The aging brain and changes in cognitive performance

Findings from morphometry and quantitative susceptibility mapping of iron

Thesis for doctoral degree (Ph.D.)

By

Ninni Persson
Stockholm University, 2015
The aging brain and changes in cognitive performance

Findings from morphometry and quantitative susceptibility mapping of iron

Ninni Persson
To all the women in academia who resist the patriarchy.
List of studies


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Other contributions


Abstract

Brain aging is a heterogeneous phenomenon, and this thesis illustrates how the course of aging can vary within individuals over time and between individuals as a function of age, sex, and genetic variability. We used two contrasts from magnetic resonance imaging (MRI), namely spin-lattice T1-weighted imaging, and quantitative susceptibility mapping (QSM) from gradient-echo images, to picture the aging brain, by means of morphometric measures and brain-iron concentrations. Within each study, the same rigorous imaging acquisitioning protocols were used over large samples sizes of 167-183 individuals, which contribute to the uniqueness of the studies. Most of the current knowledge about the aging brain rests on the foundation of cross-sectional age-related differences, and studies I and III contribute to current knowledge with longitudinal designs to investigate individual rates of change. The importance of genetic variation in relation to regional brain changes was addressed with a specific emphasis on functional polymorphisms involved in pro-inflammatory responses. These studies further shed light on the importance of bi-directional relations between structural integrity and maintained cognitive abilities over time. Study II is the largest study to date to have quantitative susceptibility estimates examined in healthy adults, and the first in-vivo report to show a lowering in overall subcortical brain iron estimates in women from midlife to old age. Studies I and III are unique by examining longitudinal differences in anatomical brain regions using high resolution images from a 4 Tesla scanner. Peripheral vascular risk factors were not strong determinants of either brain- or cognitive changes in the studied samples. The results are discussed in the context of cognitive reserve, the brain maintenance hypothesis, and potential influences of hormones, inflammation and oxidative stress.

*Keywords:* brain aging; volumes; individual differences; QSM; cognitive aging; iron; episodic memory; fluid-; crystalized abilities; sex differences; gender differences.
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## Abbreviations

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<th>Description</th>
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<tbody>
<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
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<tr>
<td>CFA</td>
<td>Confirmatory factor analysis</td>
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<td>EM</td>
<td>Episodic memory</td>
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<td>Fe</td>
<td>Iron</td>
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<td>Gc</td>
<td>Crystalized intelligence</td>
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<td>Gf</td>
<td>Fluid intelligence</td>
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<tr>
<td>ICC</td>
<td>Intra-class coefficient</td>
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<td>LCSM</td>
<td>Latent change score model</td>
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<td>MGA</td>
<td>Multiple group analysis</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
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<td>PD</td>
<td>Parkinson’s disease</td>
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<td>PM</td>
<td>Post-mortem</td>
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<td>QSM</td>
<td>Quantitative susceptibility mapping</td>
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<tr>
<td>Relaxation</td>
<td>Signals change with time</td>
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<td>ROI</td>
<td>Region of interest</td>
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<td>SEM</td>
<td>Structural equation modeling</td>
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<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
</tr>
<tr>
<td>T1w</td>
<td>T1 weighted (T1 = longitudinal relaxation)</td>
</tr>
<tr>
<td>V</td>
<td>Vocabulary</td>
</tr>
<tr>
<td>1.5 T</td>
<td>1.5 Tesla (strength of the magnetic field)</td>
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<td>4 T</td>
<td>4 Tesla (strength of the magnetic field)</td>
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“It is never too late to turn on the light. Your ability to break an unhealthy habit or turn off an old tape doesn't depend on how long it has been running; a shift in perspective doesn't depend on how long you've held on to the old view.

When you flip the switch in that attic, it doesn't matter whether it's been dark for ten minutes, ten years or ten decades.

The light still illuminates the room and banishes the murkiness, letting you see the things you couldn't see before.

It's never too late to take a moment to look.”

—Sharon Salzberg
1 Introduction

“The future is not some place we are going to, but one we are creating. The paths are not to be found, but made, and the activity of making them, changes both the maker and the destination”.

—John Schaar, futurist

Brain changes following aging can have implications for mental abilities and our functioning in everyday life. The aging brain undergoes changes in neuroanatomical regions as well as neurotransmitter systems, and brain iron levels. It is well known that in many aspects of development, each person ages at an individual rate. The phenomenon is well studied in the research area of cognitive aging, but less is known about the heterogeneity of brain aging. It is important to know more about differential age-related brain changes to prolong cognitive health, but also to learn more about differences between normal age-related versus pathology-related neurodegeneration. Aging is a dynamic phenomenon that cannot be studied in a snapshot frozen in time. Yet, most of the existing reports focus selectively on cross-sectional age-related differences in neural correlates of aging, hence ignoring individual differences in change. Another important issue is unraveling the determinants of such individual differences. Both genetic variants, health factors, and social and demographic factors may act as a reserve for maintaining sufficient structural integrity of the brain for optimal function on one hand, and to offset accelerated shrinkage on the other hand.

Most of the current knowledge in the field of brain aging has emerged from cross-sectional age-differences as a function of chronological age at a single time point. Also, most of our current knowledge about brain aging from structural magnetic resonance imaging is based on the contrast between gray and white matter or white matter integrity. Brain iron estimates from iron-sensitive contrasts provide new possibilities for describing age-related changes in the human brain, and perhaps even illustrate the mechanisms underlying aging, by influencing processes such as oxidative stress. Further, little is known about other determinants of iron accumulation beyond chronological age, and herein we focused on sex-related variations, and how iron elevation occurs in conjunction over several of the subcortical nuclei, which is a less studied phenomenon.
Brain aging is also accompanied by age-related changes in cognitive functions. Most of the current work reflects the contribution of neuronal variations to individual differences in cognitive performance, while substantially less work has focused on the bi-directional influences between cognition and brain. As science reveals how behavior also potentially shapes the brain, the relevance of further assessment of such bi-directional brain-cognition relationships becomes more obvious; people who maintain their cognitive abilities may have “vital brains.” The overall aim of this thesis is to fill the outlined gaps in knowledge.

1.1 Brain aging

Although behavior alters the brain, and virtually any experience may leave traces, certain age-related changes of the parenchyma are to be expected, although varying in degree across individuals. The brain undergoes age-related changes and declines in volume and weight after the sixth decade of life at a more gross level. This is manifested in shrinking gyri and widening sulci, as well as ventricular enlargements of the cortex (Anderton, 1997), and alterations in the vasculature (Trollor & Valenzuela, 2001). Many individual brain regions exhibit shrinkage in volume in both gray and white matter (Fjell & Walhovd, 2010; Hedman, van Haren, Schnack, Kahn, & Hulshoff Pol, 2012; Persson et al., 2014; Raz et al., 2005a; Sullivan & Pfefferbaum, 2006). In post mortem (PM) can neurochemical and more microscopic assessment of the brain be conducted; however important aspects of developmental changes are then lost. PM findings reveal morphological and molecular markers of tissue degeneration in the prefrontal- and medial temporal lobes as well as the anterior cingulum in specimens from participants free of dementia (Henstridge et al., 2015). Further, neurofibrillary tangles, amyloid plaques, and neuropil threads are present in the gray matter, primarily in the medial temporal lobe and amygdala, also in non-pathologic aging, although to a considerably lesser extent than in Alzheimer’s disease (AD) (Anderton, 1997; Mrak, Griffin, & Graham, 1997; Price, Davis, Morris, & White; Whalley, Deary, Appleton, & Starr, 2004).

Axons also demyelinate during aging (Flechsig Of Leipsic, 1901), which influences action potentials traveling along the axon (Waxman, 2006), potentially slowing cognitive processing (Ylikoski et al., 1993). Aging is also associated with alterations in several neurotransmitter systems that signal via acetylcholine and monoamines (serotonin and dopamine) (Trollor & Valenzuela, 2001), which may have implica-
tions for behavioral changes. The dopamine neurotransmitter system (Seeman et al., 1987; Severson, Marcusson, Winblad, & Finch, 1982; Volkow et al., 1998), which can alter both cognitive and motor functions (Volkow et al., 1998) is of particular interest in aging. For reasons that are poorly understood, heavy metals may accumulate in the aging brain. Both iron and copper increases are reported (Zecca et al., 2004), but only iron has sufficiently high paramagnetic influence on the magnetic resonance (MR) signal, to manifest itself as contrast enhancement in MR images (Schenck, 2003). Age-related brain iron accumulation is primarily observed in the subcortical nuclei, but also to a somewhat lesser extent in the cortex according to PM findings (Hallgren & Sourander, 1958; Ward, Zucca, Duyn, Crichton, & Zecca, 2014).

In summary, PM techniques allow for thorough assessment of the brain at a molecular level, but fail to capture age-related changes over time, which is only plausible via study of the live human brain. Non-invasive in vivo magnetic resonance imaging (MRI) provides a unique window into the aging human brain.

1.1.1 Neuroanatomical regions

Structural MRI is a powerful tool for non-invasive study of the aging brain in vivo. Shrinkage of regional brain volumes is often reported to reflect neuronal cell death or de-generation, but precise counts of synapses or neurons are not possible through in vivo MRI. One of the great advantages of MRI is that the brain can be studied in the context of development, which PM studies do not allow. Findings from structural MR contrast imaging reveal that the magnitude of shrinkage varies across brain regions (Persson et al., 2014; Raz, Ghisletta, Rodrigue, Kennedy, & Lindenberger, 2010). The literature often emphasizes that effects are greater for the prefrontal areas of the brain when measured at a point in time or over short repeated measurement intervals (Fjell et al., 2009; Raz et al., 2004), but other studies contradict this (Persson et al., 2014; Raz et al., 2010). White matter integrity is hypothesized, primarily on the basis of cross-sectional reports, to have a curvilinear relationship with age, with increasing structural integrity until early adulthood followed by a brief plateau, after which there is a decline from approximately the seventh decade of life (Westlye et al., 2010). This trend is more pronounced in regions of the frontal and the parietal lobes (Sowell et al., 2003). Recent longitudinal
work over shorter measurement intervals supports the presence of age-related decline from the fifth decade of life (Sexton et al., 2014). It would be necessary to collect repeated measurements at several time points to assess the true functional form of white matter aging, e.g., whether it is non-linear or has some other relationship. Late post-adolescent brain maturation, expressed as a reduction of cortical gray matter of the dorsal frontal cortex, has been observed up until young adulthood. Thinner cortex in cross-section has been interpreted as denoting synaptic pruning (Sowell, Thompson, Tessner, & Toga, 2001). Smaller volumes as a function of age are also observed in the structures of the limbic cortices. Significant age-related alterations in structure and function have been observed in the hippocampus, entorhinal cortices, and parahippocampal gyrus (Rodrigue & Raz, 2004; Thomann et al., 2013), as well as the amygdala formation (St Jacques, Dolcos, & Cabeza, 2009). Volume and microstructural degradation with advanced age have also been observed in phylogenetically older structures in the basal ganglia and the thalamic nuclei (Fama & Sullivan, 2015; Wenjing Li et al., 2014). Finally, significant shrinkage is observed in the cerebellar cortices over two years in healthy adults (Persson et al., 2014). Longitudinal studies suggest that the posterior regions of the brain are in general spared until advanced age in healthy adults (Fjell et al., 2009; Raz et al., 2005a).

In addition to general trends toward brain shrinkage there exist individual differences in the course of brain aging (Persson et al., 2014; Raz, Ghisletta, Rodrigue, Kennedy, & Lindenberger, 2010). See Figure 1 for an illustration of individual differences at two measurement points (adopted from Persson et al., 2014).
Figure 1. Longitudinal spaghetti plots illustrate each individual’s values over measurement occasions as a function of age at each time point. The figure is reprinted with permission from Elsevier.

1.1.2 Brain iron

Iron serves as an important cofactor in myelination and dopamine transmission (Berg & Youdim, 2006; Mills, Dong, Wang, & Xu, 2010) but can become neurotoxic when there is excessive accumula-
Iron is believed to enter the brain via the blood-brain barrier by transferrin receptor mediated endocytosis (active cell transport of molecules) in the brain capillaries and released back to circulation via absorption of cerebrospinal fluid (Andersen et al., 2014a; Andersen, Johnsen, & Moos, 2014b; Madsen & Gitlin, 2007; Moos, Skjoerringe, Gosk, & Morgan, 2006). Iron is present in various cell types in the central nervous system (CNS), but is abundant in the astrocytes (star-shaped glial cells), which has given rise to the idea that glial cells are involved in iron storage and regulation (Madsen & Gitlin, 2007).

Iron can be studied directly in the PM brain. PM findings of several decades ago show that iron accumulates as a function of chronological age in the brain (Hallgren & Sourander, 1958). Brains from individuals suffering from cerebrovascular and neuropsychiatric disorders were carefully excluded from the aforementioned study (Hallgren & Sourander, 1958). This study provides a unique contribution to the body of research, including the remarkably high number of 98 specimens.

More iron is present in the subcortical nuclei, and interestingly, iron deposits are particularly prevalent in the extrapyramidal system, which supports motor functions. Iron deposits also occur in the cortex, and to a somewhat greater degree in the motor cortex than the rest of the cortices. Prefrontal regions, the medial temporal lobes, and sensory cortices show an exponential increase in iron until early midlife, but lower total concentrations than the subcortical nuclei (Hallgren & Sourander, 1958).

With the development of iron-sensitive MRI contrasts, it has become possible to study the presence of iron in the in vivo human brain. In vivo MRI can approximate iron deposits based on enhanced contrast in the MR image, caused by interplay between iron’s paramagnetic properties and the proton relaxation resonance behavior of tissue water (Schenck, 2003). Most of the non-heme iron in the brain, which affects the MR signal, consists of ferritin or hemosiderin (Schenck & Zimmerman, 2004; Schenck, 2003).

Novel techniques in post-mortem MRI have made it possible to more carefully evaluate the association between PM brain iron levels and
MRI estimates of iron concentrations, and a novel group of iron sensitive contrasts in quantitative susceptibility mapping (QSM) show particular promise (Langkammer et al., 2012). MRI findings reveal both linear and non-linear age-related trends in iron distribution (Haacke et al., 2010; Hagemeier et al., 2013; W Li et al., 2014; Xu, Wang, & Zhang, 2008). Age-dependency of MRI estimates of iron have been studied to the greatest extent in the basal ganglia (Bartzokis et al., 1997a, 2011; Bilgic, Pfefferbaum, Rohlfing, Sullivan, & Adalsteinsson, 2012; Hagemeier et al., 2013; W Li et al., 2014; A. Pfefferbaum, Adalsteinsson, Rohlfing, & Sullivan, 2009; Xu et al., 2008), as opposed to other subcortical nuclei such as the sub-thalamic nuclei (Haacke et al., 2010; Hagemeier et al., 2013), cerebellar dentate nuclei (W Li et al., 2014), the red nucleus and substantia nigra (Bilgic et al., 2012; Haacke et al., 2010; W Li et al., 2014). Few reports focus on age dependency of iron estimates in cortical white matter and the motor cortex, but existing findings reveal that iron levels are much lower in the cortex than in the subcortical nuclei (W Li et al., 2014).

**Figure 2.** Iron (Fe) cycles in the brain. Iron crosses the blood brain barrier via the transferrin receptor pathway on the endothelium. The brain iron cycle consists of glia and neurons. Astrocytes extend long processes that enclose the brain capillaries and help to form the brain blood barrier (BBB). The divalent metal transporter 1 (DMT1) transports Fe. Near the ends of these processes a special form of the Fe oxidizing enzyme, ceruloplasmin (Cp), is expressed. The protein Cp is transported to the plasma membrane by the glycoprophosphatidylinositol anchor (a glycolipid). Adopted from Madsen and Gitlin (2007), with the permission from Annual Reviews.
1.1.3 Theories of Brain Aging

Two important theories focus on specific mechanisms underlying neurodegeneration in normative aging (i.e., aging free from neurologic diseases) have attracted significant attention in the research community: inflammation and oxidative stress. After a description of these theories, the hypotheses of brain maintenance and neuronal reserve will be introduced, followed by discussion of the frontal aging hypothesis.

1.1.3.1 Inflammation

Individuals vary in length of their lifespan, and various factors may accelerate or mitigate aging. The immune system is genetically controlled, differs among individuals, and is thought to account for the observed differences in life expectancy (Franceschi et al., 2000). Importantly, lifestyle factors, including improvement of dietary habits (increased fiber intake), can mediate the inflammatory response (Kantor, Lampe, Kratz, & White, 2013). Increased levels of inflammatory plasma markers have been associated with individual differences in brain structure. Elevated circulatory pro-inflammatory cytokines (e.g., interleukin (IL)-6, and tumor necrosis factor α), C-reactive protein (CRP), and homocysteine (Hcy) have been linked to reduced volumes in the hippocampus, cerebral cortex, and white matter, as well as increased burden of white matter hyperintensities (Bettcher et al., 2012; Taki, Thyreau, Kinomura, Sato, Goto, Wu, Kakizaki, et al., 2013; van Dijk et al., 2005).

1.1.3.2 Oxidative stress

Production of free radicals, as generated by reactive oxygen and nitrogen species (antimicrobial molecules) occurs in the normal cellular metabolism, and these free radicals are hypothesized to influence cellular degeneration in normal brains over time. These free radicals cause damage to the cellular DNA (deoxyribonucleic acid), lipids, and proteins causing cellular dysfunction and ultimately cell death (Trollor & Valenzuela, 2001). Degree of oxidative damage can vary across individuals with some people being less protected against oxygen radicals than others; such individuals are at greater risk of experiencing significant oxidative damage (Franceschi et al., 2000). Inflammation
has been proposed to play a key role in mediating cellular death and destruction via poorly liganded iron (Kell, 2009). Increased brain iron levels is one factor that can influence oxygen species (ROS), favoring oxidative stress, and cell death if levels become excessive (Andersen et al., 2014b; Dixon & Stockwell, 2014)

1.1.3.3 Brain reserve

The concept of brain reserve stems from the need to explain individual differences in resilience to trauma and neurodegeneration. Manifest neuropathology does not necessarily correspond with clinical symptoms (Snowdon, 2003). Larger brain volumes and more synaptic connections may slow brain pathology, and a certain threshold of damage is needed to manifest itself in symptoms. Greater resilience has been explained on a neuronal level as arising from a greater initial number of neurons and synapses (Katzman et al., 1988), as well as greater gross brain volume, and higher overall brain weight (Katzman et al., 1988; Satz, 1993).

1.1.3.4 Brain maintenance

The brain maintenance hypothesis posits that older people vary in age-related changes, and those whose brains resemble those of younger individuals maintain better cognitive performance (Lindenberger, 2014; Nyberg, Lövdén, Riklund, Lindenberger, & Bäckman, 2012). Brain maintenance or lack of brain pathology constitutes the foundation of maintaining cognitive abilities with increasing age (Lindenberger, 2014; Nyberg et al., 2012).

Some support for this hypothesis has been gained from associative studies investigating individual differences in morphometry and white matter integrity (Charlton, Schiavone, Barrick, Morris, & Markus, 2010; Nyberg et al., 2012; Persson et al., 2014; Raz et al., 2010), but the vast majority of studies in this field are yet cross-sectional.

1.1.3.5 Frontal aging hypothesis

The view of the prefrontal cortex as the most age-sensitive brain region is dominant in the research community and in recent years has
gained a renaissance in the “last in, first out” hypothesis in aging research. This hypothesis suggests the inverse of development: late-maturing regions of the brain are preferentially vulnerable to age-related loss of structural integrity in gray and white-matter (Fjell et al., 2009; Raz, 2000; Westlye et al., 2010). Alterations of the frontal lobes have further implications for cognitive aging. Cognitive domains, such as executive and memory functions, that depend on the prefrontal lobe are affected in aging, while cognitive functions relying on other brain regions are relatively spared (West, 1996). This localization-focused approach has been criticized in favor of a more network-oriented approach involving several brain regions (Greenwood, 2000).

1.1.4 Modifiers of brain aging

Multiple factors and their interplay may underlie the course of brain aging, and explain why some individual’s brains are more resistant to age-related changes than others. Apart from chronological age, as discussed in the previous section, other factors may include the interplay of genetic, environmental, and lifestyle factors. I will next discuss the potential influence of sex, genetics, and socio-economic factors as modifiers of the course of brain development in adulthood.

1.1.4.1 Sex differences

Sex-specific differences are sporadically reported in the literature in relation to regional brain volumes. Recent studies suggest that most of the reported sex-specific variations in regional brain volumes may be attributed to different ways of accounting for the intracranial volume (ICV) between studies (Pintzka, Hansen, Evensmoen, & Håberg, 2015; Voevodskaya et al., 2014). Sex differences may, however, be of greater importance in relation to neurotransmission (Davis, Ward, Selmanoff, Herbison, & McCarthy, 1999; Vries, 1990), and brain iron levels.

Sex differences have been reported in prevalence, age of onset, and symptom severity of neurodegenerative diseases that are related to increased iron deposits (e.g., multiple sclerosis, Parkinson's and Alzheimer's disease; Bartzokis et al., 2007; de Rijk et al., 2000; Langkammer et al., 2013; Taylor, Cook, & Counsell, 2007). Women tend to exhibit lower peripheral iron levels (e.g. Bartzokis et al., 2007; Fleming et al., 2001; Whitfield, Treloar, Zhu, Powell, & Martin,
2003), while men tend to have higher iron concentrations in the cortical white matter and subcortical nuclei according to changes in MRI contrast (Bartzokis et al., 2007, 2011; Hagemeier et al., 2013; Tishler, Raven, Lu, Altshuler, & Bartzokis, 2012, but see Xu, Wang, and Zhang 2008). Peripheral iron levels may influence brain iron deposits, according to early PM findings showing that a history of anemia can predict reduced brain iron (Hallgren & Sourander, 1958). Findings from in vivo MRI suggest that menstrual blood loss in women may contribute to sex differences in brain iron accumulation (Tishler et al., 2012). Interestingly, recent PM work indicates that women have lower levels of total brain iron than men from midlife to old age (Ramos et al., 2014). Sex steroids, whose levels change post menopause (Al-Azzawi & Palacios, 2009), could account for some of the sex-related variations in brain iron levels (Gu, Xi, Liu, Keep, & Hua, 2010). Taken together, such findings render sex of interest when studying age-related brain iron accumulation.

1.1.4.2 Genetics

The most prominent genetic polymorphism investigated in relation to cerebral aging is the APOE ε4 allelic variant in the apolipoprotein E gene. The ε4 allelic variant in this single nucleotide polymorphism (SNP) increases the risk for late-onset Alzheimer's disease (AD) (Farlow, 1997; Roses, 1996), and the ε2 variant is considered protective of longevity, while ε4 being deleterious (Brooks-Wilson, 2013). The APOE ε4 allele controls availability of the apolipoprotein E, which is a vital factor in lipid transport. The APOE ε4 allelic variant has also been linked to elevated risk of developing cardiovascular disease (Mahley & Rall, 2000; Rall & Mahley, 1992) and hyperlipidemia (Davignon, Gregg, & Sing; Rall & Mahley, 1992). However, the extant literature concerning the effect of APOE ε4 on healthy brain aging and cognition is contradictory (Reiman, 2007; Reinvang, Espeseth, & Westlye, 2013), and some studies suggest that positive findings may reflect inclusion of individuals in the prodromal stages of dementia (Cherbuin, Leach, Christensen, & Anstey, 2007). Variation in inflammatory response may serve as an important factor in explaining variability across individuals in cerebral aging, considering its importance for longevity. Several SNPs regulating pro-inflammatory responses may be of importance in individual brain aging. MTHFR C677T or rs1801133 is a polymorphism in the methylenetetrahydrofolate reductase gene (MTHFR) that controls production of the enzyme necessary for metabolizing homocysteine (Hcy) (Bathum et al.,
2007; de Lau et al., 2010), which is related to the inflammatory response (Li et al., 2015). Variants of the IL-1β gene (e.g., rs16944) are associated with release of cytokines in response to infection (Yarlagadda, Alfson, & Clayton, 2009).

The current knowledge regarding inflammation and brain integrity has all emerged from cross-sectional research studies. Studies have associated several SNPs that promote inflammation with structural brain differences. For example, the MTHFR -677 T allele has been linked to smaller white matter volumes and accelerated shrinkage in periventricular fronto-parietal and parieto-occipital brain regions (Rajagopalan et al., 2012). G homozygotes of the polymorphism of the IL-6 gene (IL-6A-174G, rs1800795) have greater hippocampal gray matter volumes than heterozygotes and A homozygotes (Baune et al., 2012). In healthy adults (Raz, Yang, Dahle, & Land, 2012), the T allelic variant of the IL-1β C-511T and CRP-286 (rs3091244) polymorphisms are associated with greater white matter hyperintensities (WMH).

1.1.4.3 Cardiovascular risk factors

Factors influencing cardiovascular health are also important for the brain. Changes in endocrine, vascular, and inflammatory systems with advancing age render the older individual at risk of numerous cardiovascular diseases (Perry, 1999; Wu, Xia, Kalionis, Wan, & Sun, 2014). Cardiovascular risk factors are important determinants of structural brain changes and are also important for maintenance of cognitive functions over time. The substantial relevance of many cardiovascular risk factors in determining cognitive individual differences, as well as cerebral alterations, are well documented (see e.g., Amenta, Di Tullio, & Tomassoni, 2003; Waldstein, Manuck, Ryan, & Muldoon, 1991, for reviews). Treatment and exercise interventions targeting such modifiable risk factors may slow brain aging. Yet such factors are often ignored in the study of aging and in the development of theories about reserve factors. Cardiovascular risk, when addressed, is often on an individual basis. A multivariate approach taking into account shared variance among factors may be of greater interest because cardiovascular risk factors often accompany each other.

Poorly regulated blood pressure is linked to decreased white matter integrity (Foster-Dingley et al., 2015; Mortamais, Artero, & Ritchie, 2013; Salat et al., 2012), as well as reduced volumes of prefrontal gray
and white matter (Raz et al., 2005a; Raz, Rodrigue, & Acker, 2003), and appears to accelerate age-related shrinkage of the hippocampus (Hc) and the striatum (Elcombe et al., 2015; Foster-Dingley et al., 2015; Fotuhi, Do, & Jack, 2012). Higher blood glucose levels in the normal range have further been associated with smaller gray and white matter volumes in the frontal cortices (Mortby et al., 2014), Hc (Cherbuin et al., 2007; Cherbuin, Sachdev, & Anstey, 2012; Convit, Wolf, Tarshish, & De Leon, 2003) and amygdala (Cherbuin et al., 2012). Further, higher plasma homocysteine levels have been linked to greater Hc atrophy in non-demented adults aged 60-90 years (den Heijer et al., 2003). Some controversy exists regarding more gross measures of regional brain volumes and higher body mass index (BMI), with studies both supporting and refuting the potential influence of higher-range BMI on whole brain and total gray matter volume (Willette & Kapogiannis, 2015; Gunstad et al., 2008). Smaller volumes of gray matter in the frontal, parietal, and temporal lobes as a function of higher BMI have been noted in middle-aged and older adults (Walther, Birdsill, Glisky, & Ryan, 2010; Willette & Kapogiannis, 2015). However, higher levels of serum lipids have been further linked to larger Hc size (Wolf et al., 2004), and higher low density lipoprotein levels (LDL) may be associated with decreased WM integrity in frontal and temporal regions (Williams et al., 2013).

Studies have also failed to find associations between cardiovascular risk factors, such as hypertension, low-density lipoprotein levels, and BMI, global and regional brain volumes, and white matter integrity (Persson et al., 2014; Raz et al., 2012; Willette & Kapogiannis, 2015). Differences in sampling strategies and participants’ ages may in part mediate such variations in results.

1.1.4.4 Socioeconomic factors

It is known that experience can alter the brain. Experiments have elucidated experience-dependent neuroanatomical changes following training in motor tasks (Sampaio-Baptista et al., 2014, 2015), repeated cognitive test administration (Bäckman et al., 2011; Bäckman & Nyberg, 2013; Dahlin, Neely, Larsson, Bäckman, & Nyberg, 2008; Engvig et al., 2014), and skill training in early life (music training) (White-Schwoch, Carr, Anderson, Strait, & Kraus, 2013). As socioeconomic disparities increase, it is important to address potential concomitants at the level of the individual. Specifically, does socioeconomic adversity contribute to inequalities concerning education,
health, stress, economy, and cognitive abilities (Cochrane, Leslie, & O’Hara, 1982; D’Angiulli, Lipina, & Olesinska, 2012; Lupien et al., 2005). Early socio-economic status (SES) disparities may indeed influence the risk of disease and limitations on activities of daily living in adulthood (Ziol-Guest, Duncan, Kalil, & Boyce, 2012). Numerous factors are thought to be of importance in defining the construct of SES, and a common proxy for SES is educational achievement, which may be a consequence of early influences of SES. Recent work stresses the importance of addressing early factors beyond an individual’s SES in adulthood, such as the individual’s parent’s education (Noble, 2014). Socioeconomic factors have been linked to thickness of the prefrontal cortex in children, white-matter integrity in adolescence (Noble, Korgaonkar, Grieve, & Brickman, 2013), and gross surface area (Noble et al., 2015). Further, hippocampal size may be altered in both children and adults (Brito & Noble, 2014; Noble et al., 2012) as a function of SES. Recent longitudinal work shows that childhood SES predicts hippocampal size, even after accounting for childhood cognitive ability, adult SES, and educational attainment (Staff et al., 2012), suggesting the possibility of long-term effects of early life socio-economic inequalities.

1.2 Cognitive aging

Human aging is accompanied by average declines in various cognitive abilities (Cattell, 1943; Horn & Cattell, 1967; Salthouse, 2010), but more importantly the trajectories of age-related change also vary among individuals (Hultsch, Hertzog, Small, McDonald-Miszczak, & Dixon, 1992; Lindenberger, 2014; Rabbitt, 1993) and age-related changes vary in magnitude over different cognitive domains (Ghisletta, Rabbitt, Lunn, & Lindenberger, 2012; Rabbitt et al., 2004; Rabbitt, 1993). Cattell (1943) further differentiated Spearman’s (1904) concept of general intelligence to include two aspects of cognitive abilities: fluid (G_f) and crystallized intelligence (G_c). The former relates to problem solving, logical reasoning, and speed of processing, while the latter is based on acquired knowledge. The development of fluid abilities was viewed as continuing until young adulthood, followed by a brief plateau, with a subsequent decline from the seventh decade of life into senescence (Cattell, 1943). In contrast, crystallized abilities improve throughout child- and adulthood, with gradual declines from the upper part of the lifespan (Cattell, 1943). Cattell’s landmark theories and early empirical work have gained extensive support from the research community (Finkel, Reynolds, McArdle,
Research conducted after Cattell’s initial proposal suggests more elaborated sets of age-sensitive cognitive domains, with the rates of change varying across different cognitive constructs. Working memory, episodic memory, processing speed, and spatial reasoning scores all exhibit pronounced age-effects, while vocabulary and verbal comprehension are spared until late life (Hultsch, Hertzog, Small, McDonald-Miszczak, & Dixon, 1992; Persson, Lavebratt, & Wahlin, 2013; Persson, Viitanen, Almkvist, & Wahlin, 2013; Rabbitt et al., 2004; Rabbitt, 1993).

1.3 Brain–cognition relationships

The vast majority of studies investigating relationships between brain and cognition are based on cross-sectional study designs. Cross-sectional designs focus on age-related differences, rather than changes within individuals. Thus, cross-sectional designs are ineffectual for generating hypotheses about changes in brain-cognition relationships over time (Ghisletta & Lindenberger, 2003; Hofer & Sliwinski, 2001; Lindenberger, 2014; Sliwinski, Hoffman, & Hofer, 2010). Further, the existing longitudinal studies focus primarily on average effects, without accounting for individual differences in changes.

Cross-sectional differences in prefrontal volume, and increased prefrontal white matter connectivity (i.e., increased fractional anisotropy, interpreted as increased fiber density and decreased diffusivity as cell growth) are related to age differences in fluid reasoning and in more specific cognitive functions, such as strategic control of episodic memory, processing speed, and executive functions (Bennett & Madden, 2014; Euston, Gruber, & McNaughton, 2012; Kane & Engle, 2002; Madden, Bennett, & Song, 2009; Rajah & D’Esposito, 2005). Additionally, both functional and structural imaging studies performed over the last decade have revealed the importance cerebellar involvement in multiple cognitive processes (Buckner, 2013; Stoodley, 2012).

Longitudinal studies of non-demented adults show that global deterioration of the brain, expressed in ventricular expansion (Grimm, An, McArdle, Zonderman, & Resnick, 2012; McArdle et al., 2004) as well as regional shrinkage of the hippocampus (Kramer et al., 2007; Rusinek et al., 2003), leads to declines in episodic memory. Changes in episodic memory are also related to changes in resting-state connec-
tivity, beyond the influence of structural shrinkage in healthy adults (Fjell et al., 2015).

Empirical findings illuminate the bi-directional relationship between brain and cognition during the adult lifespan. Smaller gross brain volume has been linked to declines in fluid intelligence among older adults (Rabbitt et al., 2008). Longitudinal evidence further links higher fluid intelligence and episodic memory scores to less shrinkage of the medial temporal lobes (MTL) (Borghesani et al., 2012; Raz et al., 2008; Rodrigue & Raz, 2004). Further, greater general cognitive ability measured in youth predicts larger brain volumes in old age (Royle et al., 2013).

1.4 Theories of cognitive aging

The distinction between non-normative and normative or non-pathological versus pathological aging is often very arbitrary. Different criteria are applied in studies to exclude participants with cardiovascular disorders and neurodegenerative diseases. One complicating factor in this context is that the prenominal phase of AD is very extensive (Small, Mobly, Laukka, Jones, & Bäckman, 2003). Recent findings suggest that there might be little age-related cognitive decline in old people relative to those who will die within approximately 42 months (Wilson, Beckett, Bienias, Evans, & Bennett, 2003; Wilson, Beck, Bienias, & Bennett, 2007), and studies also reveal that elderly individuals can actually improve or maintain their cognitive abilities (Dahlin et al., 2008; Persson et al., 2016; Rönnlund, Nyberg, Bäckman, & Nilsson, 2005). Such findings make it interesting to further investigate the possibility of successful cognitive aging and cognitive reserve factors.

1.4.1 Successful aging

Rowe and Kahn (1987) made a distinction between successful and unsuccessful normal aging, and challenged earlier more deterministic traditions focusing selectively on decline in aging. Successful aging was characterized by maintenance of high physical and cognitive function, and sustained engagement in social and productive activities (Rowe & Kahn, 1997). These extrinsic factors were hypothesized to play a neutral or positive role in successful aging. Their early claim
that the negative aspects of the aging processes was exaggerated, while mitigating effects of diet, exercise, personal habits, and psychosocial factors were underestimated has been very influential in the development of different notions of reserve. The successful aging paradigm has received substantial criticisms in recent years for empirical and methodological limitations that ignore social inequality, health disparities, and age relations (Katz & Calasanti, 2015; Rubinstein & de Medeiros, 2015). There is also great inconsistency among studies in conceptualizing and operationalizing the notion of successful aging (Katz & Calasanti, 2015). The theories of successful aging as conceptualized by Rowe and Kahn (1987, 1997) also lack a clear neurobiological foundation in terms of neural underpinnings.

1.4.2 Cognitive reserve

The influential notion of cognitive reserve holds that some individuals cope better than others with maintaining cognitive performance over time (Stern, 2002, 2003), and individuals vary in how well they can make use of available brain reserve (Stern, 2002). Moreover, there is a distinction between passive and active reserve that can be summarized as “having” more versus “doing more” (Staff, 2012; Stern, 2002). The already available brain reserve can mitigate the expression of neurodegeneration. The active component of cognitive reserve refers to the person’s ability to compensate for age-related changes through maintaining well-functioning cognitive abilities, acquisition of alternative coping skills, and the tendency to adhere to lifestyles and practices that promote maintenance of neural circuit function (Stern, 2002; Tucker & Stern, 2011). The theory includes better efficiency in general cognitive ability and knowledge, and causal variables associated with the level of cognitive functioning. Standard proxies for cognitive reserve are education, intelligence, literacy, occupational attainment, engagement in leisure activities, and integrity of social networks (Tucker & Stern, 2011). Recent work suggests that aspects of personality (conscientiousness) contribute to cognitive reserve (Tucker & Stern, 2011; Wilson, Schneider, Arnold, Bienias, & Bennett, 2007). Often cognitive reserve factors are operationalized at a single level, although more multivariate approaches exist (Satz, Cole, Hardy, & Rassovsky, 2011; Scarmeas et al., 2003). Recent work casts doubt on the link between various factors included under the cognitive reserve umbrella of causal variables, but cognitive activity or engagement exhibits a more direct mitigating influence on neurodegeneration (Bennett, Arnold, Valenzuela, Brayne, & Schneider, 2014).
2 Goals

Previous work has primarily focused on cross-sectional age-differences in describing the cognitive neuroscience of aging. The existing longitudinal structural MRI studies focus predominately on average effects and fail to take into account individual differences in changes. Further, the vast majority of studies consider the structural integrity of gray and white matter. Modern iron-sensitive contrasts provide new opportunities and may illuminate novel mechanisms underpinning neuronal aging. Moreover, the existing work focuses on how brain integrity predicts cognitive functions, rather than their bi-directional relationship over time. The goal of this thesis is to reduce these outlined gaps in knowledge.

The specific goals were the following:

Study I: examined changes on average, as well as individual differences in change in neuroanatomical brain volumes, and the influence of determinants of individual rates of change were examined. Genetic and cardiovascular risk factors were considered. A specific focus was placed on genetic polymorphisms influencing pro-inflammatory responses.

Study II: examined the functional form of age-related differences in brain iron distributions. Further, we assessed whether brain iron increased in synchrony across the brain structures of interest. A final important goal was to unravel potential sex differences in subcortical brain iron concentrations.

Study III: assessed whether brain changes could be coupled with cognitive changes to unravel the bi-directional relationships between anatomical brain regions and three cognitive domains: episodic memory (EM), fluid intelligence (Gf), and vocabulary (V). The effects of socio-economic status, and genetic and cardiovascular risk, were accounted for.
3 Method

3.1 Participants

For study I and III, volunteers were recruited through media advertisement and flyers in a major metropolitan area in the mid-western United States. People who reported a history of cardiovascular, neurologic or psychiatric diseases, head trauma with lost consciousness in excess of five minutes, thyroid dysfunction, or history of treatment for drug and/or alcohol abuse or habitual consumption of alcohol (three or more drinks per day), as well as individuals taking anti-seizure medication, anxiolytics or antidepressants were excluded from the study sample. Claustrophobic individuals were advised not to participate. People with a reported diagnosis of hypertension were included, if they were taking anti-hypertensive medications, such as beta-blockers, calcium channel blockers, angiotensin-converting enzyme inhibitors or potassium-sparing diuretics. All participants were screened for dementia and depression using the Mini Mental State Examination (MMSE; Folstein, Folstein, & McHugh, 1975), using a cut-off of 26; Center for Epidemiologic Studies Depression Scale (CES-D; Radloff, 1977), with a cut-off of 15. All participants were right-handed. The magnetic resonance (MR) scans were examined by a neuroradiologist for suspected space-occupying lesions and signs of pathology. Out of 1055 respondents, 360 were eligible to participate in the study. See Figure 3 for details.
For study III, participants were recruited from hospital personnel in the city of Dalian, China. Participants were free of neurological, psychiatric, or cardiovascular diseases (diabetes, stroke, and hypertension), drug or alcohol abuse, and history of concussion or brain surgery. In all studies, MRI images were screened by an experienced radiologist for brain abnormalities. Absence of brain resection, infarction, focal lesions, and large hyperintensities were further confirmed by a radiologist. All participants were right-handed.
Participants in studies I, II, and III gave their informed consent, and the studies were subject to ethical approval by the regional ethics committees.

3.2 MRI methods

Structural magnetic resonance imaging was used in the studies, specifically spin-lattice relaxation rates and quantitative susceptibility mapping.

3.2.1 T1-weighted imaging (spin lattice)

In T1-weighted (or spin lattice) imaging, the T1 parameter reflects how the MR signal changes over time according to an exponential relaxation curve that describes how the magnetized spinning proton returns to its equilibrium state and realigns with the main magnetic field, after excitation by a radiofrequency pulse (that is turned on and off). The energy that is generated when the protons return to the location determined by the external magnetic field of the scanner is detected via the head coil of the scanner. T1 images, which contrast solid and fluid properties as well as white versus gray matter, are used for morphometric analyses of neuroanatomical brain volumes. Differences in contrast are caused by differential density of protons (Hornak, 1997; Lipton, 2008).

For study I and III, all image acquisition was performed at the Magnetic Resonance Research Facility at Wayne State University, USA, on a 4-Tesla scanner (Bruker Biospin, Ettlingen, Germany) using an 8-channel radio frequency coil. Magnetization-prepared rapid gradient echo (MPRAGE) T1-weighted images in the coronal plane were acquired for volume measurements. The following acquisition parameters were used: echo time (TE) = 4.38 ms, repetition time (TR) = 1600 ms, inversion time (TI) = 800 ms, field of view (FOV) = 256 × 256 mm², resolution = 0.67 × 0.67 × 1.34 mm³, matrix size = 384 × 384 and flip angle (FA) = 8°. The regions of interest were manually outlined on the T1-weighted images, based on contrast differences and neuroanatomical boundaries defined by a previously described manual tracing protocol (Persson et al., 2014; Raz et al., 2010).


3.2.2 Quantitative susceptibility mapping

Quantitative susceptibility mapping (QSM) is a novel technique that incorporates both phase and magnitude information from flow-compensated gradient echo (GRE) images (Wang & Liu, 2015). A gradient-recalled echo (GRE) pulse sequence is generated to acquire images. This method exploits the susceptibility differences between tissues and uses the phase image (phase shifts of the protons in response to the gradient), in addition to signal magnitude (protons fall out of phase, transverse magnetization decreases). QSM allows for short scan times, and provides a better solution to non-local field effects that are prominent in phase-contrast imaging (J. Li et al., 2012). QSM is better at visualizing the iron distribution in the brain than other iron-sensitive contrasts (Langkammer et al., 2012). \( T_2^* \) and \( R_2^* \) (\( R_2^* = 1/ T_2^* \)) from magnitude data are more sensitive to changes in imaging parameters such as echo time (TE) (Brown, Cheng, & Haacke, 2014). Morphology-enabled dipole inversion (MEDI) (de Rochefort et al., 2010; Wang & Liu, 2015), a QSM algorithm incorporating both phase and magnitude information, holds much promise with its sensitivity to both para-magnetism (iron) (Langkammer et al., 2012; J. Li et al., 2012) and diamagnetism (e.g., calcium, white-matter) (Schweser, Deistung, Lehr, & Reichenbach, 2010), and shows good reproducibility over different scanner manufacturers and magnet strengths (Deh et al., 2015). For study II, we used a modified version of the MEDI Laplacian (1) algorithm (Persson et al., 2015). An example of the reconstruction of MEDI QSM is illustrated in Figure 4.

**Figure 4.** T1 recovery (spin-lattice relaxation), involving recovery of the longitudinal magnetization (yellow) because of the energy release (green) into the environment. The lattice is indicated in tan. Adopted from Bitar et al. (2006). Reprinted with permission from RSNA.
The MRI examination for study II was performed on a 1.5-T scanner (GE Signa EXCITE 14.0) using an 8-channel head coil, via a 3D spoiled gradient echo sequence with flow compensation. The following imaging parameters were used for the gradient echo sequence: true axial plane, echo time (TE) = 40 ms, repetition time (TR) = 53 ms, flip angle (FA) = 20º, slice thickness = 3 mm, bandwidth = +/- 31.25Hz/pixel, field of view (FOV) = 24 cm, matrix size = 512 × 512 × 40.

The regions of interest were manually outlined on the QSM images, based on contrast differences as described in Persson et al. (2015).

**Figure 5.** Illustrating an outline of the MEDI QSM reconstruction using edge information from the magnitude image, and the tissue field from the local field map, using morphology-enabled dipole inversion (MEDI), here illustrated in 3-T images of the transverse plane. Reprinted with permission.
3.3 Design

Both cross-sectional and longitudinal study designs were used in this thesis. A longitudinal design was applied in Studies I and III (Persson et al., 2014; Persson et al., 2016), while study II was cross-sectional (Persson et al., 2015).

A cross-sectional study design can only account for age-related differences among individuals, while a longitudinal design also captures individual differences in change trajectories (Hofer & Sliwinski, 2001). Studies addressing individual variation show that individuals do indeed evidence substantial heterogeneity in brain development and cognitive functions (McArdle et al., 2004; Rabbitt, 1993; Rabbitt et al., 2008), and some studies suggest that such heterogeneity increases particularly in older age (Rabbitt, 1993, but see Salthouse, 2011). Nonetheless, many longitudinal studies that focus on brain aging and its relation to cognitive aging often solely examine average change rates. In the context of latent variable modeling, variance in change can be specified as latent or random effects that reflect a random probability distribution around the fixed effect (average) (Curran, Obeidat, & Losardo, 2010).

In the longitudinal study of cognitive aging, retest effects are important because healthy people tend to learn from repeated task exposure. To more accurately determine the true effects of chronological age, it is important to account for bias due to retest effects (Ferrer, Salthouse, McArdle, Stewart, & Schwartz, 2005). The assessment of retest effects requires several measurement intervals, and such effects could be underestimated over just two test occasions. This issue will be discussed in greater detail in relation to study III in the General discussion.

3.4 Statistical methods

Structural equation modeling (SEM) was used in all the studies of this thesis. Confirmatory and second-order factor models and structural equation models with covariate effects were estimated in study II, while univariate and bivariate latent change score models (LCSMs) were fitted to the data in studies I and III. The SEM algorithms for the specified models can be viewed in Persson et al. (2015, 2016). All
models were estimated using the Mplus software (Muthén, & Muthén, 1998, 2015).

3.4.1 Structural equation modeling

In structural equation modeling (SEM) latent variables can be specified to make the measurement error less influential (Jöreskog, 1970; McArdle, 1996). The basic model assumption is that the latent variables and the residuals are normally distributed (Jöreskog, 1970). Analysis of variance (ANOVA) and traditional regression techniques are more sensitive to bias from measurement error, and estimates of effect size may be underestimated. Measurement error can attenuate correlations and regression slopes, and increase standard errors of the parameter estimates (Rigdon, 1994).

Other important advantages of SEM include a greater ability to model complex multivariate relations with multiple dependent and independent variables, and assessment of moderation and mediation. SEM with latent variables can focus on a measurement model, which is analogous to common factor or a confirmatory factor model, where the common factors (also called latent variables) are represented by various measured (or manifest or observed) variables, and a structural model in which variance in several measures can be explained by a set of predictors/covariates.

3.4.1.1 Measurement model

In specifying models for study II, I first fit a multivariate measurement model, or confirmatory factor analysis model (CFA) to the data. The model was comprised of seven latent variables, each represented by average susceptibility over left and right hemispheres. The left hemisphere was set to the value of one across the latent variables for model identification. The common factors were further interrelated by twenty-one covariance relationships between them. Dentate nucleus images were available for 88 participants, so this ROI was estimated as a single latent variable. The residuals were constrained to be equal for local identification, and the model was evaluated by the strength of the factor loadings; values ≥ .852 were considered strong (cut-off, .70, Kline, 1994). In study III, two measurement models were specified to reflect socioeconomic status and cardiovascular risk, based on two and four
manifest or observed variables, which were included as predictors in the longitudinal models described in section 3.5.2.

A second order latent variable can be added to the model to represent another hierarchical level for conceptualizing a further construct level. In study II, such a hierarchical factor model was specified to represent total brain iron via a second-order latent variable that was represented by the susceptibility measurements of iron estimates in the seven subordinate latent variables (see Figure 6).

**Figure 6.** The path diagram shows the second-order factor model. The first order factors ($\eta_1, \ldots, \eta_7$) reflect the susceptibilities of the seven ROIs. $\lambda$ are the factor loadings of susceptibilities in left and right hemispheres on the latent variable $\eta$, and $\varepsilon$ is the residual error term of each manifest indicator (e.g., Cdl = left caudate nucleus). $\xi$ is the second-order latent variable reflecting total estimated iron concentration across the subcortical nuclei. $\Gamma$ denotes the regression coefficient between the second-order latent variable $\xi$ and the subordinate factors $\eta_1, \ldots, \eta_7$. The figure is reprinted with permission from Elsevier.
3.4.1.2 SEM with covariates

As described in the previous sections, SEMs with age and sex as predictors of the level of susceptibility were estimated in a second step in study II.

3.4.1.3 Multiple group analysis

In study II, we performed a multiple group analysis (MGA) to simultaneously derive parameter estimates for men and women, to compare parameter estimates between the groups, and to evaluate the effects of the sex by age interaction on susceptibility counts. Measurement invariance was first established to make certain that the same measurement model held across the two groups, by comparing a model with all parameters estimated freely across groups, with two models with gradually imposed equality constraints on the parameters. The models were estimated under the assumption of strict factorial invariance, and the parameter constraints did not indicate substantial loss of fit when the difference in $\chi^2$ ($\Delta \chi^2 = \chi^2_{\text{restricted}} - \chi^2_{\text{unrestricted}}$ with $df = df_{\text{restricted}} - df_{\text{unrestricted}}$) (see e.g., Bentler & Bonett, 1980) was calculated upon parameter constraints.

3.4.1.4 Model fit

Model fit statistics were used to evaluate the mathematical models. Through all evaluations of model fit, we recognize that all models are approximations, rather than the final truth: all models are wrong, but some are useful. Joint criteria based on several model-fit indices were used, as follows: comparative fit index (CFI) $> 0.95$, standardized root mean square residual (SRMR) $< 0.08$, and root-mean-square error of approximation (RMSEA) $< 0.08$ (Browne, Cudeck, & Bollen, 1993; Hu & Bentler, 1998; Hu & Bentler, 1999).
3.4.2 Latent change score modeling

Latent-change score models (LCSMs) were specified in study I and III. Based on the extant literature, we expected to find heterogeneity in change, in addition to average trends in change (Rabbitt et al., 2004; Rabbitt, 1993). More specifically, a latent change score was used to examine change in sample averages as well as variance across individuals, after accounting for baseline level mean differences and variance. For instance, the latent score at time point two of one participant was constructed as the unit-weighted sum of the latent scores at baseline and a latent score representing change between baseline and follow-up. Further, the covariance between initial levels and rates of change were specified. In study III, the multivariate construct episodic memory (EM) was specified in a second order latent variable represented by two subordinate latent constructs: episodic recall and recognition at time point 1 (T1) and T2. Specification at the latent level assures that the change estimation is reliable and not contaminated by measurement error, unlike, for example, simple difference scores between measured indicators (Cronbach & Furby, 1970). See Figure 7 for an illustration of the LCSM for EM from study III.

When creating common factors from repeated measures, it is important to ascertain that the same common factors are measured at each measurement occasion, so that changes in the observed mean can be represented by change in factor means, and to avoid bias in the estimation of mean change (Grimm & Ram, 2009; McArdle & Grimm, 2010; McArdle & Nesselroade). Tests of factorial invariance can be applied by freeing and adding parameter constraints, ranging from weak to strict factorial invariance (Gregorich, 2006; Meredith & Horn, 2001; Meredith, 1993; Persson et al., 2015). Once the presence of invariance is established, various covariates can be added as determinants of individual differences in change of regional brain volumes and cognitive performance scores, as in the analyses performed in studies I and III. Specific covariances of the measurements over time were further specified to address the possibility of additional confounds from measurement error (McArdle, 2009), and initial level versus change correlations were added to the model.

Lead-lag relationships can be established to evaluate dynamic expressions in bivariate LCSMs (Grimm, McArdle, Zonderman, & Resnick, 2012; McArdle, & Grimm, 2010). In study III, such bivariate models were specified with initial levels in test scores or regional brain volumes predicting change in the other. Further, coupled changes were
specified if both cognitive scores and volumetric measures exhibited significant variance in change.

**Figure 7.** A latent change score model for the estimation of a two-occasion change in episodic memory (EM), with first-order factors recall and recognition. Squares represent observed variables, circles are latent variables. The triangle indicates that the model has means. All free parameters are marked by an asterisk. Constrained equality is denoted by an equals sign and the same subscript. Change ($\Delta$) is specified in a second order latent variable (EM), represented by two subordinate latent variables: recall and recognition. Notations: $\delta =$ mean at baseline, $\alpha =$ variance at baseline, $\beta =$ mean change, $\gamma =$ variance in change, $\epsilon =$ covariance between individual differences in EM at baseline and individual differences in changes between baseline and follow-up. The model has 21 degrees of freedom and contains eight observed variables. The figure is reprinted with permission from Elsevier.
4 Summary of studies

The following section summarizes the three empirical studies included in this thesis. All reports are reprinted with permission from Elsevier.

Table 1. Overview of the studies included in the thesis.

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>MRI</th>
<th>N</th>
<th>Age</th>
<th>Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>I*</td>
<td>Longitudinal</td>
<td>T1w</td>
<td>167</td>
<td>19-79</td>
<td>10 ROIs, 4 SNPs, age, HBP</td>
</tr>
<tr>
<td>II</td>
<td>Cross-sectional</td>
<td>T1w</td>
<td>183</td>
<td>20-69</td>
<td>8 ROIs, age, sex</td>
</tr>
<tr>
<td>III*</td>
<td>Longitudinal</td>
<td>QSM</td>
<td>167</td>
<td>19-79</td>
<td>6 ROIs, 1 SNP, age, SES, VR</td>
</tr>
</tbody>
</table>

Note. MRI = magnetic resonance imaging, T1w = T1-weighted (spin-lattice), N = number, QSM = Quantitative susceptibility mapping, Age = age in years at baseline, ROI = region of interest, HBP = diagnosis of hypertension at baseline (0 = no, 1 = yes), SNPs = single nucleotide polymorphism, SES = socio-economic status (parental), VR = Vascular risk (pulse pressure, body mass index, low density lipoprotein, fasting blood glucose). * Sex was omitted from the equation due to lack of significance.
4.1 Study I


4.1.1 Background

Brain volumes decrease with age and significant reductions are observed in several brain regions. The vast majority of studies are cross-sectional and many existing studies focus predominately on average rates of change (but see Raz et al., 2005b, 2010). The prefrontal cortices, medial temporal lobes, limbic cortices, and cerebellum all exhibit average declines (Fjell, McEvoy, Holland, Dale, & Walhovd, 2013; Resnick, Pham, Kraut, Zonderman, & Davatzikos, 2003; Scahill et al., 2003), and the subcortical basal ganglia shrink to a somewhat lesser extent (Fjell et al., 2009; Raz et al., 2005b). Interestingly, individuals also vary in rates of change across many neuroanatomical regions (Raz et al., 2005b, 2010). Several brain biomarkers, including circulatory plasma markers and single nucleotide polymorphisms (SNPs) influencing pro-inflammatory responses, have been linked to individual differences in hippocampal volume and cerebral white and gray matter (Bettcher & Kramer, 2014; Taki, Thyreau, Kinomura, Sato, Goto, Wu, Kawashima, et al., 2013; van Dijk et al., 2005).

4.1.2 Objective

The first aim was to assess regional brain changes occurring over two years in healthy adults and to replicate previous findings obtained using weaker systems (1.5T). Based on the extant literature, we expected to observe mean shrinkage of the cerebellum, hippocampus, striatum, and prefrontal cortices, but no substantial changes in the volume of the primary visual cortex, because this region is known to be more resistant to age-related changes. We further examined individual rates of
change across regional brain volumes. Based on previous studies, we hypothesized that individuals would show variance in changes across all regions of interest (ROIs). Finally, we assessed determinants of brain-aging trajectories in regional brain volumes. Specifically, we considered the effects of vascular risk factors such as arterial hypertension, and genetic variants associated with increased pro-inflammatory response, namely \textit{IL-1\beta}C-511T, rs16944; \textit{CRP}-286C>A>T, rs3091244; and \textit{MTHFR} C677T, rs1801133. In addition, we accounted for confounding effects by considering the effect of a common genetic risk factor for AD (\textit{APOE} \varepsilon4) on rates of change in regional brain volumes.

4.1.3 Method

Data were analyzed from 167 healthy adults over approximately two years (average interval between assessments: two years and 24 days). Participants suffering from dementia, psychiatric illnesses, cardiovascular diseases, or exhibiting brain abnormalities were excluded. Individuals with medically controlled hypertension were included. The regions of interest (ROIs) were segmented using a manual tracing protocol on images from a 4-Tesla scanner (Bruker Biospin, Ettingen, Germany). Raters were blind to participants’ demographic characteristics. The intra-class coefficient (ICC) between the raters exceeded .90.

A series of latent change score models (LCSMs) were fitted to the data with parameters for both average changes and individual variance in changes in the following regions of interest (ROIs): lateral prefrontal cortex (LPFC), orbital frontal cortex (OF), prefrontal white matter (PFw), hippocampus (Hc), parahippocampal gyrus (PhG), caudate nucleus (Cd), putamen (Pt), insula (In), cerebellar hemispheres (CbH), and primary visual cortex (VC). LCSMs allow for estimation of longitudinal age-related changes, simultaneously taking into account cross-sectional effects. Second, predictors of change were added to explain individual rates of change across the ROIs. We accounted for the influence of pro-inflammatory genetic polymorphisms by considering the influence of chronological age, arterial hypertension, and the \textit{AP\OE} \varepsilon4 polymorphisms.
4.1.4 Results

Greatest magnitudes of change were noted in In and CbH, followed by PhG, Hc, and OF; very small average effects were observed in LPFC, PFw, VC, Cd, and Pt. Larger proportions of individual variance emerged in the In, and CbH, followed by PhG and VC. More moderate levels of individual variance were observed in Hc, Pt, PFw, LPFC and Cd. A smaller degree of variance was evident in OF. Larger baseline volumes of the CbH and In were associated with greater shrinkage. No other baseline versus change relationships emerged.

Age was a strong predictor of cross-sectional age-related differences in volumes, whereby advanced age was associated with smaller neuroanatomical brain regions. However, baseline age showed no significant relationship with the course of individual rates of change over two years. None of the covariates showed significant relationships with neuroanatomical ROIs at baseline. Possession of two copies of the T allele in the *IL-1β*-511 SNP predicted greater shrinkage over two years in the PhG, and the CbH (see Figures 8A and 9). At least one copy of the T allelic variant in the *MTHFR*-677 SNP indicated increased decline in volume in the PhG (see Figure 8B).
Figure 8. The bar charts illustrate the effect of two pro-inflammatory single-nucleotide polymorphisms $IL-1\beta$ C-511T (A) and $MTHFR$ C677T (B) on regional shrinkage in the parahippocampal gyrus. The T allelic variant marked in dark gray in A is associated with higher circulatory interleukin-1\beta, contrasted with carriers of any C allele. B shows carriers of the $MTHFR$ T allele are linked with higher plasma homocysteine levels compared to homozygote C carriers. Shrinkage is indexed by the mean expected value of latent change scores computed from the estimated means of the models while taking into account the effects of covariates. The error bars represent 95% confidence intervals around the means. Reprinted with permission from Elsevier.

Figure 9. The figure shows the effect of a pro-inflammatory SNP, $IL-1\beta$ C-511T, on shrinkage of the cerebellar hemispheres in normotensive participants. Shrinkage is indexed by the mean expected value of latent change scores computed from the estimated means of the models while taking into account the effects of covariates. The error bars represent 95% confidence intervals around the means. Reprinted with permission from Elsevier.
4.1.5 Conclusion

These results confirm previous findings of average and differential rates of shrinkage in brain aging, but counteract previous reports in one important respect: we found no average shrinkage in the volume of the lateral prefrontal cortex. These findings cast doubt on the unique role of the prefrontal cortex in aging. Further, in conflict with the brain reserve hypothesis, we observed that larger volumes at baseline predicted greater shrinkage of the cerebellar hemispheres and the insula. Unlike other reports, age and hypertension did not predict shrinkage in brain volumes. This may be related to our selection of individuals in optimal health. Our findings highlight the importance of investigating the role of inflammation in brain aging, but warrant replication in future studies.
4.2 Study II


4.2.1 Background

Age-related brain iron accumulations accelerate across the lifespan according to post mortem (PM) findings by Hallgren and Sourander (1958). Substantially more brain iron is observed in the subcortical nuclei than in the cortex (Gelman, 1995). Age-related iron distributions assessed by in vivo MRI have been studied to a greater degree in the striatum and pallidus (Bartzokis et al., 1997b; Bilgic et al., 2012; Cherubini, Peran, Caltagirone, Sabatini, & Spalletta, 2009; Haacke et al., 2005) as compared with the substantia nigra, red nucleus, subthalamic nucleus, and cerebellar dentate nucleus (Bilgic et al., 2012; W Li et al., 2014). Further, sex-related variations in sub-cortical brain iron estimates have also been studied to a lesser degree. Recent PM work provides clarity by revealing lower brain iron levels in women than men from midlife (Ramos et al., 2014). Various contrasts exist for generating brain iron estimates in vivo, but there are several limitations to be noted. Both field-dependent relaxation rates (FDRI) (Bartzokis, Aravagiri, Oldendorf, Mintz, & Marder, 1993), magnitude ($R_2^*$) (Gorell et al., 1995; Haacke et al., 2005), and phase signal (Haacke et al., 2007) have been used. FDRI requires two field strengths and is time consuming. Both phase and $T_2^* / R_2^* (R_2^* = 1/T_2^*)$ suffer from non-local field effects. $R_2^*$ is also sensitive to echo time, field strength, and object orientation (Brown et al., 2014). Quantitative susceptibility mapping (QSM) is a novel method that can address non-local field effects in the phase information and retain the tissue magnetic susceptibility. It also preserves magnitude information in the MEDI(morphology enabled dipole inversion) algorithm (de Rochefort et al., 2010; Wang & Liu, 2015).
4.2.2 Objective

The main objective was to study age- and sex-related distributions of subcortical brain iron concentrations. We aimed to replicate previous findings of age-related differences in brain iron of the striatum, but also to add to the current state of knowledge by studying smaller, less studied subcortical nuclei, such as the sub-thalamic pulvinar complex, substantia nigra and red nucleus, and dentate nucleus in the cerebellum. We examined intercorrelations among subcortical nuclei to discover if a uniform pattern of correlations would emerge, potentially reflecting an age-related mechanism. We further assessed sex-related differences in the subcortical nuclei. We also investigated potential lowering of brain iron levels from expected menopause age, because recent postmortem work reveals smaller iron deposits in women compared to men from midlife.

4.2.3 Method

One hundred and eighty-three healthy participants were included in the study (20-69 years, 49% women). Phase and magnitude information were derived from a three dimensional flow-compensated gradient echo sequence on a 1.5-Tesla scanner (GE Signa EXCITE 14.0). QSM images were constructed using a modified version of MEDI QSM (de Rochefort et al., 2010). The gradient mask was based on filtering the first echo magnitude image. QSM in CSF was set to zero to allow comparison across participants (Dong et al., 2015; Persson et al., 2015). The following ROIs: were segmented manually on the QSM image by experienced raters: caudate nucleus (Cd), putamen (Pt), globus pallidus (Gp), substantia nigra (Sn), red nuclei (Rn), thalamus (Th), pulvinar (Pul), and dentate nucleus (Dn). To minimize inter-subject variability, each ROI was segmented by a single radiologist. Average susceptibility was measured within each ROI. A series of structural equation models (SEMs) were fitted to the data. Both multivariate confirmatory factor models (CFA) and hierarchical factor models were evaluated. Sex differences were evaluated in the context of multiple group analysis (MGA). Age and sex were added as covariates and both linear and curvilinear effects of age were evaluated. Total subcortical brain iron levels were represented by a second order factor to examine the influence of post-menopause age (women at the average age of menopause onset 51 years were coded as 1). The dummy variable was added as a predictor to the model.
4.2.4 Results

The distributions of iron varied across structures, consistent with previous post-mortem findings (see Figure 10). All models showed good fit to the data according to conventional thresholds for joint criteria of fit indices (described in greater detail in the Method section). Strict factorial invariance was established by careful evaluation of model constraints so that parameter estimates and constraints could be evaluated across men and women. Brain iron levels were correlated across many ROIs: twenty out of twenty-eight correlations were significant and positive. The strongest linear effects of age on susceptibility increase were observed in the striatum (Figure 11). Curvilinear effects of age on susceptibility increase was particularly strong in the RN ($\Delta f^2_{12} = 0.344$, proportion of variance accounted for linear age), moderate for the Pul (0.131), and small for the other curves (<0.15) (Co-
Men showed higher overall brain iron concentrations as a function of age according to mean proportions of explained variance, and sex-specific effects emerged in Sn and the Pul whereby men had higher levels of susceptibility. A trend in the same direction was present for susceptibility levels in the Rn ($p = .059$). The second-order factor analysis revealed that women expected to be post-menopause had a lower total subcortical susceptibility level than men and younger women, after accounting for individual age (Figure 12). Women expected to be post menopause were 169 parts per billion (ppb) lower in susceptibility than the rest of the subjects, according to the unstandardized parameter estimates.

Figure 11. Scatter plots illustrating linear increment of susceptibility values as a function of chronological age. High values are indicative of high iron load. The outer gray lines represent the 95% prediction limits. Susceptibility is presented in parts per billion (ppb). ©Elsevier 2015, reprinted with permission.

4.2.5 Conclusion

The findings relating to age-dependent brain iron accumulation are consistent with previous reports, but also add to the current state of knowledge by reporting age-related changes in less studied, smaller subcortical nuclei. We suggest that the many positive correlations over the subcortical nuclei may reflect an age-related mechanism. A potential explanation is that excessive accumulation of intracellular non-
Heme iron promotes reactive oxygen species (ROS), oxidative stress, and cell death, resulting in neurodegeneration (Andersen et al., 2014b; Dixon & Stockwell, 2014; Floyd & Carney, 1993). This report is, to the best of our knowledge, the first to show in vivo that women have lower total subcortical brain iron levels after expected menopause onset. Age-related changes in estrogen levels may be a mediating factor in such associations. The current study illustrates that age and sex are important co-factors to take into account when establishing a baseline level to differentiate pathologic neurodegeneration from healthy aging. Longitudinal evaluation is necessary to determine how the age-related differences reported herein evolve over time.

**Figure 12.** Decrease in susceptibility in women, after average age of menopause onset (51 years). Total susceptibility is reflected in a second order latent variable representing the susceptibility of seven ROIs: caudate nucleus (Cd), putamen (Pt), globus pallidus (Gp), thalamus (Th), pulvinar (Pul), red nucleus (Rn), and the substantia nigra (Sn). Susceptibility is presented in parts per billion (ppb). ©Elsevier 2015, reprinted with permission
4.3 Study III


4.3.1 Background

Aging is characterized by shrinkage in multiple brain regions, although individuals also exhibit substantial heterogeneity in change rates. Aging is further characterized by growth, decline, and stability of cognitive functions, depending on the measured domain. Fluid abilities tend to decline on average, while crystalized abilities tend to remain stable until late in the lifespan (Rabbitt, 1993). As the brain ages, it is likely that cognitive functions are affected. Most studies focusing on the neural correlates of cognitive changes rely on cross-sectional designs, which are insufficient for generating hypothesis about brain-cognition relationships over time. Longitudinal studies have related ventricular expansion to declines in episodic memory, and higher fluid intelligence and episodic memory scores to slower shrinkage of the medial temporal lobes (Borghesani et al., 2012; McArdle et al., 2004; Raz et al., 2008). Further, various structural and functional MRI studies shed light on the important role of the cerebellum in various cognitive functions (Stoodley, 2012), and variations in prefrontal volumes are related to age differences in fluid reasoning and in strategic control of episodic memory (Kane & Engle, 2002). Further, higher intelligence scores in childhood predict greater brain volumes later in life (Royle et al., 2013). Hence the relationship may be bi-directional rather than unidirectional, and studies investigating reciprocal longitudinal relationships between brain and cognition are warranted.
4.3.2 Objective

The main objective of this study was to examine the relationship between regional heterogeneity of brain shrinkage and change in performance in age-sensitive cognitive domains. Brain regions of interest (ROIs) were chosen based on their theoretical and empirical relevance to the studied cognitive domains. ROIs consisted of the prefrontal cortices, the medial temporal lobes, and the cerebellar hemispheres. The visual cortex was used as control region, as this ROI generally shows slower age-related changes. Two age-sensitive cognitive constructs, episodic memory (EM) and fluid ability (Gf), and one age-resilient construct, vocabulary (crystallized ability), were chosen. Parameters representing both mean change and variance in change were specified in latent change score models (LCSMs). We further evaluated bidirectional lags between baseline levels and subsequent changes in brain volumes and cognitive performance scores. Finally, we tested the role of putative modifiers of change in the brain and cognition by adding the following variables: cardiovascular risk, a common genetic variant associated with dementia risk (APOE ε4), and socioeconomic status, in addition to chronological age as predictors of individual differences in neuroanatomical and cognitive changes.

4.3.3 Method

Data were analyzed from 167 healthy adults over approximately two years (average interval between assessments: two years and 24 days). Participants suffering from dementia, psychiatric illnesses, cardiovascular diseases or exhibiting brain abnormalities were excluded. Individuals with medically controlled hypertension were included. The following regions of interest (ROIs) were manually segmented following a previously described protocol (Persson et al., 2014): lateral prefrontal cortex (LPFC), prefrontal white matter (PFw), hippocampus (Hc), parahippocampal gyrus (PhG), the cerebellar hemispheres (CbH), and primary visual cortex (VC; calcarine fissure). Cognitive tasks were used from the Culture Fair Intelligence Test (CFIT; Cattell & Cattell, 1960), the Memory for Names subtest of the Woodcock-Johnson Psychoeducational Battery – Revised (Woodcock & Mather, 1989), and the Logical Memory (LM) subtest from the Wechsler Memory Scale – Revised (Wechsler, 1987). Word knowledge was measured by vocabulary tests generated from the ETS Kit of factor-
referenced cognitive tests (Ekstrom, French, Harman, & Dermen, 1976). The memory and CFIT tasks were identical over the test occasions, while list one of the vocabulary (V) lists was identical and list 2 was replaced by list 3 (the correlation of stability was perfect, $r = 1.000$). LCSMs were fitted to the cognitive tasks and the ROI data. Separate confirmatory factor analyses (CFAs) were applied to the data for latent representation of vascular risk (BMI, fasting glucose, pulse pressure, and LDL cholesterol) and socioeconomic status (maternal and paternal education). Age in years was treated as a time-invariant interval-scaled variable, and $APOE \varepsilon4$ was coded as 1.

4.3.4 Results

The univariate LCSMs revealed individual differences in change across all ROIs. Individuals with larger brain volumes did not exhibit slower rates of shrinkage over two years. Moreover, higher $Gf$ baseline scores were associated with smaller gains. The participants exhibited improvement in episodic memory (EM) and vocabulary (V) performance, but not fluid ability ($Gf$). Of the cognitive scores, variance in change only occurred for $Gf$, while EM and V scores showed only changes in average trends. Hence baseline EM and V predicted change in ROIs but not vice versa, because there was no variance in EM and V. Bidirectional lags could only be specified for the ROIs and $Gf$ because this cognitive domain showed significant variance in change.

After accounting for the covariates, larger baseline volumes of four regions (PFw, Hc, PhG, and CbH) predicted positive change in $Gf$. Only one region (PFw) had less shrinkage and was associated with greater gains in $Gf$ ($r = .346$). Higher EM scores at inception predicted slower shrinkage of LPFC ($p = .013, \alpha' = .017$), with $Gf$ showing a trend ($p = .051$) and V a non-significant relationship ($p = .13$) in the same direction.

Sex was not associated with any of the measures (all $p$’s > .05), and was excluded from further analyses on the basis of model parsimony. The bivariate models revealed that older baseline age was associated with smaller volumes of all ROIs, independently of socioeconomic status (SES), vascular risk (VR), and $APOE \varepsilon4$ status. Older age was further related to lower memory, lower $Gf$ scores, and better V performance. Higher SES status indicated greater initial V scores and fluid intelligence, but no statistically reliable relationship was established with EM. Of all ROIs measured at baseline, only Hc volume
was associated with SES, and the effect rendered significant after statistical correction selectively in one of the three models were the ROI was included. Neither vascular risk nor APOE ε4 variant displayed significant associations with any of the baseline volumes or cognitive factors. The analyses of bivariate models for Gf and regional volumes revealed that in the model that included CbH, younger age and higher SES were associated with greater gains in Gf. No independent significant effects of age, SES, VR, or APOE ε4 variant on the rate of shrinkage of any ROI were observed, although trends for VR and APOE ε4 were noted. However, in at least one model (with CbH and Gf as target variables), the combined influence of baseline age, SES, VR, and APOE ε4 was significant ($R^2 = .551$), with the direction of change suggesting faster shrinkage for older participants, carriers of the APOE ε4 allele, and individuals with lower SES.

4.3.5 Conclusion

The results suggest that although larger brain volumes were not associated with slower rates of shrinkage, they may be important for maintaining high performance in important age-sensitive cognitive domains. Notably, brain and cognition exert a bi-directional influence on each other: better cognitive performance predicts better maintenance of structural integrity in an important age-sensitive region, and larger brain volumes at inception predict greater gains in fluid intelligence scores. Our findings contribute to better understanding of the neural underpinnings of cognitive aging and highlight the importance of maintaining cognitive functioning in older age. The specific mechanisms of the observed effects of cognitive and brain reserve remain to be elucidated.
5 Overall discussion

Brain aging is a heterogeneous phenomenon, and this thesis illustrates how the course of aging can vary within individuals over time and between individuals as a function of age, sex, and genetic variability. We used two contrasts from magnetic resonance imaging (MRI), namely spin-lattice T1-weighted imaging, and quantitative susceptibility mapping (QSM) from gradient-echo images, to picture the aging brain, by means of morphometric measures and brain-iron concentrations. Within each study, the same rigorous imaging acquisition protocols were used with large samples sizes of 167-183 individuals, which contribute to the uniqueness of the studies. Most of the current knowledge about the aging brain rests on a foundation of cross-sectional age-related differences, and studies I and III contribute to current knowledge by using longitudinal designs to investigate individual rates of change. The importance of genetic variation in relation to regional brain changes was addressed with specific emphasis on functional polymorphisms involved in pro-inflammatory responses. These studies further illuminate the importance of bi-directional relationships between structural integrity and preserved cognitive abilities over time. Study II is the largest study to date to obtain quantitative susceptibility estimates from healthy adults, and the first in vivo report to show a lowering in overall subcortical brain-iron estimates in women from midlife to old age. Studies I and III are unique in their examination of longitudinal differences in anatomical brain regions using high resolution images from a 4-Tesla scanner. Peripheral vascular risk factors were not strong determinants of either brain or cognitive changes in the studied samples. The results are discussed here in the context of cognitive reserve, the brain maintenance hypothesis, and potential influences of hormones, inflammation, and oxidative stress.

5.1 Mean change and individual differences

The latent change score models (LCSMs) of study I and III allow us to make inferences about change, and parameters reflecting sample average trends as well as heterogeneity in change rates were specified, based on empirical findings of age-related heterogeneity (Hertzog, Lindenberger, Ghisletta, & Oertzen, 2006; Rabbitt, 1993; Raz et al., 2010; Raz et al., 2008). Both mean differences and variance in change were present across many of the neuroanatomical regions. The observed shrinkage on the images from the 4-Tesla scanner concurred reasonably well with previous reports using weaker magnets (1.5 T).
(Raz et al., 2005a, 2010). Importantly, the average shrinkages varied in magnitude over the neuroanatomical regions of interest (ROIs). The pronounced shrinkage of the cerebellar hemispheres, shown in study I and III, replicates previous findings (Raz et al., 2005a, 2010). Declines in the volumes of the medial temporal lobes described herein (hippocampus and the parahippocampal gyrus) have been previously reported in longitudinal studies involving repeated scans after six months to five years (Pfefferbaum, 1998; Raz et al., 2005a, 2013; Resnick et al., 2003). In addition, the observed stability in average trends of the visual cortices (calcarine fissure) replicates previous studies (Fjell et al., 2009; Raz et al., 2005a, 2010). Relative stability of the striatum (putamen and caudate nucleus) both confirms (Raz et al., 2010) and refutes (Fjell et al., 2009) previous longitudinal findings.

In contrast, the insula showed a much greater magnitude of average shrinkage than previously reported (Raz et al., 2010). The lack of mean shrinkage in the lateral prefrontal cortices and the subsequent prefrontal white matter observed in studies I and III contrasts with previous longitudinal work using weaker magnets (Driscoll et al., 2009; Fjell et al., 2009; Raz et al., 2013; Resnick et al., 2003), with one exception (Raz et al., 2010). These findings challenge the “last in, first out” view of aging, namely that the regions developing later in life are the first to show age-related deteriorations, and further contradict the prefrontal-aging hypothesis that has dominated the field over the last two decades (West, 1996). This result needs further replication using high-field MRI, and shrinkage may be more prominent over longer periods of time.

In addition to shrinkage on average, the analyses also revealed a prominent pattern of significant individual differences in the rates of change of the ROIs, with all ROIs but the orbitofrontal cortex exhibiting substantial variance. The heterogeneity in developmental trends replicates previous work on similar study populations, with individual differences in change being most pronounced in the insula and cerebellum, followed by parahippocampal gyrus, hippocampus, striatum, lateral prefrontal cortex, prefrontal white matter, and the visual cortices (Raz et al., 2005a, 2010).

The mechanisms underpinning the observed regional brain shrinkages remain unclear because there is no well-established neurobiological basis of volume differences and changes observed on MRI. Volume variation in some regions has been linked to neuronal attrition in the brains of patients suffering from neurodegenerative disease (Bobinski et al., 2000). Experimental evidence suggests that MRI-derived vol-
ume differences may reflect changes in neuropil (unmyelinated axons, dendrites, and glial cells); in rodents volume changes closely track expansion and loss of neuropil during the estrous cycle (Qiu et al., 2013). Volume loss is further associated with loss of neuropil following chronic treatment with antipsychotic drugs (Vernon et al., 2014) or cardiac arrest (Suzuki et al., 2013). Loss of Purkinje cells in a mouse model of autoimmune encephalomyelitis has been related to smaller cerebellar volume (MacKenzie-Graham et al., 2009), as well as a decrease in numbers of layer V pyramidal neurons, and a decrease in length of the apical dendrites (of single trunk, which branches in the upper layers of the neocortex), whereas the remaining neurons have been related to atrophy in MRI studies (Spence et al., 2014).

5.2 The influence of age

Replicating previous work (Raz & Kennedy, 2009), chronological age was a strong determinant of cross-sectional age-related differences across all ROIs (studies I and III), with older individuals having smaller brain regions at baseline. The cross-sectional component of the analyses further revealed that older adults had lower fluid intelligence and episodic memory scores, but better vocabulary performance than younger participants, which also replicates earlier work (Horn & Cattell, 1967). The lack of correspondence between the reported cross-sectional age differences and longitudinal age-effects suggests that the latter cannot always be inferred from the former. Age-heterogeneous cross-sectional designs have high commonality between age and multiple measures of interest, thereby increasing the likelihood of confounds of between-individual age trends (Hertzog et al., 2006; Hofer & Sliwinski, 2001; Lindenberger & Pötter, 1998; Robitaille et al., 2013; Sliwinski et al., 2010), which can lead to spurious inferences concerning interdependencies among age-related functions (Hofer, Berg, & Era, 2003).

In study II, we showed that the distributions of brain iron varied over structures, consistent with earlier postmortem findings (Hallgren & Sourander, 1958). The shape of the age-function in the striatum over the ages 20-69 years was linear for the susceptibility distributions, which replicates previous reports using various iron-sensitive contrasts (Bartzokis et al., 1997a; Cherubini et al., 2009; Haacke et al., 2010; Westlye et al., 2010). All other structures showed non-linearity in the age-trends that varied in degree across the ROIs. The strongest curvilinear fit in susceptibility rise was observed in the red nucleus, followed by the sub-thalamic nuclei and substantia nigra. Less marked
nonlinear trends were observed in susceptibility counts for the thalamus, dentate nucleus, and putamen. Non-linear susceptibility trends in those structures have been reported previously, yet studies also exist that suggest more linear trends in thalamic iron distributions (Haacke et al., 2010; Hagemeier et al., 2013). These differences may arise from the use of high-pass filtered gradient-echo phase images in the aforementioned studies. Different settings for filter kernel size can change the sensitivity of the measure to detect iron, and the impact of this depends on the size and shape of the structure (Haacke, Xu, Cheng, & Reichenbach, 2004; Pfefferbaum et al., 2009). A recent paper further demonstrates that when using high-pass filtering, anatomical changes due to gray matter atrophy may introduce a phase shift seemingly indicative of increased iron concentration, even though the biophysical tissue composition has not changed (Schweser, Dwyer, Deistung, Reichenbach, & Zivadinov, 2013). These results further highlight the need for longitudinal evaluations due to the issues of commonality in age-heterogeneous cross-sectional designs discussed earlier. As reproducibility of iron-sensitive contrasts becomes more consistent (Deh et al., 2015), the investigation of age-related changes and variance therein becomes plausible.

High iron concentrations were positively correlated among several subcortical structures, which may reflect an underlying age-related process. A potential mechanism could be that excessive accumulation of intracellular non-heme iron promotes reactive oxygen species (ROS), oxidative stress, and cell death, resulting in neurodegeneration (Andersen et al., 2014a; Dixon & Stockwell, 2014; Floyd & Carney, 1993). Iron distributions in several of the structures assessed followed the same inverted U-shape pattern as myelination in brain aging (Westlye et al., 2010). Changes in ferritin may follow the same curvilinear shape as myelination through life. Iron is a cofactor in the synthesis of myelin (Piñero & Connor, 2000) and there is evidence that iron undergoes translocation between brain regions (Barkai, Durkin, Dwork, & Nelson, 1991; Dwork et al., 1990). Myelin breakdown in the surrounding regions may interact with the release of ferritin in the subcortical gray matter. Iron is stored in oligodendrocytes (Madsen & Gitlin, 2007) and many of the subcortical gray matter structures both contain and border deep white matter tracts (see e.g., Bartzokis et al., 1997a; Mitrofanis & Guillery, 1993). Myelin breakdown has been associated with ferritin in Alzheimer's disease (Quintana et al., 2006), and late-life myelination has also been encountered in phylogenetically older structures (Benes, 1994).
5.3 Determinants of age-related changes

The strongest predictors of longitudinal brain changes were pro-inflammatory genetic variants that mediated greater shrinkage in the parahippocampal gyrus (PhG) and the cerebellar cortices (CbH) over two years. This is indeed interesting given that the examined allelic variants (IL-1β C-511T: PhG, CbH; MTHFR C677T, PhG) promote a greater systemic pro-inflammatory response (IL-1β 511T: greater circulatory interleukin-1β; MTHFR T allele: higher plasma homocysteine levels). It is unclear why shrinkage in the parahippocampal gyrus and cerebellum is particularly pronounced in carriers of T alleles in two of the examined pro-inflammatory genetic variants. The literature on regional effects of pro-inflammatory cytokines on the brain is still scarce. Response to neuroinflammation has been linked to shrinkage of the medial temporal lobes in rats (Hauss-Wegrzyniak, Galons, & Wenk, 2000), and IL-1β may affect glutaminergic and GABAergic transmission in the cerebellum (Mandolesi et al., 2013).

Further, recent cross-sectional studies highlight the relationship between higher levels of plasma IL-1beta (Sudheimer et al., 2014), genetic variants regulating pro-inflammatory responses, and smaller volumes in the medial temporal lobe structures (Raz et al., 2015). The MTHFR-677 T allele has been associated with decreased density of the parahippocampal gyrus in schizophrenia (Zhang et al., 2013). Those, however, were cross-sectional studies, and their findings are not directly comparable with the longitudinal observations reported herein. Our findings are consistent with both the possibility that the major alleles of IL-1β and MTHFR polymorphisms have neuroprotective influence on the parahippocampal cortex, and that variant alleles promote shrinkage. However, all the mentioned studies are cross-sectional, and the observational nature and limited statistical power concerning the allelic variants of the current report preclude a more definitive conclusion about causality.

In study I and III, the covariates socio-economic status (SES), the APOE ε4 allelic variant and vascular risk had limited importance regarding changes in both brain and cognition. The effects of age and SES on regional brain volumes (e.g., hippocampus), and cognition (fluid intelligence) were sporadic, observed in few models, and we found no specific effects of vascular risk or APOE ε4. Further, only individuals with controlled hypertension were included in the studies, and those suffering from cardiovascular pathologies were excluded. Elevated cardiovascular plasma markers may be more important for
brain and cognition, if the former reach clinical levels. The APOE ε4 variant may have greater influence amongst individuals with Alzheimer’s disease (AD). However, in combination, risk-relevant covariates (advanced age, lower SES, APOE ε4 allele, and vascular risk) were associated with significantly greater shrinkage of at least one region, namely the lateral prefrontal cortex. Such cumulative influence of multiple interrelated risk factors, rather than independent effects of such factors, may be plausible in a sample selected for better than typical health. Unfortunately, the sample size precludes us from investigating interactive effects, given the relatively small number of individuals possessing variants of the risk allele.

5.4 Sex related differences in brain iron

Sex was a poor predictor of regional brain volumes and was therefore eliminated from analyses in studies I and III. Sporadically reported sex differences in regional brain volumes may have arisen due to differences in accounting for the intracranial volume, as suggested by recent studies (Pintzka et al., 2015; Voevodskaya et al., 2014). Sex differences may be less influential in brain morphology than in molecular or neurochemical functions. Sex-specific variations are present in neurodegenerative diseases (Bartzokis et al., 2011; Taylor et al., 2007), often accompanied by an accumulation of iron deposits (Bartzokis et al., 2007; Langkammer et al., 2013), which makes sex differences important in relation to iron accumulation. Study II showed that greater proportions of variance in susceptibility can be attributed to age in men than women. Further, women post expected menopause age had lower total sub-cortical susceptibility counts, which replicates recent post mortem findings of lower tissue brain iron levels in women than men from midlife to old age (Ramos et al., 2014). These findings require replication, but are of importance: as various MRI studies consider brain iron elevations in various neurodegenerative diseases, the potential effect of sex must be considered (Bartzokis et al., 1997a; Langkammer et al., 2013).

5.5 Bidirectional brain-cognition relationships

The results from study III demonstrate that larger brain volumes predict lesser decline in fluid abilities (Gf) and that better cognitive attainment early in life may mitigate short term shrinkage of an important age-sensitive brain region: the lateral prefrontal cortex. Showing such reciprocal relationships between brain and cognition in a lon-
A longitudinal study is important as such a design circumvents problems with interpreting changes in cognition alone.

These results can be interpreted in the context of reserve. Our findings support recent more complex and nuanced interpretations of brain-cognition reserve than initially proposed by Satz (1993), and are more consistent with Stern’s (2009) recent conceptualization of the phenomenon. The former brain reserve hypothesis posits that larger brains containing more neurons and synaptic connections among them (Katzman et al., 1988; Satz, 1993) are better prepared for compensating for acute and chronic insults. Stern (2009) incorporated both neuronal aspects and cognitive aspects into the concept of reserve. This reconceived cognitive reserve refers to the advantage conveyed by higher cognitive ability and education in coping with cognitive expressions of brain pathology, presumably through efficient use of cognitive processes to resist cognitive decline, regardless of their specific connection to identifiable neural changes (Stern, 2009; Tucker & Stern, 2011). Cognitive reserve can arise from multiple sources and multiple processes can contribute to expression of cognitive reserve, including compensation based on existing cognitive skills, acquisition of alternative coping strategies, life or propensity to adhere to lifestyles, as well as socioeconomic status and practices that benefit maintenance of healthy neural circuits (Hertzog & Kramer, 2008; Tucker & Stern, 2011).

Evidence for the cognitive reserve hypothesis has been generated primarily from cross-sectional observational and epidemiological studies, and as mentioned, study III provides some support for this hypothesis via our finding of the mitigating effect of better episodic memory (EM) performance at baseline. The effect generalized to all age-sensitive cognitive domains to some extent. Baseline Gf scores had a similar positive trend on change in the tertiary cortices as EM ($p = .051, \alpha' = .017$). One explanation for the lack of significance may be that the relationship between Gf and tertiary volumes was attenuated by the underlying correlation between socio-economic status (SES) and Gf, essentially reflecting different aspects of cognitive reserve. Similar attenuations emerge from the commonality between intelligence and SES (Deary & Batty, 2007).

Study III illustrated that the rate of cognitive change may depend on initial brain volume, which can aid in characterizing bias problems when cognitive decline is diagnosed using neuropsychological assessments. Some studies suggest that cognitive reserve may simply reflect initial levels of performance, with highly educated individuals
approaching the diagnostic threshold later than those with poorer educational attainment and lower baseline cognitive performance scores (Tuokko, Garrett, McDowell, Silverberg, & Kristjansson, 2003).

Moreover, in our sample, as in some of the extant studies (e.g., McArdle, 2009), those with higher baseline fluid abilities actually exhibited smaller gains after the second administration of the tests. Thus, the advantage of higher cognitive endowment in slowing cognitive decline is likely to be a real, albeit complex, phenomenon, based on reciprocal influences between the brain and behavior. This is consistent with the “brain maintenance” hypothesis, which stresses the importance of structural integrity for preservation of high cognitive function in aging (Nyberg et al., 2012).

5.6 Methodological considerations

One of the major strengths of the current reports is the large sample sizes in the context of imaging studies. Low statistical power and subsequent risk of failure in detecting effects is often a major issue for most MR studies that use smaller sample sizes. Further, all participants in each study were scanned according to the same image acquisition protocol. Larger studies often comprise several pooled samples combining different acquisition protocols, which may potentially induce between scan variability to the measures. Study II is one of the largest samples of healthy adults to have in vivo MRI estimates of iron, and the largest study applying QSM maps on adults of a wide age range.

QSM is a relatively new technique, and researchers may prefer more traditional measures. However, QSM has advantages over more traditional methods, as will be discussed. Recent work further supports the validity and reproducibility of quantitative susceptibility maps. The association between magnetic susceptibility and its estimation using QSM has been validated by phantom experiments (de Rochefort et al., 2010; Liu, Xu, Spincemaille, Avestimehr, & Wang, 2012). Recent evidence from postmortem evaluation of QSM (via mass spectrometry) supports the validity of the measure by showing that iron is the dominant source of magnetic susceptibility (Langkammer et al., 2012). Further QSM maps show good reproducibility between scans, and among scanner manufacturers and magnet strengths (Deh et al., 2015). Compared with gradient-echo phase images, QSM provides a more accurate spatial depiction and a clearer image reconstruction,
and suppresses the blooming artifacts observed in phase (J. Li et al., 2012).

Additionally, latent variable modeling holds many advantages over, for example, regression and other general linear model approaches. By fitting the observed scores to a theoretical model comprised of latent variables, using likelihood estimation, we gain better insight into many of the artifacts observed in traditional linear regression models, such as measurement error, residual error, and regression to the mean, by accounting for the covariance between initial levels and changes (McArdle, Nesselroade, 2003).

In study I and II, a rigorous segmentation protocol was applied. Manual segmentation is often considered a gold standard in the clinical context, and it is used for quantitative evaluation of automatic segmentation (see e.g., Destrieux, Fischl, Dale, & Halgren, 2010 for FreeSurfer). Semi-automated methods may sacrifice anatomically valid manual measurements (Kennedy et al., 2009) and for some brain regions, introduce age-related bias (Wenger et al., 2014). However, with manual segmentation techniques there is also the risk of introducing subjective error. We addressed this by using tracing protocols in which the operators were blind to participant age and sex. Further, intra class correlations between raters were assessed in studies I and III. To counteract potential inter-subject variability in study II, each region was traced by a single tracer with training in neuroanatomy. The neuroanatomical regions were segmented on the 3 mm slice that was most prominent, and we cannot know if the results would be the same if the structure was examined in its entirety. The rank order of iron distributions followed previously reported iron concentrations from post-mortem work, suggesting that this limitation is unlikely to be a major issue (Hallgren & Sourander, 1958).

The results of these studies should be interpreted in the context of several limitations. For all studies, we relied on a convenience sample. Hence, the results suffer from limited generalizability due to the non-random recruitment procedures. For instance, prevalence of hypertension in American adults (age 20 and above) is 33% compared to 20% in this sample, and prevalence of diabetes is 8% compared to 0%, respectively (Go et al., 2014).

However, we make no claims regarding generalization of the results to the general population. The findings may further be contaminated by selection bias. Rigorous exclusion was applied in the sampling procedure for studies I and III, and only people free of cardiovascular, psy-
chiatric, or neurodegenerative diseases, or substance abuse were recruited for study II. The purpose, however, was to study non-pathological aging, which is a necessity in this field.

Despite exclusion criteria, sub-clinical influence of disease may be an issue. Even younger participants may harbor early precursors of neurological and vascular diseases but do not express them at a detectable level. The prodromal stage of Alzheimer’s disease is known to be extensive, and we cannot completely exclude the possibility that some of the participants were in early stages of the disease, despite our screening procedures. We know from a study of nuns (i.e., religious order studies) that individuals with preserved mental functioning may also have considerably progressed neuropathology post-mortem, despite high cognitive achievement through senescence (Snowdon, 2003).

The higher prevalence of cardiovascular pathology in the older populations of studies I and III may have led to even more selective recruiting of optimally aged older adults. However, we also included individuals with medically controlled hypertension to counteract such bias. Selection for good health could have different effects on the younger and older segments of the age continuum, and reduce possible age-related differences in the variables of interest. Thus, a sampling strategy aimed at minimizing confounding effects of low education and age-related diseases could have introduced more selection bias and hence further reduced generalizability of the findings.

A few limitations should be mentioned regarding imaging procedures. One single gradient echo was acquired in study II, and $R_2^*$ maps could not be calculated. A comparison of QSM with $R_2^*$ would have been useful for assessing age-related dependency in ROIs with high iron concentration. QSM is still a primarily research methodology, although recent work has shown good reproducibility (Deh et al., 2015) and validity (Langkammer et al., 2012), and its clinical use is growing in popularity (Haacke et al., 2015; Wang & Liu, 2015). Previous work has shown that $R_2^*$ and susceptibility yield similar results regarding age dependency in estimates of iron deposits in the subcortical gray matter (W Li et al., 2014), and the unavailability of $R_2^*$ maps is unlikely to be a major limitation.

A potential weakness of study III is the lack of assessment of practice or retest effects. The single-sample study design with only two measurement occasions precluded estimation of retest effects. This could confound the results by masking declines and exaggerating gains. Because of the influence of one prior test experience (Salthouse, 2013),
and re-test effects (Salthouse, 2014) on subsequent cognitive performance, changes in scores on all cognitive measures observed in study III might be underestimated. Future studies with three or more repeated measures, or a control group comparison, should address the issue of retest-effects in the context of neural correlates of cognitive aging.

In study II, we approximated the onset of menopause by using the reported average age of menopause onset in Chinese women (Cheung et al., 2011). Although studies have shown relatively small standard deviations in the age of menopause onset in Asians (Cheung et al., 2011; Gold et al., 2001), we do not know the exact variance of menopause age among the particular cohort of Chinese women included in the study. Information about hormone replacement therapy was not available. This should be of minor concern because large studies report that few Chinese women use hormone replacement therapy (0.8% or less) (Lundberg, Tolonen, Stegmayr, Kuulasmaa, & Asplund, 2004; Yang et al., 2008).

5.7 Future directions

The cross-sectional work in study II should be further evaluated in the context of age-related changes to evaluate the true iron accumulation in aging, in addition to the age-related differences reported herein. The longitudinal relationship between susceptibility and cognitive change is important, given recent cross-sectional findings of associations between cognitive functions and iron accumulation (Ghadery et al., 2015; Pinter et al., 2015). Such studies are still scarce (but see Penke et al., 2012). The brief time window of two occasions precludes us from drawing conclusions about change over longer periods of time. Long-term assessments over several test occasions are uncommon (but see Raz et al., 2010 for a 3 occasion design), and several repeated imaging occasions would allow for the testing of different trajectories over time. Phylogenetically motivated models, as the “last in, first out” hypothesis, could be tested appropriately, rather than in current approaches, i.e., those primarily assessing age-related differences between individuals. The bi-directional effects reported in study III needs replication, and it would be interesting to see if high cognitive performers maintain structural integrity over longer periods of time than the brief time window of two years studied herein. The influence of, and neuronal underpinnings of, test-retest effects need further investigation using several repeated measurement intervals.

The relationship between structure shrinkage and regional brain iron levels needs further investigation and quantitative susceptibility mapping holds much promise as a more reliable measure for determining adequate biophysical tissue composition of iron in the presence of atrophy in the gray matter (Schweser et al., 2013). Little is known about the specific mechanisms of iron accumulation in the brain. The age-related iron distributions of many of the structures followed the same curvilinear shape as white matter development, and as oligodendrocytes store iron, iron release following myelin breakdown may be an important co-factor in the study of myelination (Piñero & Connor, 2000). Future work incorporating iron-sensitive contrasts with white-matter imaging could aid in quantifying such relationships, and high-resolution diffusion spectrum imaging may allow more detailed investigation of brain white matter.

5.8 Concluding remarks

The thesis assessed age-related changes in the healthy human brain by means of structural MRI correlates from spin-lattice images and quantitative susceptibility mapping, as well as changes in age sensitive and age-resilient cognitive domains. The contributions of demographic-, genetic-, and health-related factors were further evaluated. The results may aid in establishing a baseline distinguishing pathological neurodegeneration from healthy aging, and emphasize the importance of addressing both risk promoting and mitigating factors in healthy aging. The following points are highlighted as concluding remarks:

- Average shrinkage and individual differences in change were observed in regional brain volumes over a brief time window of two years. The findings from studies I and III are unique, and the existent longitudinal literature looking at individual differences in change are vast, but the findings would benefit from further replication over longer periods of time, to examine different trajectories (e.g., various non-linear relationships).

- No mean shrinkage was noted in the lateral prefrontal cortex, in a sample of adults in good health, which challenges the “last in, first out” hypothesis of brain aging. We may have to re-evaluate the current dominant role of the frontal aging hypothesis in healthy aging, and intact prefrontal functions may be a key factor in successful aging.
- Pro-inflammatory single nucleotide polymorphisms predicted greater shrinkage in the cerebellum and the parahippocampal gyrus (PhG). These findings from study I are novel, and need further replication. It is uncertain why the hippocampus was spared, while other medial temporal lobe structures were not. Future work focusing on hippocampal subfields segmented via ultra-high resolution in vivo MRI may be of importance since effects might be specific to certain bordering subfields communicating with the PhG rather than the structure in its entirety.

- The distributions of susceptibility varied across the subcortical nuclei, consistent with previous post-mortem findings which provide validation of our results in the absence of PM specimens. A gradual linear rise with age was observed in the striatum and varying degrees of curvilinearity were observed among the other nuclei. The results from study II were consistent with previous work using QSM and R* maps; when deviations emerged these were predominately related to the high-pass filtering phase, which is known to show greater variability at different kernel sizes.

- A novel finding of study II was that a positive manifold of correlations of susceptibility were observed across several of the subcortical nuclei supporting the dopaminergic system. Iron serves as a co-factor in dopamine signaling and it would be interesting to investigate whether the synchronicity among regions maintains over time, and how iron relates to age-related dopamine alterations, by applying a multimodal imaging protocol.

- Sex could influence degree of susceptibility, and the most prominent difference emerged in total susceptibility across the regions of interest in women post menopause relative to men and younger women. This is an interesting new finding, which replicated previous PM work showing that women have lower brain iron levels from midlife to old age relative to their male counterparts. Changing estrogen levels may mitigate brain-iron decrease in women post menopause. Experimental work shows that estrogen can influence cellular iron and mitigate brain damage, but the associative nature of the current report precludes definite conclusions about causality. The results would benefit from further replication and the neurobiological mech-
anisms underlying lower brain iron levels in humans post menopause need to be addressed.

- Gains were noted in memory, vocabulary, and individual differences in change in $G_f$ scores in study III, showing that repeated cognitive tests indeed improve measured cognitive performance over brief measurement intervals. Because of the influence of prior test experience and test-retest effects on subsequent cognitive performance, changes in scores on all cognitive measures observed in this study are positively biased and should be viewed as underestimation of the true change over the two-year period. The two-period design used herein precludes separating re-test from time effects. Future studies with three or more repeated measures should address the issue of retest practice effects in the context cognitive aging and its neural correlates.

- Study III showed that better baseline cognitive performance mitigated individual prefrontal gray matter shrinkage, highlighting the need to maintain good cognitive performance to better maintain structural integrity in an important age-sensitive region. The mechanisms by which higher cognitive performance at baseline may mitigate individual prefrontal shrinkage or even promote volume growth and thus serve as a neuroprotective factor are unclear.

- Larger brain volumes at baseline predicted greater gains in $G_f$. Unfortunately; we did not find similar results for episodic memory and vocabulary scores. Could we have found additional brain-cognition and cognition-brain associations? This question cannot be answered empirically, and study III benefitted from a relatively large sample size. We estimated latent variance in change to better account for measurement error and regression to the mean effects; the findings are thus likely robust. Designs incorporating several measurement occasions (3+) may be helpful in elucidating individual differences in episodic memory and vocabulary scores, as individuals were closer to the sample mean effects in the current work.

- The findings from study III contribute to better understanding of the neural substrates of cognitive aging and the importance of maintaining cognitive functioning in older age. The specific mechanisms of the observed effects of cognitive and brain reserve remain to be elucidated.
6 Summary in Swedish

Hjärnans åldrande är en heterogen företeelse, och denna avhandling illustrerar hur åldrandets förlopp varierar inom individer över tid, men också mellan individer beroende på ålder, kön, och genetisk variation. Det mesta av det som är känt om hjärnans åldrande idag baseras på studier som gjorts vid ett enda mättillfälle. Longitudinella studier med flera mättillfällen är dock nödvändiga för att ta reda på hur åldrandet tar sig uttryck över tid. Två magnetic resonance imaging (MRI) kontraster, T1 weighted imaging och quantitative susceptibility mapping (QSM), användes för att avbilda den åldrande hjärnan och kvantifiera volymer och koncentrationer av järn i vävnaden. Magnet styrkan i MRI scannern varierade från 1.5 Tesla till 4 Tesla. De inkluderade stick proven på 167-183 individer är stora urval i hjärnabildnings sammanhang. Den här avhandlingen bidrar med longitudinala undersökningar i studie I och III avseende dels hjärnans subsets, men också kognitiva förmågor. I studie II undersökte med hjälp av tvärsnitts data hur koncentrationen av järn i hjärnans subcortikala strukturer förändras som en funktion av ålder och kön. Studie I visar att flertalet strukturer i mediala temporal loben, orbitofrontal cortex, samt cerebellum (lill-hjärnan) krymper över tid, medan striatum och den visuella cortex är mer stabila. Prefrontala cortex däremot krymper inte signifikant över tid, vare sig avseende vit eller grå substans. Detta fynd motsäger dominerande idé strömningar, om att just denna region är särskilt känslig för åldersmässiga förändringar. Det är viktigt att poängtera att även individuella skillnader förekom så tillvida att det fanns signifikanta avvikelser från medelvärdes trender i alla de mätta strukturer bortsett orbitofrontal cortex. Vidare visade innehavare av genetisk risk variant som ökar pro-inflammatorisk respons mer omfattnande förändringar i delar av mediala temporal loberna samt lillhjärnan, jämfört med personer som bar en skyddande genetisk variant. I studie II där effekter av ålder och kön på MRI-estimat av järn mättes framkom att halterna av järn i striatum steg linjärt per levnadsår från 20 till 69 års ålder. I övriga strukturer var denna utveckling icke linjär, med platå-liknande förhållande från cirka 30 års ålder, för att sedan avta från det sextionde levnadsåret. Dessa icke linjära samband var starkast i red nucleus i hjärnstammen, följt av det subthalamiska komplexet och substantia nigra, även denna senare region lokaliserad i hjärnstammen. Marginella icke-linjära effekter återfanns i sambandet mellan järn nivåer och ålder avseende thalamus, dentate nucleus i cerebellum (lill-hjärnan), samt globus pallidus. Kvinnor hade överlag lägre nivåer av järn i hjärnan än män, och kvinnor från 51 års ålder och äldre hade lägre total subcortikal järn-nivå jämfört med både män
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