter 5.

To fully appreciate findings in phase images of diseases, further studies into the mechanism of phase contrast, especially at field strengths of 7T and higher are needed. The applications of such work will be highly valuable for finding ways to monitor disease progression and treatments outcomes in neurodegenerative disease such as Alzheimer and Parkinson.


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