Neurocognitive Plasticity:
A Longitudinal Study of Memory Training and White Matter Microstructure

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2. General summary

The assumption that the brain’s structure can be influenced by our own conscious effort is not only intriguing; it may have significant implications related to cognitive and brain health throughout the lifespan. The brain is highly vulnerable to the impact of age, and thus knowledge about the potential to influence age-related decline is of importance to help increase the number of well-functioning years in the ageing population. During the last decade, the field of neuroimaging has been marked by considerable interest in the effects of cognitive training on both cognition and the brain. Several studies involving cognitive training paradigms have shown positive training effects in both young and older adults (Burki, Ludwig, Chicherio, & de Ribaupierre, 2014; Engvig et al., 2012; Jones et al., 2006; Lovden, Bodammer, et al., 2010; Nyberg et al., 2003; Scholz, Klein, Behrens, & Johansen-Berg, 2009). However, a number of questions remain unanswered: Why do some individuals benefit more from cognitive training relative to others? How does plastic potential change with age? Are structural brain alterations a neurobiological substrate for behavioural improvements? Is continuous cognitive training a premise for the persistence of training-related brain changes?

The objective of this thesis was to address these questions through a longitudinal memory-training study including young and older participants. Paper I focuses on the brain’s white matter microstructure as a potential explanatory factor for the large individual variation in training gains observed in older adults. Paper II centres on age differences in plasticity, in addition to examining relationships between cognitive and microstructural plasticity. Paper III provides novel evidence of the temporal dynamics of WM microstructural plasticity in ageing. In summary, the results from the three papers indicate that 1) individual differences in microstructural brain characteristics influence the ability to benefit from memory training in older age, 2) plasticity is preserved into older age, and microstructural alterations may be part of a neurobiological substrate for cognitive improvements in ageing, 3) cognitive training has the potential to moderate the magnitude of age-related decline in white matter microstructure, but 4) continuous training appears to be a premise for the persistence of such effects.
3. List of papers


III. de Lange, A.M.G., Bråthen, A.C.S., Rohani, D.A., Fjell, A.M & Walhovd, K.B. Age-related Decline in Brain Structure Moderated by Cognitive Training: The Temporal Dynamics of Brain Plasticity in Aging (*to be submitted*)
4. Introduction

4.1 The adaptable brain

For centuries, the consensus of mainstream science and medicine was that the adult brain anatomy was fixed or ‘hardwired’, such that the brain only underwent developmental alterations during childhood and deteriorations in older age, remaining unaltered throughout adulthood (Pascual-Leone, Amedi, Fregni, & Merabet, 2005). However, some scientists already began to challenge this view in the late nineteenth century. In Principles of Psychology (1890), William James introduced the term brain plasticity from a surprisingly modern perspective. He linked the property of plasticity to behaviour, wrote about the habilitation of brain pathways through repeated use, and suggested that brain components that were active at the same time could form new association paths (Berlucchi & Buchtel, 2009). In 1949, Donald O. Hebb proposed his theory of neuronal adaptation during learning, in which he described how co-activation of pre- and postsynaptic neurons would lead to a potentiated connection between these neurons, increasing synaptic efficacy (Hebb, 1949). However, it was not before the late 1960s that a line of empirical evidence gradually convinced scientists to accept the idea that the adult brain is modifiable in response to the environment.

Early evidence of synaptic plasticity was demonstrated by Terje Lømo in 1966, when he discovered that repeated stimulation of hippocampal neurons resulted in long-term enhancement of signal transmission (Lomo, 1966). Around the same time, evidence of cortical plasticity was revealed by David Hubel and Torsten Wiesel, who discovered that if new-born kittens were deprived of visual stimulation in one eye during a critical, early life period, they failed to develop vision in that eye. Thus, the kittens’ brain development clearly depended on experience. Moreover, the part of the visual cortex associated with the blind eye had begun to process visual input from the functioning eye, suggesting that the brain had ‘rewired’ itself in response to the change in input (Hubel & Wiesel, 1963; Wiesel & Hubel, 1965). The discovery of critical periods was groundbreaking. However, most scientists, including the authors, still opposed the idea that plasticity could occur in the adult brain. Later, Michael
Merzenich and his colleagues provided evidence demonstrating that the somatosensory cortex was plastic in adult monkeys (M. M. Merzenich et al., 1983). Together with Bill Jenkins and others, Merzenich later demonstrated adult plasticity in relation to learning and memory (M. Merzenich et al., 1996; M. M. Merzenich & Sameshima, 1993) and recovery after brain injury (Jenkins & Merzenich, 1987).

With the emergence of magnetic resonance imaging (MRI) studies in the late 1900s, new discoveries of structural brain plasticity triggered the beginning of a decade of extensive investigation into the plastic potential of the human brain. Eleanor Maguire and colleagues (Maguire et al., 2000) showed that the hippocampi of London taxi drivers were larger relative to controls, indicating that memorising the streets of London had led to structural changes in a brain region known to be particularly relevant for spatial memory and navigation (O'keefe & Nadel, 1978). In 2004, Bogdan Draganski and colleagues showed grey matter increases in young adults after three months of juggling (Draganski et al., 2004), which was later shown to also affect the brain’s white matter (Scholz et al., 2009).

Although subject to long-lasting controversy, the term plasticity has been used in the brain and behavioural sciences for a long time in reference to the neural changes which may account for behaviour modifiability such as learning, adaption to shifting environments, maturation and compensation in response to brain damage or ageing (Berlucchi & Buchtel, 2009). The meaning of the term has, however, undergone substantial proliferation and evolution during the last century (Lovden, Backman, Lindenberger, Schaefer, & Schmiedek, 2010).

4.1.1 How can plasticity be defined?

One general definition for plasticity is ‘the capacity for being moulded or altered’ (Merriam-Webster, 2016). Clearly, many types of alterations take place in both our mental capacity range and in the brain, over very different time scales, and in response to variable environmental conditions. Thus, plasticity is a concept that pervades contemporary neuroscience and psychology with various connotations (Lovden, Backman, et al., 2010).
Lövdén and colleagues propose a theoretical framework for the study of plasticity, where alterations are regarded as plastic only when the observed changes in behaviour are accompanied by structural brain alterations (Lovden, Backman, et al., 2010). Plastic alterations in brain and behaviour are thought to take place when there is a mismatch between intrinsic capacity, i.e. supply, and the environmental demands. Hence, plasticity is initiated by changes in the range of processing capacity (e.g. after brain injury), or in the environmental demands (as with cognitive training). Plastic responses are however not reactions to changes in supply or demand alone, but induced only in the presence of a mismatch between the functional capacity and the environmental demands (Lovden, Backman, et al., 2010). The capacity for variations in behaviour that do not require structural brain changes is referred to as flexibility. Flexibility can generate improvements in performance, but does not require structural brain alterations as opposed to plasticity (Noack, Lovden, Schmiedek, & Lindenberger, 2009). This framework is highly useful for interpreting findings from cognitive training studies, as the concept of plasticity is clearly defined. In the recent literature however, a distinction is often drawn between brain plasticity and cognitive plasticity, where cognitive plasticity refers to the potential to improve performance in response to training (P. B. Baltes & Lindenberger, 1988; Burki et al., 2014; Fernandez-Ballesteros et al., 2012; Jones et al., 2006; Karbach & Schubert, 2013; Willis & Schaie, 2009).

In the present thesis, the term cognitive plasticity is used to refer to the extent of cognitive benefit in response to memory training, independent of co-occurring changes in brain structure. White matter microstructural plasticity refers to the observed changes in white matter measures in response to training. Some findings are however discussed in relation to the framework proposed by Lövdén and colleagues (Lovden, Backman, et al., 2010), with particular focus on the supply-demand concept.

### 4.1.2 How does the brain manage ageing?

Although ageing may not be genetically programmed (Kirkwood, 2005), most would agree that advancing age involves accumulation of changes in forms of wear and tear that can lead to deteriorations in brain and cognition. The senescent decline in a number of cognitive functions is well known (C. Grady, 2012), and a common view is
that at least some portion of the age-related changes in cognitive function can be explained by structural brain changes (Bennett & Madden, 2014; Fjell, Sneve, Grydeland, Storsve, & Walhovd, 2016; Fjell et al., 2015; Nyberg, Dahlin, Stigsdotter Neely, & Backman, 2009). A number of neuroimaging studies have shown structural brain changes with older age, such as reductions in brain volume, cortical thinning and decreases in the integrity of white matter microstructure (Bennett, Madden, Vaidya, Howard, & Howard, 2010; Buckner, 2004; Fjell & Walhovd, 2010; Fjell et al., 2009; C. Grady, 2012). However, the ageing population is characterised by substantial individual variation in cognitive function, and while some individuals suffer from cognitive impairment, Alzheimer’s disease and other types of dementia, others tend to maintain cognitive functions into old age (Nyberg, Lovden, Riklund, Lindenberger, & Backman, 2012). The large individual variation in older samples has led to various theories of ageing, focusing on why some older people show age-related decline in cognition while others show little or no evidence of decline.

Reserve theories refer to the hypothesised capacity of the brain to cope with pathology, or be resistant to the symptomatology of pathology. The idea is based on observations of the lack of direct relationships between the degree of brain pathology and the clinical symptoms related to that type of pathology (Driscoll et al., 2006; Snowdon & Nun, 2003; Stern, 2002). The brain reserve theory suggests that individual differences in the brain itself allow some individuals to cope better with brain pathology than others (Stern, 2009). In this view, the amount of intact brain in terms of for example synaptic density, neuronal counts and size, is a better predictor of individual variance in cognitive function compared to the amount of pathology. Pathology will affect behaviour only when the brain’s resources fall below a certain threshold (Nyberg et al., 2012). In contrast, the cognitive reserve theory does not suggest that the brains of well functioning individuals are substantially different from the brains of those who show lower cognitive performance, but that the well functioning individuals are able to process tasks in a more efficient way that allows them to cope better with brain pathology (Stern, 2002). One mechanism proposed to underlie efficient task processing is neural compensation (Stern, 2009), which refers to engagement of new, compensatory brain networks to process tasks in the presence of pathology (Bartres-Faz & Arenaza-Urquijo, 2011). Cognitive functions such as memory, attention and processing speed rely on the interaction between distributed
brain regions rather than isolated areas (Buckner, Andrews-Hanna, & Schacter, 2008; Jeong, Chung, & Kim, 2015), and a number of functional MRI (fMRI) studies have reported differential engagement of cortical networks in older versus adults during task performance (see Grady, 2012 for a review). Such age differences in brain activity are suggested to reflect compensatory mechanisms including additional recruitment of task relevant regions, which has been associated with enhanced task performance in older individuals (Bartres-Faz & Arenaza-Urquijo, 2011). However, it is unclear whether or how age-related differences in engagement of brain regions during task performance have implications for white matter microstructure, which is the subject of the present thesis.

Brain maintenance is another concept that can be viewed as complementary to the reserve theories (Nyberg et al., 2012). Whereas the reserve theories seek to explain why some individuals maintain cognitive functioning in the presence of brain pathology, the concept of brain maintenance focuses on the conditions that promote the preservation of brain integrity in old age. Thus, the maintenance of a ‘younger’ brain, or the relative lack of age-related changes including pathology, is a better predictor of cognitive function compared to how individuals cope with present pathology (Nyberg et al., 2012).

It has been suggested that education, intelligence and occupational attainment function as predictors of cognitive reserve, such that those with high levels of intelligence and education are more likely to sustain greater brain pathology before demonstrating cognitive symptoms (Bartres-Faz & Arenaza-Urquijo, 2011). However, these factors may also influence cognitive performance by preserving brain integrity (Hertzog, Kramer, Wilson, & Lindenberger, 2008; Nyberg et al., 2012). One study reported that participants who were physically active, had higher education, women and those who lived with someone were more likely to maintain memory function into older age (Josefsson, de Luna, Pudas, Nilsson, & Nyberg, 2012). A cognitively active lifestyle has been suggested to protect against dementia and Alzheimer’s disease (Fratiglioni, Paillard-Borg, & Winblad, 2004), and factors such as nutrition and physical activity have been associated with cortical sparing in older age (Walhovd, Storsve, Westlye, Drevon, & Fjell, 2014). In light of evidence pointing
to lifelong potential for experience-dependent brain plasticity (Lovden, Bodammer, et al., 2010; Wenger et al., 2012), engagement in stimulating activities may influence brain and cognitive trajectories throughout the lifespan. However, remarkable lifespan stability of cognitive function has been reported in a study by Deary and colleagues (2004), where intelligence (IQ) at the age of 11 correlated largely with IQ measured at the age of 80 (Deary, Whiteman, Starr, Whalley, & Fox, 2004). Hence, starting point seems to be a prominent predictor of cognitive function in older age, and genetics and early life factors such as birth weight and parental education have been suggested to influence brain and cognition continuously across the lifespan (Finkel, Reynolds, McArdle, & Pedersen, 2005; Lyons et al., 2009; Walhovd et al., 2016).

In cognitive training studies, the individual differences in training outcome tend to be considerable in older adults (Bherer et al., 2006; West, Murphy, Armilio, Craik, & Stuss, 2002), suggesting that the potential for cognitive improvement also varies substantially among older individuals (Bherer, 2015; West et al., 2002). Little is known about the brain characteristics that may account for the individual differences in cognitive improvements, and whether this variation can best be explained by differences in reserve mechanisms, differences in preservation of brain integrity or differences in starting point. Current evidence suggests that these perspectives are likely to be complimentary rather than mutually exclusive (Nyberg et al., 2012), emphasising the complexity researchers are faced with in their attempts to understand the ageing brain.

4.1.3 What are the age differences in plastic potential?

Although evidence suggests that the brain is able to change in response to the environment throughout the lifespan (Boyke, Driemeyer, Gaser, Buchel, & May, 2008; Draganski et al., 2004; Draganski et al., 2006; Driemeyer, Boyke, Gaser, Buchel, & May, 2008; Engvig et al., 2012; Johansen-Berg & Duzel, 2016; Lovden, Bodammer, et al., 2010; Lustig, Shah, Seidler, & Reuter-Lorenz, 2009; Nyberg et al., 2003; Scholz et al., 2009), the human reliance on accumulated experience across decades suggests that major neural replacements in the adult brain are not feasible (Rakic, 1985; Walhovd et al., 2015). Hence, advancing age involves accumulation of changes in forms of wear and tear, and one might expect older adults to exhibit lower
capacity for structural brain change relative to younger adults. This is consistent with animal models, where increasing age is associated with a lower magnitude of structural changes (Blumenfeld-Katzir, Pasternak, Dagan, & Assaf, 2011; van Praag, Shubert, Zhao, & Gage, 2005). Young adults also tend to show larger cognitive training gains relative to older adults (M. M. Baltes, Kuhl, & Sowarka, 1992; Burki et al., 2014; E. Dahlin, Nyberg, Backman, & Neely, 2008), suggesting that the potential for cognitive plasticity decreases with older age.

However, the few studies that have focused on human age differences in structural plasticity show mixed findings. While one cognitive training study (Lovden, Bodammer, et al., 2010) reported similar magnitudes of WM microstructural plasticity in young and older adults, another study found that spatial navigation training yielded cortical changes in young adults only (Wenger et al., 2012). Juggling exercise has been reported to affect grey matter in both young and older adults, although to a smaller extent in older adults (Boyke et al., 2008). Further complicating conclusions are the possible effects of factors related to participation, such as general cognitive activity (Gallucci et al., 2009). As modest cognitive improvement has been observed in active control groups (Legault et al., 2011), the inclusion of such groups is necessary to determine the specific effects of training (Hart, Fann, & Novack, 2008; Law, Barnett, Yau, & Gray, 2014). Hence, some training studies include active control groups (Barnes et al., 2013; Fabre, Chamari, Mucci, Masse-Biron, & Prefaut, 2002; Legault et al., 2011; Lövdén et al., 2010; Oswald, Gunzelmann, Rupprecht, & Hagen, 2006; Schwenk, Zieschang, Oster, & Hauer, 2010; Suzuki et al., 2012; Zelinski et al., 2011), however, the inclusion of both active and passive control groups appears to be lacking in studies comparing young and older adults.

4.1.4 Is experience-dependent plasticity transient or enduring?

The idea that cognitive training can improve brain structure and help mitigate age-related brain decline is certainly appealing. However, training effects may have limited durability, as some evidence suggests that the effects do not last beyond the intervention periods (Draganski et al., 2004; Driemeyer et al., 2008). Although the time course of experience-dependent plasticity is largely unknown, one study reported that the effects of juggling exercise on grey matter had decreased three months after the termination of the training (Draganski et al., 2004), indicating that neural
alterations do not persist in the absence of exercise. A later study demonstrated that changes in grey matter in response to juggling were detectable after only one week of training, but that subsequent training to improve the skill further did not result in structural changes (Driemeyer et al., 2008). Two months after the end of the training period, the structural effects had receded. However, the participants’ juggling skills were still fluent, indicating that the learning of a new skill may be a more critical aspect of brain plasticity relative to practicing already learned tasks (Driemeyer et al., 2008).

Although no studies have thus far examined the time course of brain plasticity across extended periods with multiple intervention phases, the Draganski et al. and Driemeyer et al. studies do indicate temporal limitations of structural training effects. However, they do not necessarily imply that training cannot mitigate age-related decline, as such an inference would require a direct comparison of the condition of the ageing brain during times of training and no training. Hence, it is unknown whether trajectories can be systematically influenced by cognitive training, and whether continuous training is a premise for the persistence of structural brain alterations.

4.2 Why memory training?

Structural brain plasticity in response to training of episodic memory, a cognitive function known as particularly challenging in older age (C. Grady, 2012; Nyberg et al., 2012), is largely unexplored. Episodic memory refers to the ability to remember personal experiences by mentally travelling back in time (Tulving, 2002). Thus, episodic memories are immensely important for our identities, as well as helpful for solving present problems and planning for the future by enabling the recall of perceptions and emotions accompanying past experience. This unique memory system is highly susceptible to age-related decline in brain structure (Buckner, 2004; Charlton, Barrick, Markus, & Morris, 2010; Fjell & Walhovd, 2010; Nilsson et al., 1997; Nyberg et al., 2012). Age-related deterioration in white matter microstructure has been associated with episodic memory decline (Bennett & Madden, 2014; Charlton et al., 2010), and differential activity patterns in young and older adults during episodic memory tasks have been found in fMRI studies (Cabeza et al., 2004; C. L. Grady, McIntosh, & Craik, 2003; C. L. Grady, St-Laurent, & Burianova, 2015;
The majority of older individuals report memory problems (Reid & Maclullich, 2006). However, as episodic memory is a limited cognitive resource in general, most people, regardless of age, are certainly familiar with memory lapses from time to time. Hence, techniques for improved memorising, such as mnemonic strategies, have been used since the ancient Greeks to facilitate recollection in the absence of external sources of reference (Yates, 1967).

One of the oldest mnemonic strategies is the Method of loci (MoL) (Bower, 1970). MoL involves constructing a mental travel route through a well-known place, such as one's childhood home or work place, and placing items or visual imagery along that route to encode them as memories. To recall the items, one imagines taking a walk through the travel route again, seeking out the items along the way (Legge, Madan, Ng, & Caplan, 2012). Thus, this method involves the engagement of episodic memory. In memory-training studies, the visual images are often associated with words on a list, and the use of a travel route enables the words to be recollected in a specific order. MoL is one of the most utilized techniques among world-class mnemonists, who are known to remember exceptionally large amounts of information learned in a short period of time (Foer, 2011).

Although the mechanisms underlying the effectiveness of MoL are not fully understood, as the subjective, internal processes (i.e. imagining oneself walking through a personal environment) make it challenging to study (Legge et al., 2012), the technique has been shown to improve serial recall substantially in both young and older adults (de Lange et al., 2016; Engvig et al., 2012; Kliegl, Smith, & Baltes, 1990; R. Li et al., 2014; Nyberg et al., 2003). One study has demonstrated differential brain activation for young and older adults during encoding (Nyberg et al., 2003). However, the effects of mnemonic strategy training on structural plasticity have not been systematically investigated across age groups. Moreover, the temporal aspects of performance improvement and structural changes after this type of training are thus far unexplored. Mnemonic strategies are likely to involve multiple brain regions, as they rely on visual imagery, episodic memory, navigation and strategising, thus, efficient integration and transfer of information between these regions may be critical for performance.
4.3 White matter microstructure - connecting the brain

The white matter of the brain controls the signals between neurons by modulating the distributions of action potentials. Thus, white matter influences cognitive functions such as learning and memory by coordinating the communication between widespread areas of the brain (Fields, 2008). White matter is mainly composed of axons connecting individual neurons, in addition to glial cells such as oligondendrocytes and astrocytes. White matter is named according to its light appearance, which originates from the myelin wrapped around the axons. The myelin acts as electrical insulation by increasing the transmission speed of nerve signals, allowing information to pass quickly from one region to another. The bundles of axons connecting the various brain regions constitute three types of white matter pathways or tracts: Association tracts connect regions within the same hemisphere of the brain. Commissural tracts are inter-hemispheric, and cross from one hemisphere to the other. Projection tracts connect the cortex to subcortical structures such as the basal ganglia, spine and cerebellum (Andronikou, 2012). Figure 1 shows a three-dimensional rendering of probabilistic white matter tracts.

Figure 1 Probabilistic tracts from the Mori atlas (Mori, Wakana, Van Zijl, & Nagae-Poetscher, 2005) including association tracts (e.g. Superior longitudinal fasciculus shown in yellow), commissural tracts (e.g. Corpus callosum shown in blue and dark green) and projection tracts (e.g. the Cortico-spinal tract shown in purple). The figure is adapted from (Krogsrud et al., 2016).

For decades, theories about learning and memory focused on molecular action inside the neurons and at the synapses. Scientists exhibited limited interest in the white matter of the brain, which was mainly perceived as passive pathways that played no significant part in cognitive functions (Fields, 2008). In contemporary neuroscience however, the importance of white matter is well established. Research has
documented that the integrity of white matter microstructure undertakes a central role in cognitive functioning (Johansen-Berg, 2010) both in early development (Barnea-Goraly et al., 2005; Dubois et al., 2014; Krogsrud et al., 2016) and in ageing (Cremers et al., 2014; de Groot et al., 2000; Kennedy & Raz, 2009a), and that decline in white matter microstructure is one of the hallmarks of normal brain ageing (Barrick, Charlton, Clark, & Markus, 2010; Bender, Volkle, & Raz, 2015; Bennett & Madden, 2014; Bennett et al., 2010; Burzynska et al., 2010; Cremers et al., 2014; D. H. Salat et al., 2005; Sexton et al., 2014). Decline in white matter microstructure has further been associated with Alzheimer’s disease (Bozzali et al., 2002; Genc et al., 2016; Huang, Friedland, & Auchus, 2007; Rose et al., 2000) and psychiatric conditions such as schizophrenia (Kyriakopoulos, Bargiotas, Barker, & Frangou, 2008; Kyriakopoulos & Frangou, 2009; Thomason & Thompson, 2011). In short; white matter matters.

Diffusion tensor imaging (DTI) is commonly applied in studies of human white matter microstructure in vivo. DTI is based on magnetic resonance imaging principles, where magnetic properties of hydrogen nuclei in brain tissue are measured in order to provide contrast between brain tissue such as white matter, grey matter and cerebrospinal fluid. MRI enables detailed images to be acquired in a short space of time in a non-invasive way, achieving image resolutions at the millimetre level. DTI provides indices of white matter integrity within neural networks by measuring diffusion of molecular water (C. Pierpaoli, Jezzard, Basser, Barnett, & Di Chiro, 1996). Within spaces filled with fluid in the brain, such as the ventricles, diffusion is nearly unbounded and thus isotropic. In grey matter, diffusion is relatively isotropic as well, due to the non-uniform molecular movement restricted by microstructures such as dendrites and cell bodies (Bennett & Madden, 2014). In contrast, the diffusion within white matter is anisotropic, i.e. more directional, because molecular movement is restricted by microstructures such as axonal cell membranes and myelin. Figure 2 illustrates isotropic and anisotropic tensor shapes.

DTI provides diffusion measurements in multiple directions and extracts the diffusivities parallel and perpendicular to the fibres using tensor decomposition (Assaf & Pasternak, 2008). Diffusion can be characterised by six parameters quantifying the size and direction of diffusion along three orthogonal axes. This information is best described by an ellipsoid, which represents the probability for a
water molecule to move in a particular direction within a voxel (Roberts, Anderson, & Husain, 2013). The integrity of white matter structures is inferred from the rate and directionality of the molecular diffusion, resulting in metrics such as fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD). These diffusion metrics relate to the value of the three eigenvalues of the tensor, \( \lambda_1, \lambda_2 \) and \( \lambda_3 \), as illustrated in Figure 2.

**Figure 2** Tensor shapes: The diagram on the left shows an anisotropic diffusion profile, where the diffusion is more directional along the primary axis. The figure on the right shows an isotropic diffusion profile, where the tensor has equal eigenvalues along each axis.

AD represents the rate of the diffusion along the primary axis of the diffusion ellipsoid, \( \lambda_1 \), which runs parallel to axons (C. Pierpaoli et al., 1996). RD represents the average of \( \lambda_2 \) and \( \lambda_3 \). MD provides an average of all three \( \lambda \) terms, and thus measures the mean molecular motion independent of tissue directionality. FA measures the relative difference between the largest eigenvalue as compared to the others. The mathematical relations between the metrics are shown below.

\[
\begin{align*}
\lambda_1 &= \text{Axial diffusivity (AD)} \\
(\lambda_2 + \lambda_3)/2 &= \text{Radial diffusivity (RD)} \\
(\lambda_1 + \lambda_2 + \lambda_3)/3 &= \text{Mean diffusivity (MD)} \\
\sqrt{\frac{(\lambda_1 - \lambda_2)^2 + (\lambda_1 - \lambda_3)^2 + (\lambda_2 - \lambda_3)^2}{2(\lambda_1^2 + \lambda_2^2 + \lambda_3^2)}} &= \text{Fractional anisotropy (FA)}
\end{align*}
\]

Evidence suggests that diffusion along the primary axis (AD) is associated with levels of axonal integrity (Budde et al., 2007), whereas RD has been associated with
myelination (Song et al., 2002). MD is suggested to relate to cellular properties such as size and integrity (Basser, 1995; C. Pierpaoli et al., 1996), while FA has been linked to restricted molecular motion caused by directionally oriented microstructures such as myelin sheaths and axonal cell membranes (Beaulieu, 2002; C. Pierpaoli et al., 1996). However, in areas of crossing fibres, FA measures may be reduced as axons from different white matter pathways are less closely collimated (Jeurissen, Leemans, Tournier, Jones, & Sijbers, 2010). Thus, the interpretation of the underlying changes in diffusion metrics depends upon the local fibre architecture.

Although the physiological basis for the anisotropic diffusion of water in white matter is not yet fully understood (Beaulieu, 2002), computer based methods known as tractography have been highly useful for mapping the white matter pathways of the brain. Hence, tractography using DTI-derived data have contributed significantly to the understanding of brain development throughout the lifespan. DTI studies suggest that white matter development follows an inverted U shape pattern with protracted growth extending into midlife, followed by subsequent accelerated decrease (Courchesne et al., 2000; Ikram et al., 2008; Jernigan et al., 2001; Resnick, Pham, Kraut, Zonderman, & Davatzikos, 2003; David H Salat et al., 2009; Westlye et al., 2010). As cognitive functions such as memory rely on the interaction between distributed brain regions rather than isolated areas (Buckner et al., 2008; Jeong et al., 2015), cortical disconnection theories posit that cognitive changes across the adult lifespan can be partly attributed to alterations in white matter pathways that coordinate the communication between brain regions (Andrews-Hanna et al., 2007; Antonenko & Flöel, 2014; Bennett & Madden, 2014; David H. Salat, 2011). In support of this theory, white matter microstructure has been associated with a wide range of cognitive functions (Bennett & Madden, 2014; Johansen-Berg, 2010). Some studies have emphasised the effect of age-related white matter decline on reduced processing speed (Kerchner et al., 2012; Salami, Eriksson, Nilsson, & Nyberg, 2012), which in turn is likely to influence performance in a range of cognitive domains (Salthouse, 1996).

The possible underlying mechanisms for the white matter changes observed throughout the lifespan are numerous, including myelination of fibre tracts and axonal rewiring in development (Lebel, Walker, Leemans, Phillips, & Beaulieu, 2008;
Tamnes et al., 2010), shrinkage and loss of myelinated fibres (Marner, Nyengaard, Tang, & Pakkenberg, 2003; Peters, 2002) and accumulation of redundant myelin in older age (Wozniak & Lim, 2006). Can these white matter trajectories be modified, and if so, by what means?

Recent DTI studies have shown that targeted cognitive training can alter white matter microstructure in both young and older adults (Engvig et al., 2012; Lovden, Bodammer, et al., 2010; Scholz et al., 2009). Although the exact neurobiological underpinnings of such training effects cannot be directly inferred, evidence from animal studies has shown physiological effects of spatial memory training, such as increased immunofluorescence staining of myelin basic protein (MBP) in co-occurrence with increased FA (Blumenfeld-Katzir et al., 2011; Sampaio-Baptista et al., 2013). Changes in the activation of astrocytes have also been observed after training (Blumenfeld-Katzir et al., 2011; Sagi et al., 2012), which have been suggested to underlie reductions in MD through intra/extracellular ratio alterations or cellular tissue swelling (Le Bihan et al., 2001; Theodosis, Poulain, & Oliet, 2008). Thus, recent evidence suggests that DTI may be sensitive to underlying cellular changes of large enough volumetric contribution (Fields, 2015; Sagi et al., 2012).

White matter microstructure is the measure of interest in the present thesis, due to 1) its essential role in cognitive functioning throughout the lifespan and susceptibility to decline in older age, and 2) existing evidence pointing towards training-related modifiability of white matter microstructure in both young and older adults.

### 4.4 Which brain-cognition relationships can be expected?

The evidence for concurrent changes in brain and cognition after training interventions is mixed. Some studies have reported relationships between the magnitude of white matter microstructural alterations and the degree of cognitive improvement (Engvig et al., 2012; Hofstetter, Tavor, Tzur Moryosef, & Assaf, 2013; Mackey, Whitaker, & Bunge, 2012; Nordvik et al., 2012; Schlegel, Rudelson, & Tse, 2012; Scholz et al., 2009). Conversely, other studies report a lack of such relationships (Lovden, Bodammer, et al., 2010; Lovden et al., 2012; Lovden, Wenger,
Martensson, Lindenberger, & Backman, 2013). Thus, the extent to which white matter microstructural alterations correlate with changes in cognitive improvement is unclear.

Age represents a central factor for the understanding of brain-cognition relationships. Importantly, the extent to which relationships between white matter microstructure and cognitive performance are retained when age is included as a covariate in statistical analyses varies considerably between studies (Charlton et al., 2010; Lockhart et al., 2012; Sasson, Doniger, Pasternak, Tarrasch, & Assaf, 2013). Thus, when age is corrected for, effects are often reduced (Salthouse, 2011). However, a number of studies have reported age-independent relationships between white matter microstructure and cognition throughout the lifespan (Johansen-Berg, 2010). Although the evidence for brain-cognition relationships in training studies is mixed, moderate age-independent relationships can be expected.

In general, research has yielded various perspectives regarding the origin of brain-cognition relationships throughout the lifespan. It has been hypothesised that brain-cognition relationships are driven by the concurrent, accelerated decline in both of these factors with advancing age, which is referred to as the *neuropsychological hypothesis* (Van Petten, 2004). According to this view, brain-cognition relationships are predicted to increase in strength with advancing age due to decline, and to be stronger in older adults relative to young adults. Another perspective referred to as the *developmental hypothesis*, emphasises the relationships between the development of cognitive abilities and brain structure during childhood and adolescence (Van Petten, 2004). Thus, this hypothesis predicts that early development of brain structure modulates the development of cognitive function. A meta-analysis by Van Petten (2004), which included studies relating memory function to hippocampal volume, showed support for both the neuropsychological and the developmental hypothesis. Hence, these hypotheses need not be mutually exclusive, but may indicate differential brain-cognition relationships during different phases of the lifespan. However, as the majority of the studies supporting the neuropsychological hypothesis included patients with Alzheimer’s disease, other types of dementia and mild cognitive decline (Van Petten, 2004), the results may not necessarily generalise to healthy ageing.
Brain-cognition relationships in healthy individuals were recently investigated in a longitudinal study by Walhovd et. al (2016). The results showed that brain-cognition relationships were remarkably stable throughout the lifespan, and that highly functional individuals showed lifespan trajectories in brain structure parallel to those with lower cognitive function. Thus, brain-cognition relationships may be largely influenced by early life factors (Karama et al., 2014; Walhovd et al., 2016).

4.5 Recap

To summarise, accumulating evidence suggests that the brain is able to change in response to experience throughout the lifespan. However, few studies have directly compared plastic potential in young and older adults. Moreover, active control groups are often lacking in studies focusing on age differences, precluding the possibility to differentiate specific training effects from effects related to participation in general. Groups of older adults are often characterised by large individual differences in cognitive function, and a complex interplay of genetic, biological and environmental factors is likely to underlie this individual variation. This variation is also observed in the extent of cognitive gain after training interventions, and it is unknown whether brain characteristics such as white matter microstructure contribute to this variation. As the temporal nature of training-induced plasticity is largely unexplored, it is unknown whether training needs to be continuously upheld in order for structural brain changes to persist.
5. Main objectives

The overall objective of the thesis was to investigate the effects of memory training on white matter (WM) microstructure, with the focus falling on four main research questions:

I. Are the cognitive and WM microstructural effects of the memory training specific for the training group, relative to active and passive control groups?

II. Can WM microstructural brain characteristics explain individual differences among older adults in regards to cognitive training gains?

III. What are the age differences in cognitive and WM microstructural plasticity in response to memory training?

IV. Can memory training systematically moderate the magnitude of age-related decline in WM microstructure?
6. Methods

6.1 Participants

The sample was drawn from the project Neurocognitive Plasticity at the Research Group for Lifespan Changes in Brain and Cognition (LCBC), Department of Psychology, University of Oslo. Participants were recruited through multiple newspaper and webpage adverts, which ran between one and seven days. All participants were screened with a health interview. Participants were required to be either young or older (in or around their 20s or 70s, respectively) healthy adults, right handed, fluent Norwegian speakers, and have normal or corrected to normal vision and hearing. Exclusion criteria were history of injury or disease known to affect central nervous system (CNS) function, including neurological or psychiatric illness or serious head trauma, being under psychiatric treatment, use of psychoactive drugs known to affect CNS functioning, and MRI contraindications. Participants were required to score above 25 on the Mini Mental State Examination (MMS) (Folstein, Folstein, & McHugh, 1975) and have scores less than 2 standard deviations (SD) below mean on the five minutes delayed recall subtest of the California Verbal Learning Test II (CVLT II) (D. C. Delis, Kramer, Kaplan, & Ober, 2000). Three individuals in the older group were excluded based on these criteria. All participants further had to achieve an IQ above 85 on the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999)). All scans were evaluated by a neuroradiologist and deemed to be free of significant injuries or conditions.

The participants were assigned to one of three intervention groups at registration. Group 1 (ABAB) started with ten weeks of memory training, group 2 (BABA) started with ten weeks as passive controls and group 3, the active control group (AC), received an active control intervention (the design is described in section 6.2). Pools of around 20 participants were recruited at a time, with continuous data collection for all three conditions simultaneously. At the time of the present study, 126 older adults and 91 young adults - a total of 217 participants - fulfilled the inclusion criteria. Sample demographics are listed in Table 1. A flow chart representing the recruitment process including numbers of subjects included and drop out rates is provided in Appendix I.
Table 1 Sample demographics

Sample demographics as measured at baseline. Abbreviations: Edu = education, MMS = Mini mental status, CVLT L = learning trials, CVLT R = five minutes recall, Word recall = total words recalled on a word list test (described in section 6.4). Group 1 (ABAB) started with ten weeks of memory training, group 2 (BABA) started with ten weeks of no intervention, and group 3 (AC, active control group) received ten weeks of the active control intervention. One-way analysis of variance (ANOVA, Bonferroni corrected) showed differences between the young and the older adults across groups on MMS ($t = 3.3, p = 1.0 \times 10^{-3}$), IQ ($t = 6.2, p = 2.9 \times 10^{-9}$), CVLT learning ($t = 11.1, p = 5.3 \times 10^{-23}$) and CVLT five minutes recall ($t = 10.3, p = 2.2 \times 10^{-20}$). No differences were found in sex and education. Separate ANOVAs for the young and older sample showed a difference in sex in the young sample ($F(2) = 5.3, p = 6.9 \times 10^{-3}$). Pairwise comparisons (Bonferroni corrected) showed a significant mean difference of 0.46 between the active and the passive control groups ($p = 5.1 \times 10^{-5}$). No other differences were found between the experimental groups.

In the older sample, 15 of the participants dropped out after the first assessment session (13 in the training group, 1 in the active control group and 1 in the passive control group). 19 of the younger participants dropped out after the first assessment session (12 in the training group, 5 in the active control group and 2 in the passive control group). The reasons for dropping out included that the participation was too time consuming or that the particular time frame for assessment was inconvenient. The young participants who dropped out after the first assessment session performed
lower than the rest of the young sample in terms of IQ (mean ± SD for the drop outs = 107.0 ± 10.0, for the included sample = 113.0 ± 8.7; t(71) = 2.3, p = 0.02). The group of older participants who dropped out after the first assessment session performed lower than the rest of the older sample in terms of IQ (mean ± SD for the drop outs = 114.4 ± 10.5, for the included sample = 120.5 ± 10.2; t(109) = 2.2, p = 0.03) and CVLT five minutes recall (mean ± SD for the drop outs = 8.3 ± 3.7, for the included sample = 10.2 ± 3.2; t(109) = 2.1, p = 0.04) and showed a trend towards lower MMS score (mean ± SD for the drop outs = 27.7±1.4, for the included sample = 28.7 ± 1.3; t(109) = 2.0, p = 0.06).

Lower cognitive performance among dropouts is commonly observed in longitudinal studies, resulting in a selection bias effect towards higher functioning individuals (Salthouse, 2014). To control for selection bias, a repeated measures analysis of covariance (ANCOVA) was performed to test whether a group of included participants, who matched the participants who dropped out, differed from the rest of the sample in terms of memory improvement. Age and sex were used as covariates. 12 young participants were matched with the young trainers who dropped out based on IQ (mean ± SD for the drop outs = 107.2 ± 9.5, for the matched group = 107.0 ± 9.0). 13 older participants were matched with the older trainers who dropped out on IQ (mean ± SD for the drop outs = 112.3 ± 9.6, for the matched group = 112.8 ± 9.3). The results showed that the matched groups did not differ from the rest of the training group in terms of memory improvement (F(1) = 0.38, p = 0.5 for the young adults and F(1) = 0.14, p = 0.7 for the older adults).

Across the remaining duration of the study, 27 young participants and 18 older participants discontinued their participation. Details are provided in Appendix I.
6.2 Design and memory-training program

The experimental design is illustrated in Figure 3. The participants were examined with MRI and cognitive testing, with a ten-week interval between each assessment.

![Design Illustration](image)

**Figure 3 Design**
Illustration of the experimental design. N includes all young and older participants who fulfilled the inclusion criteria. Drop out information is provided in Section 6.1.

The memory training included practicing the mnemonic technique MoL (Bower, 1970). The training program included a single course session each week and eight weekly home assignments. The first group session included a presentation of the project, an introduction of the MoL, and an initial word list task consisting of 15 words. The following weekly group sessions included updating of the strategy and a word list task, which was increased by five words each week to ensure a continuous challenge. However, the participants were encouraged to individually adjust the difficulty level to match their capacity, with the aim of achieving a challenging but manageable training level across all the participants. Eight home assignments were sent out weekly, with a minimum requirement that four be completed. The home assignments consisted of word lists with various themes and followed the level of difficulty set in the group session the same week, with options for individual adjustment including increasing/decreasing the number of words on the weekly tasks, performing the tasks within individual time limits and recollection of the word lists in
reverse order. The proportion of tasks completed during the first training period was on average 74% in the older group and 45% in the young group. The home assignments were completed online and registered to a database. The two training periods followed the same course structure and included the same strategy training. The level of difficulty was increased each week, and the initial level in the second training period corresponded to the middle level of the first period. Thus, the final level of difficulty was higher in the second training period. Four home assignments were sent out weekly, as the assignments in period two were expected to be more time consuming relative to those in period one, due to higher level of difficulty. During the second training period, 68% of the tasks were completed in the older training group and 45% in the young training group.

The active control program involved attending popular scientific lectures and completing home assignments. None of the tasks or lectures in the active control program involved any specific form of memory training. Modest improvement in cognitive function has been found in active control groups (Legault et al., 2011), indicating that increased cognitive activity and social engagement may affect cognitive function in older people (Gallucci et al., 2009). Thus, the inclusion of such groups is necessary to determine the specific effects of training (Hart et al., 2008; Law et al., 2014). The number of tasks, group meetings, and contact with staff were matched between the training intervention and the active control intervention. Furthermore, test sessions and time intervals were held identical for all participants, in order to ensure that test-retest effects would not differ across groups. The number of total tasks completed in the active control group was on average 70% for the older adults and 39% for the younger adults. Independent samples t-tests showed that the number of tasks completed did not differ between the training groups and the active control groups (mean ± SD = 32.7 ± 20.1 for the young training group, 30.8 ± 19.7 for the young active control group, t(43) = -0.29, p = 0.8, and mean ± SD = 57.6 ± 15.1 for the older training group, 55.5 ± 21.1 for the older active control group t(60) = -0.45, p = 0.7). Test sessions and time intervals were held identical for all participants, in order to ensure that test-retest effects would not differ across the groups.
6.3 Ethics

All procedures were approved by the Regional Ethical Committee of Southern Norway, and written consent was obtained from all participants. The research was conducted in line with the Norwegian law for medical and health research (http://www.lovdata.no/all/hl-20080620-044.html), which states that medical and health research should be grounded in respect for the participants’ human rights and dignity. Thus, the participants’ integrity takes precedence over scientific interests. Providing sufficient information on the requirements of participation was strongly emphasised at recruitment, as well as during the project period. Written protocols included that the participants could withdraw from the study at any time, in addition to detailed information about the challenges and possible discomfort associated with MRI and cognitive assessments. Although MRI has been used in research and clinical investigation for several years and there are no known side effects, scanning may be uncomfortable due to noise and possible claustrophobia related to lying in a scanner for around an hour. Thus, individuals were discouraged from participating if they had known claustrophobia. Trained radiographers conducted screenings for MRI contraindications such as pregnancy, pacemakers or metal implants in the body. If there was doubt regarding possible contraindications, MRI assessment was not performed. Although a history of injury or disease known to affect the central nervous system was set as an exclusion criterion, unexpected findings may be uncovered on MRI in normal populations. Thus, all MRI assessments included sequences sensitive for detecting brain abnormalities that were evaluated by a neuroradiologist. Sensitive data was subject to confidentiality and treated in line with the Norwegian Personal Data Act (http://www.lovdata.no/all/hl-20000414-031.html). Thus, all sensitive data was stored in high security servers.

6.4 Cognitive measures

All participants completed cognitive testing with a ten-week interval between each assessment. A full neuropsychological test battery was administrated, including the Wechsler Abbreviated Scales of Intelligence (Wechsler, 1999), as a measure of general verbal and performance ability, Mini mental state (MMS), as a measure of
cognitive state (Folstein et al., 1975), different versions of the California Verbal Learning Test (Mckee, Delis, Kramer, Massman, & Gettman, 1990; Woods, Delis, Scott, Kramer, & Holdnack, 2006) as a measure of verbal long term memory, versions of the Rey-Oesterrieth Complex Figure as a measure of visuospatial memory, measures of verbal working memory (digit span forward and backward), as well as experimental tests of executive function hypothesised to index three target functions, namely updating, inhibition and shifting, and experimental tests of word recognition and source memory.

Memory performance was measured using an experimental word list test developed to measure verbal recollection. The test enabled the MoL to be applied, such that the measure of memory performance after the strategy training was closely related to the utilized technique, and thus convenient for measuring training gains. Participants were presented with the full word list, and given five minutes to learn as many words as possible in the correct list order. Immediately after the learning trial, they were given 10 minutes to recall the words. The learning trial was supervised by a test administrator. To avoid potential ceiling effects, the word list consisted of 100 words. The word lists varied across participants for each testing session. Thus, the words in the list differed between time points. The composition of words in each list was matched on criteria of frequency using the The Oslo Corpus of Tagged Norwegian Texts (http://www.tekstlab.uio.no/frekvensordlister/index.html), in addition to complexity and how easily they were assumed to transfer to visual imagery. The total number of words recalled from the word list test was used as the score of memory performance before and after the training intervention.

6.5 Image acquisition and analyses

A Siemens Skyra 3T MRI scanner with a 24-channel head-coil was used (Siemens Medical Solutions; Erlangen, Germany), and a diffusion-weighted echo-planar imaging (EPI) sequence was applied for each subject (FOV$_{xy} = 252 \times 256$ mm, dimensions = $128 \times 130 \times 70$, voxel size = $1.9626 \times 1.9626$ mm, slice thickness = $2$ mm, repetition time = $9300$ ms, echo time = $87$ ms). Sixty-four unique diffusion weighted volumes were collected at b-value = $1000$ s mm$^{-2}$ in addition to two non-
diffusion-weighted (b-value = 0 s mm\(^{-2}\)) volumes, one acquired with an opposite k-space traversal direction for the purpose of correcting susceptibility artefacts.

All scan-sets were manually checked for gross motion artefacts. The susceptibility-induced field was estimated using the FSL tool topup (J. L. Andersson, Skare, & Ashburner, 2003) and corrected for along with subject motion and eddy current-induced fields using the eddy tool (Andersson & Sotiropoulos 2016). Signal dropout caused by subject motion during the diffusion encoding was also detected and corrected (Andersson & Sotiropoulos 2014). Each acquired slice was compared with a model free prediction, and if the observed signal was statistically different (three standard deviations) from the prediction, it was replaced by the latter. Non-brain tissue (skull etc.) was removed using Brain Extraction Tool (Smith, 2002), employing a mask based on the non-diffusion-weighted volume. Fractional anisotropy (FA) images were created by fitting a tensor model to the pre-processed diffusion data using FMRIB’s Diffusion Toolbox (FDT) (Behrens et al., 2003).

All participants’ FA data were processed with the FSL software package Tract-based spatial statistics (TBSS) (Smith et al., 2006), which is widely used for deterministic tractography. In this approach, the diffusion ellipsoids (described in Section 4.3) are compared in neighbouring voxels, and connections formed by reconstructing entire pathways. The subjects FA images were aligned into a common space using the nonlinear registration tool FNIRT (Andersson et al., 2010), which uses a b-spline representation of the registration warp field (Rueckert et al., 1999). Next, the mean FA image was calculated and thinned to create a mean FA skeleton, which represents the centres of all tracts common to the group. The threshold for the mean FA skeleton was set at 0.2. Each participant’s aligned FA data were then projected onto this skeleton. The nonlinear warps and skeleton projection stages were repeated using the mean diffusivity (MD), radial diffusivity (RD) and axial diffusivity (AD) measures.
6.6 Statistical analyses

6.6.1 Memory improvement

*Papers I and II*

To investigate main effects of the training and group differences in memory improvement, repeated measures analyses of variance (ANCOVA) were conducted on the memory scores at baseline and follow up. Pairwise comparisons were performed with Bonferroni corrections. The total number of words recalled from the word list test (described in Section 6.4) was used as the outcome variable for training effects. Age, sex and baseline memory performance were included as covariates, and Greenhouse-Geisser corrections for violation of sphericity were used. Additional repeated measures ANCOVAs were run for the training group, active control group and passive control group separately, testing the change in memory performance from baseline to follow up.

In the analyses of relationships between memory improvement and WM microstructure, standardised residuals of memory scores were used as the main measure of training gains. Hence, the baseline variance is removed, representing the training gain independent of the baseline performance (discussed in section 8.4.3). The residuals were calculated from a linear regression analysis, using memory performance at follow up as the dependent variable and memory performance at baseline as the independent variable. In paper II, additional analyses using difference scores were included for comparison.

*Paper III*

The temporal dynamics of cognitive plasticity was analysed using a non-linear mixed effects model (using the maximum likelihood estimations with full covariance matrix; the matrix logarithm), as implemented in Matlab (http://uk.mathworks.com/help/stats/nlmefit.html). The memory scores for each subject at each time point were described using the following logistic function:

\[
\text{Memory}(t) = \frac{\beta_0}{1 + e^{-(t - \beta_1)/\beta_2}} + \beta_3 \times \text{age} + \beta_4 \times \text{sex} \quad (1)
\]
This function was chosen to describe an initial slow gain in memory improvement during the rest phase due to practice effects (TP0 - TP1), followed by exponential growth during the first training phase (TP1 - TP2), with an eventual plateau in performance over the remainder of the time points. The logistic function used contained three parameters, each of which was modelled as the sum of a fixed effect and a random effect. The fixed effects described general group behaviour across all subjects, while the inclusion of random effects allowed inter-subject variation to be modelled, reducing the overall error term in the model fit. The term $\beta_0$ in Equation 1 represents the plateau value of the logistic function, which measured the maximum memory performance achieved. The term $\beta_1$ determined the point at which exponential growth in memory performance first begun, while $\beta_2$ determined the overall slope of the memory improvement during this exponential phase. The remaining beta terms modelled the fixed effects of age and sex across all time points, and were included to account for variation in memory performance as a function of these variables.

6.6.2 White matter microstructure

*Paper I*

Patterns of age differences in WM microstructure measured at baseline were analysed by performing voxel-wise general linear model (GLM) analyses on the skeletonised FA and MD values. To test whether individual variability in WM microstructure at baseline could predict cognitive training gains, voxel-wise GLMs were run across all voxels in a region of interest (ROI) based on the areas showing age differences in both FA and MD. To test specificity, the analyses were repeated in the areas not showing age effects in FA and MD, and on the full skeleton. Permutation-based statistics were performed with 10,000 permutations (Nichols and Holmes, 2002) as implemented in *randomise*, part of FSL (Winkler, et al., 2014). The significance threshold was set at $p < 0.05$, corrected for multiple comparisons across space using threshold-free cluster enhancement (TFCE) (Smith and Nichols, 2009). Age, sex and motion were used as covariates. Motion was estimated as the mean of the average Root Mean Square displacement value (RMS) across each diffusion-weighted volume derived from the *eddy* procedure (Andersson and Sotiropoulos, 2015). To assess
effect sizes, mean MD was extracted from significant clusters and correlated with change in memory performance, with age, sex, and motion as covariates.

To examine whether the magnitude of age differences in MD and FA showed a posterior to anterior gradient, the mean $t$-scores were calculated across all skeleton voxels for each coronal slice, excluding the slices with fewer than 450 voxels (Sexton, et al., 2014). A linear regression model using the least squares method was fit to the mean $t$-scores. To investigate whether the magnitude of the posterior-anterior slopes was related to memory improvement, the individual slopes in MD and FA were correlated with the change in performance on the word list test (standardised residuals), using age, sex and motion as covariates.

**Paper II**

To investigate group differences in mean WM microstructural change, GLM analyses were performed on the mean of the skeletonised tensor-derived values, using age, sex, motion (RMS) and baseline WM values as covariates. Voxel-wise GLMs were then performed using the values from follow up as the dependent variable, and the values from baseline as a per-voxel regressor, testing the differences between the intervention groups within the young and the older sample, respectively. To investigate relationships between change in WM microstructure and change in memory performance, voxel-wise GLMs were performed on the full skeleton using the values from the MRI assessment after training as the dependent variable, and the values from the assessment before training as a per-voxel regressor. Improvement in memory performance was measured by standardised residuals calculated from a linear regression analysis, using memory performance at baseline as the dependent variable and memory performance at follow up as the independent variable. Permutation-based statistics with TFCE were performed with 5000 permutations, correcting for multiple comparisons across space, as implemented in `randomise`. The significance threshold was set at $p < 0.05$, as for all analyses.

**Paper III**

To investigate training effects on WM microstructure during the first training period, a voxel-wise GLM analysis of group differences was performed on the difference
maps of FA, MD, RD and AD, using age, sex and motion (RMS) as covariates. Permutation-based statistics were performed with 5000 permutations as implemented in randomise. The significance threshold was set at \( p < 0.05 \), corrected for multiple comparisons across space using TFCE. The group differences in MD change from baseline to follow up was then used to create a ROI used in the time course analyses. Thus, trajectories of WM microstructural change were analysed within areas showing training effects on general diffusivity. As a follow up, the analyses were repeated using the areas showing group differences in FA as a ROI.

In a similar fashion to memory changes, WM microstructural trajectories were analysed using a non-linear mixed effects model, as implemented in Matlab (http://uk.mathworks.com/help/stats/nlme.html). For each subject at every time point, mean values of FA, MD, RD and AD were calculated within the ROI based on the group differences in MD changes between the first two time points. Mean values were calculated separately for each subject at every time point. The following function was used to describe the time course of WM microstructural changes (for simplicity, only FA is referred to in the text):

\[
FA(t) = \beta_0 + \beta_1 \times t + \beta_2 \times \cos(\pi t) \times e^{-\beta_3 t} + \beta_4 \times \text{age} + \beta_5 \times \text{sex} + \beta_6 \times \text{motion} \tag{2}
\]

Several terms appear in this expression, each of which describe different physical characteristics of FA evolution. The first term \( \beta_0 \) is an intercept, describing the value of FA at the beginning of the study (time point 0). This term was modelled as the sum of a fixed effect and a random effect for each subject, which described an overall group effect while allowing for individual variation in baseline FA across subjects. The second term \( \beta_1 \) models a linear change in FA over the duration of the study. The term \( \beta_2 \) appears within a periodic cosine function. This term models the amplitude of a time-varying FA change, which allows changes to occur in an estimated direction during rest periods, followed by subsequent changes in the opposite direction during training periods. \( \beta_1 \) and \( \beta_2 \) were also modelled as the sum of a fixed effect and a random effect, allowing the slope and the cosine of FA changes to vary across subjects.
The term $\beta_3$ models exponential decay of the time-varying FA changes, describing a general decrease in the amplitude of plastic changes over time. The non-linear model was shown to improve with the inclusion of $\beta_3$ relative to a more basic model describing $\beta_1$ and $\beta_2$ only (loglikelihood increase from 1438 to 1445 and lower Akaike information criteria -2851 to -2862). The remaining terms in Equation 2 modeled the fixed effects of age, sex and motion across all time points. Missing data were handled within the Matlab LME framework using the method of Little and Rubin (Little & Rubin, 2014). Independent samples $t$-tests were used to compare the estimated annual change in the current sample to the estimated annual change in a matched sample of participants from a non-training study (Storsve, Fjell, Yendiki, & Walhovd, 2016), who were measured across more extended intervals (mean = 3.2 yrs, SD = 0.2 yrs).

7. Summary of results
7.1 Paper I

In paper I, we investigated the effects of memory training related to active and passive control conditions in 104 older adults, and tested whether WM microstructure at baseline was predictive of training benefits. We hypothesised that 1) memory improvement would be restricted to the training group, 2) widespread areas would show greater mean diffusivity (MD) and lower fractional anisotropy (FA) in older adults relative to a group of young adults ($N = 52$), and 3) within these areas, variability in WM microstructure in the training group would be predictive of training gains.

The results showed that only the group receiving memory training improved their memory performance. Significant age differences in MD and FA were found in widespread areas, with increasing magnitude of age effects from posterior to anterior regions. Within the areas showing age differences, voxel wise analyses showed a negative relationship between MD and memory improvement in 3 clusters, as shown in Figure 4. Furthermore, the older individuals who showed similar FA values to the young adults in frontal areas showed larger memory improvement than the individuals showing lower FA values relative to the young adults. The results demonstrate that individual differences in WM microstructure, particularly in regions vulnerable to
age-effects, may serve as a marker for the ability to adapt in response to cognitive challenges in ageing.

![Figure 4](image)

**Figure 4** *MD clusters related to memory improvement* (A) Cluster 1 (172 voxels) and cluster 2 (53 voxels) are shown in blue. Axial view of Talairach coordinates x 90, y 137, and z 94. (B) Cluster 3 (17 voxels) is shown in blue. Axial view of Talairach coordinates x 109, y 136, and 82. Clusters are overlaid on the areas showing age differences in both FA and MD (in red), the mean FA skeleton (in green), and the standard MNI152 T1 1-mm³ brain template. The results are thresholded at *p* < 0.05 and corrected for multiple comparisons. Significant areas are dilated for illustrative purposes.

7.2 Paper II

In paper II, we investigated changes in WM microstructure in response to memory training relative to passive and active control conditions in 184 young and older adults. We hypothesised that 1) only the groups receiving the memory training intervention would show improved memory performance and alterations in WM microstructure after ten weeks of memory training, 2) the young adults would show larger memory improvement and a higher degree of WM microstructural alterations as compared to the older adults and 3) improvements in memory performance would relate to changes in WM microstructure.

The results showed that both the young and older participants who received memory training improved their performance. The young adults improved their memory to a larger extent than the older adults. Group differences in memory improvement are
shown in Figure 5, which includes the results on group differences in the young sample in addition to group differences within the older sample as published in paper I (de Lange et al., 2016).

![Graph showing memory improvement for young and older adults](image)

**Figure 5** Memory improvement measured by the word list consisting of 100 words is shown for the young and older intervention groups. Memory scores are shown on the Y-axis.

In the older sample, the training group showed less age-related decline in WM microstructure compared to the control groups, in areas overlapping the corpus callosum, the cortico-spinal tract, the cingulum bundle, the superior longitudinal fasciculus and the anterior thalamic radiation. Less microstructural decline was related to a higher degree of memory improvement, indicating that microstructural alterations may be part of a neurobiological substrate for behavioural improvements in ageing. Interestingly, no training-related changes in WM microstructure were found in the young adults (as visible in Figure 6). The observed divergence of behavioural and microstructural responses to memory training with age may imply that the demands of the memory training, despite being dynamically adapted to performance levels, did not exceed the young adults’ existing range of processing capacity (Schmiedek, Lövdén, & Lindenberger, 2010). Thus, improvements in performance did not require WM microstructural plasticity, which is further discussed in Section 8.3.2.
Figure 6 Group differences in microstructural changes are plotted separately for young and older adults. The means of the skeletonised diffusion metrics are shown on the Y-axis. The axis ranges are of equal size for young and older adults (such that the slopes can be directly compared), but the values vary due to age differences in the diffusion metrics.

7.3 Paper III

In paper III, changes in WM microstructure were investigated in 107 older adults across periods of memory training and no intervention, hypothesising that training would systematically moderate age-related decline. First, we hypothesised that decline in WM microstructure could be observed in older adults over a relatively short period of less than a year. These trajectories were compared to the changes observed in an independent non-training sample of older adults followed for a substantially longer interval of 3.2 years, hypothesising that the age effects would occur at a similar rate to the effects estimated over longer time periods. Second, we proceeded to test whether experimental interventions given in a time-locked manner could systematically affect the magnitude of this age-related decline, hypothesising that: a) periods of targeted memory training would moderate decline in WM microstructure, b) neural training effects would not be upheld in the absence of training, but c) such effects could be re-invoked with a new training period, although d) effects on microstructural changes would be of larger amplitude during the first training period relative to the second.
The results showed a general decline in WM microstructure across 40 weeks, confirmed to occur at a similar rate as that observed in an independent sample across more extended intervals. Cyclic age-related reductions were followed by training-related improvements as a function of being on versus off training. Thus, the training systematically moderated the age-related decline observed across the duration of the study. Decreases in FA during the rest periods were followed by periodic increases during the training periods, and the changes in MD, RD and AD showed periodic variations corresponding to the inverse of those of FA during training and rest periods, as visible in Figure 7.

![Figure 7](image1)

**Figure 7** Mean changes in FA (left) and MD (right) for all participants across time points (time point 0-1 = rest period, 1-2 = training period, 2-3 = rest period, 3-4 = training period, 4-5 = rest period).

The amplitude of the changes decreased over time, indicating a larger effect of the first training period relative to the second. Although both periods of training led to moderation of microstructural decline, the temporal dynamics of memory performance (as shown in Figure 8) showed a divergent pattern where memory performance reached a plateau and stabilised after the first training period. Thus, the time course of changes in memory performance did not rely on continuous training to the same degree as the changes in WM microstructure, which is consistent with previous findings (Erika Dahlin, Nyberg, Bäckman, & Neely, 2008; Driemeyer et al., 2008; S. C. Li et al., 2008).
Figure 8 Left: The mean FA values for each subject are plotted separately at each time point as open circles. The slope and the cosine function are displayed as solid lines. The mean FA values across all subjects are displayed as red crosses. Right: The memory performance values for each subject are plotted separately at each time point. The fixed effects logistic function, describing the net behaviour of the entire group, is displayed as a solid line. The mean memory values across all subjects are displayed as red crosses.

The results demonstrate that targeted cognitive training has the potential to moderate the magnitude of age-related deterioration in WM microstructure. However, continuous training is likely to be a premise for the attenuation of neural decline, and the initial learning of a new skill may promote brain plasticity to a larger extent than practicing well-known tasks.
8. General discussion

8.1 What are the premises of plasticity?

8.1.1 Intervention specificity

In correspondence with previous research, the results showed that both young and older individuals have the potential to benefit from cognitive training (Boyke et al., 2008; Burki et al., 2014; Draganski et al., 2004; Engvig et al., 2012; Gross et al., 2014; Johansen-Berg & Duzel, 2016; Jones et al., 2006; Lustig et al., 2009; Rebok, Carlson, & Langbaum, 2007). The difference between the training group and the passive- and active control groups demonstrated that the memory training intervention had a unique effect on memory performance measured by the word list test.

The inclusion of an active control group strengthens the validity of the findings, by allowing comparison of effects related to general components of participation and effects related to the specific components of the memory training (Hart et al., 2008; Law et al., 2014). Modest improvement in cognitive function has been found in active control groups (Legault et al., 2011), indicating that increased cognitive activity and social engagement may affect cognitive function in older people (Gallucci et al., 2009). Some, but relatively few, cognitive training studies include active control groups receiving different interventions as a means of comparison (Barnes et al., 2013; Fabre et al., 2002; Legault et al., 2011; Oswald et al., 2006; Schwenk et al., 2010; Suzuki et al., 2012; Zelinski et al., 2011). However, exposure to intervention often varies between conditions (Law et al., 2014), which may compromise the control of factors such as expectations, social contact and cognitive activity (Hart et al., 2008; Safer & Hugo, 2006; Schwenk et al., 2010). In the current study, the number of tasks, group meetings and contact with staff was matched between the training group and the active control group, controlling for the possible effect of these factors on memory performance. Furthermore, test sessions and time intervals were held identical for all participants, in order to ensure that test-retest effects would not differ across groups.
8.1.2 Younger brain – more to gain

The results from paper I showed a relationship between memory improvement and WM microstructure within age-vulnerable WM regions, such that lower MD was associated with greater performance gains in the older adults. Furthermore, the older individuals who showed similar FA values to the young sample in frontal areas showed larger memory improvement than the individuals showing lower mean FA values compared to the young sample. On average, higher MD and lower FA were found in widespread areas in the older group relative to the young group, corresponding to previous cross-sectional results (Madden, Bennett, & Song, 2009; Marstaller, Williams, Rich, Savage, & Burianova, 2015; A. Pfefferbaum et al., 2000; Voineskos et al., 2012; Westlye et al., 2010). Evidence from longitudinal studies has shown similar patterns of increased MD and decreased FA with advancing age (Barrick et al., 2010; Bender et al., 2015; Engvig et al., 2012; Sexton et al., 2014).

These results indicate that WM microstructure, particularly in anterior regions showing prominent age differences, is to some extent predictive of the ability to benefit from cognitive training in older age. The young adults improved their memory to a larger extent than the older adults as shown in the results from paper II, and such age differences in training gains are commonly observed in training studies (M. M. Baltes et al., 1992; Brehmer, Westerberg, & Backman, 2012; Burki et al., 2014; E. Dahlin et al., 2008). Together, these results indicate that a younger brain has more to gain, and that a high extent of cognitive improvement in response to training in older age may be associated to the preservation of a ‘younger’ looking brain. Thus, the results support the brain maintenance theory, suggesting that the maintenance of a younger brain is predictive of cognitive function in older age (Nyberg et al., 2012). However, as the relationship between WM microstructure at baseline and memory improvement was not investigated in young adults, it is unknown whether this relationship was unique for older adults.

8.1.3 Mismatch between supply and demand

Interestingly, the memory training affected WM microstructure in the older adults only, even though the younger adults still managed to improve their memory performance more than the older adults. This divergence of behavioural and brain responses to training with age may be interpreted within the supply-demand
framework described in section 4.1.1, where structural brain alterations are thought to take place only when there is a mismatch between functional capacity and the environmental demands (Lovden, Backman, et al., 2010). The capacity for variations in behaviour that do not require structural brain changes, referred to as flexibility, can generate improvements in performance, but does not require changes in intrinsic capacity as opposed to plasticity (Noack et al., 2009). Thus, flexibility depends on the pre-existing range of processing capacity, and plasticity takes place only when the demands placed exceed the existing capacity, resulting in a mismatch between supply and demand (Lovden, Backman, et al., 2010).

In view of this theory, the improvement of performance in response to environmental demands may have been within the functional capacity of the young adults, while the demands may have exceeded the functional capacity of the older adults, thus, even more modest improvements would require brain changes. Although the training posed increasing demands and the individuals could adjust the tasks to their own level, the intervention itself may not have provided a demand that substantially exceeded the young adults’ capacity (Schmiedek et al., 2010). The nature of the training is also likely to have imposed a larger overall change in environment for the older adults. Indeed, the younger adults were in a phase where memory training may be more intrinsic to their everyday life, with studies and new work tasks typically posing continuous demands. The training was thus more likely to represent a considerable environmental change for the group of older adults, of which the majority were retired. Hence, microstructural plasticity in response to memory training may depend on whether the level of the training exceeds the pre-existing range of processing capacity (Lovden, Backman, et al., 2010), and whether the nature of the training induces a more considerable change in the environment.

Interpreting age differences in plastic responses within the theoretical framework proposed by Lövdén and colleagues (Lovden, Backman, et al., 2010) may add an important dimension for understanding the premises for plasticity. Thus, a matched training program adapted to individual performance level for young and older adults may promote specific cognitive improvements for all, yet fail to promote structural
plastic alterations in both age groups, due to age-related differences in flexibility, or perhaps the greater overall changes in experience and environment for older adults.

**8.1.4 Continuous training**

In concurrence with previous studies showing that structural training effects do not transfer in time (Draganski et al., 2004; Driemeyer et al., 2008), the results from paper III suggest a temporal specificity of training-related plasticity, as the periods of training and no intervention involved differential changes in WM microstructure. Ageing studies do show associations between lifestyle factors such as physical, cognitive and social activity and maintained cognitive function in older age (Fratiglioni et al., 2004; Josefsson et al., 2012; Walhovd et al., 2014). However, as recent evidence suggests that brain-cognition relationships are largely stable throughout the lifespan (Lyons et al., 2009; Walhovd et al., 2016) (discussed in section 4.4), it seems unlikely that an active lifestyle can mitigate age-related decline in brain structure unless it is continuously upheld. The results from paper III indicate that targeted cognitive training has the potential to moderate the magnitude of age-related deterioration in WM microstructure, but continuous training is likely to be a premise for the attenuation of neural decline. Thus, these findings bridge the existent literature showing relations between a cognitively active lifestyle and maintained cognitive and brain function in ageing, and the literature pointing to potential for training-induced plasticity in older adults. The evident message may be that one needs to be continuously and consistently immersed in cognitive engagement in order to mitigate age-related decline.

**8.2 Is microstructural plasticity a substrate for memory improvement?**

**8.2.1 Flexibility versus plasticity**

The observed divergence of behavioural and brain responses to training with age might indicate age-related differences in flexibility. Thus, if flexibility is high, improvements in performance do not require microstructural alterations. In the older adults, where flexibility is expected to be lower relative to the young adults, the degree of WM microstructural changes was related to the degree of cognitive
improvement, such that the older participants who improved their memory performance to the largest extent showed a decrease in MD (as shown in Figure 9). Thus, in light of the supply-demand framework, microstructural changes may be part of a neurobiological substrate for the behavioural improvements, but only when there is a mismatch between existing capacity and environmental demands, which is considered critical for the initiation of structural plasticity (Lovden, Backman, et al., 2010).

**Older adults**

![Figure 9](image.png)

**Figure 9** Areas showing relationships between memory improvement and MD (left) and FA (right) in the older sample. Sagittal and coronal views of Talairach coordinates $x = 74$, $y = 120$, $z = 85$, overlaid on the mean FA skeleton (green) and the standard MNI152 T1 1 mm³ brain template. The results are thresholded at $p < 0.05$ and corrected for multiple comparisons. The plots show the relationships between MD and FA change and memory improvement measured by standardised residuals.

In paper III, the results showed that the training intervention had a larger effect on WM microstructure during the initial training period, despite the general increase in level of difficulty in the second period. Thus, continuous training on the same tasks may have less effect on brain plasticity relative to learning new skills (Driemeyer et al., 2008). Although the two periods of training led to moderation of WM microstructural decline, the temporal dynamics of memory performance showed a divergent pattern where memory performance reached a plateau and stabilised after
the first training period as shown in Figure 8. Hence, the time course of changes in memory performance did not rely on continuous training to the same degree as the changes in WM microstructure, which is consistent with previous findings (Erika Dahlin, Lars Nyberg, et al., 2008; Driemeyer et al., 2008; S. C. Li et al., 2008). Hence, learning a new skill may affect brain structure to a larger extent relative to practicing those already learned, as the initial training may have enhanced the individuals’ intrinsic capacity for these types of tasks (Lovden, Backman, et al., 2010).

8.2.2 Which white matter regions are relevant?

The results from paper I showed that individual differences in WM microstructure in regions of the anterior corpus callosum (Hofer & Frahm, 2006), the left anterior thalamic radiation and the right inferior fronto-occipital fasciculus were particularly predictive of memory improvement, as shown in Figure 4. In paper II, the results showed that memory improvement in the older training group was related to WM microstructural changes in relatively widespread areas (as shown in Figure 9).

The cognitive processes involved in mnemonic strategies are likely to rely on multiple brain areas, and efficient transfer and integration of information between these areas may be critical for performance. The regions referred to above may represent areas of importance for information transfer that is beneficial for cognitive improvements from mnemonic training. However, the degree of regional specificity in the relation between cognitive functions and WM microstructure is debated (Salthouse, 2011). While some training studies have reported associations between cognitive improvement and altered WM microstructure in highly specific regions (Bennett, Madden, Vaidya, Howard, & Howard, 2011; Engvig et al., 2012), the overall evidence does not currently demonstrate a high degree of regional specificity in the relationship between WM microstructure and cognition (Madden et al., 2009; Salthouse, 2011). Although the results in paper I showed statistically significant relationships between WM microstructure in particular regions and memory improvement, an additional analysis (provided in the supplementary material) showed that the association between WM microstructure at baseline and memory improvement in a set of extracted WM tracts showed a clear tendency of general rather than specific effects, as shown in Figure 10.
Correlations between WM microstructure and memory improvement in a set of extracted WM tracts.

Although the individual differences in WM microstructure that predicted cognitive training gains were not necessarily restricted to specific regions, the relationships were stronger in anterior regions showing prominent age differences. The deterioration in WM microstructure observed in ageing has been suggested to follow a posterior to anterior gradient with a greater magnitude of change in frontal regions (Bennett et al., 2010; Burzynska et al., 2010; Davis et al., 2009). The suggestion that anterior brain areas are particularly vulnerable to age-related decline has gained support (Barrick et al., 2010; Bartzokis, 2004; Brickman et al., 2012; D. H. Salat et al., 2005), however, some evidence indicates that age-related changes follow an inferior-superior gradient rather than a posterior-anterior gradient (Sexton et al., 2014; Sullivan, Rohlfing, & Pfefferbaum, 2010a, 2010b; Zahr, Rohlfing, Pfefferbaum, & Sullivan, 2009), and that such gradients may be region specific rather than general (Sexton et al., 2014). As the overall evidence of regional specificity in relationships between WM microstructure and cognition are unconvincing (Salthouse, 2011), specific regional correlates to training effects cannot be established, and widespread WM regions rather than specific areas may be relevant for improvements in performance in response to memory training.
8.3 Methodological considerations

8.3.1 Sample selection

8.3.1.1 Sample size

Low sample size in studies can lead to a reduced chance of detecting true effects, and also reduces the likelihood that any results reaching statistical significance are genuine (Button et al., 2013). Although the presented study included a relatively large sample size in total, the active control group was of smaller size relative to the other groups due to practical constraints during data collection. As no significant improvements in memory were found in either of the control groups, we performed a power analysis to control for a possible lack of power (Faul, Erdfelder, Lang, & Buchner, 2007). The effect size measured within the training group was $f = 0.7701$, corresponding to a power of 0.96, with 9 subjects required to detect an effect given this $f$-value. Thus, the lack of training effects in the control groups, consisting of 77 and 31 participants, respectively, was unlikely to be caused by a power issue.

It is however important to note that despite remarkable advancements in neuroscience over the last decades, average sample sizes have not changed considerably over time (Vesterinen et al., 2011). Thus, as researchers seek smaller, more subtle effects than before without an accompanying increase in power, the likelihood of spurious findings increases (Button et al., 2013). Although the power analysis above suggests that this was not a limitation in the present study, future research should strive to achieve sample sizes that provide sufficient power to detect subtle effects.

8.3.1.2 Sex differences

All analyses in the present work included sex as a covariate in order to examine relationships independent of sex. In addition, the sample was relatively gender balanced with 59 % female participants in the young group and 56 % females in the older group. However, the young active control group had significantly larger proportion of female participants (85 %). Although sex differences were not a focus in the presented work, it is worth noting that recent evidence has reported sex differences in performance across cognitive domains in older adults, and greater resilience to age-related cognitive decline in women relative to men (McCarrey, An,
Kitner-Triolo, Ferrucci, & Resnick, 2016). Furthermore, interactions between age and sex have been reported in some cross-sectional white matter studies (Kochunov et al., 2012), and linear relationships between FA and age has been shown to be stronger for men relative to women in selected regions (Sexton et al., 2014). However, other studies have reported a lack of such associations (Hsu et al., 2008; Sullivan et al., 2001). As such, the relationships between sex and WM microstructural brain trajectories are not yet fully understood, and may represent a key area for future research.

8.3.1.3 Age range

The sample included participants in the age range of 20-30 years for young adults and 70-80 years for older adults. Although comparison of young and older adults was the main objective in the present work, adults in their midlife would be a useful additional group for comparison. As DTI studies suggest that white matter development throughout the lifespan follows an inverted U shape pattern with protracted growth extending into midlife (Courchesne et al., 2000; Ikram et al., 2008; Jernigan et al., 2001; Resnick et al., 2003; David H Salat et al., 2009; Westlye et al., 2010), the young adults included in the sample were likely to still be under some white matter development. For instance, one cross-sectional study reported that total white matter volume peaked around the age of 50, while global FA peaked around the age of 30 and was followed by a stable (but small) linear decrease until around the age of 65 (Westlye et al., 2010). Thus, including three age groups for cross-sectional comparison would allow the investigation of training-induced plasticity across larger parts of the lifespan.

8.3.1.4 Representativeness

The project was advertised in local newspapers and on Facebook, where participants signed up on their own initiative. In practice this implies that the individuals signing up are likely to be particularly interested in the research project, fit to attend weekly courses at the University and in some cases active online. Individuals who signed up for the project were on average well educated, had above average IQ and were physically in relatively good shape. The high level of functioning, perhaps
particularly evident in the group of older participants, implies that the sample is unlikely to be representative of the general population. However, between-subject variation was evident in the presented sample, despite the average high function of the participants.

Furthermore, as research usually involves attempts to control for as many variables as possible, the present study operates with rather strict inclusion criteria. One of the intentions was to exclude possible cases of mild cognitive decline or other groups with injuries or diseases, who would represent clinical sub-groups rather than ‘normal’ ageing. However, it can be argued that brain pathology, cognitive decline and/or diseases or injury to the nervous system, as well as worries about memory decline, are all parts of normal ageing. Clearly, these conditions are frequently associated with older age. Older age is also the main risk factor for Alzheimer’s disease, with a considerable increase in risk after the age of 60 (Kawas, Gray, Brookmeyer, Fozard, & Zonderman, 2000). Alzheimer’s disease is characterised by symptoms including accumulation of amyloid protein (Morris et al., 2010), brain atrophy (Pini et al., 2016; N. Raz et al., 2005) and impaired episodic memory (van Geldorp et al., 2015). These symptoms are often also seen in presumably healthy older individuals, however, and there is an on-going debate as to whether dementia is quantitatively distinct from normal ageing, known as a categorical view (Gavett & Stern, 2012), or better conceptualised as one extreme on a continuum of normal ageing (Walters, 2010), a dimensional view.

Hence, one advantage of including more variation in samples such as the present one is the possibility of studying a group of older adults who represent the ageing population more accurately. Recruiting a sample fully representative of the general population for an extensive training study is unfortunately unlikely. However, although the sample in the presented work is more heterogeneous than what reflects the population, considerable between-subject variation was observed. The presented study also takes advantage of the longitudinal nature of the design, which allows valuable assessment of training effects within subjects over extended time periods.


8.3.2 Design

8.3.2.1 Cross-sectional and longitudinal research

While the training intervention study presented in this thesis was longitudinal and included five assessment sessions across 40 weeks, the nature of the age differences in WM microstructure examined in paper I is cross-sectional. Importantly, the reported age differences do not provide evidence of age-related change, as they are based on comparison of WM microstructural characteristics across age groups rather than repeated measures of the same individuals across the lifespan. Cross-sectional and longitudinal studies have been shown to vary in regards to estimates of age-related changes in cognitive function and brain structure in adulthood: cross-sectional studies have been shown to overestimate age-related cognitive changes (Rönnlund, Nyberg, Bäckman, & Nilsson, 2005; Salthouse, 2011) and underestimate structural changes (Naftali Raz et al., 2005) relative to longitudinal studies. One source of the observed discrepancies between cross-sectional and longitudinal studies can presumably be explained by environmental differences across generations such as educational attainment, which makes cross-sectional studies susceptible to cohort effects (Nyberg et al., 2012). Thus, only longitudinal studies, where individuals can be used as their own controls, provide a measure of change rather than drawing inferences from descriptions of group differences.

Although the observed age-differences in WM microstructure relied on cross-sectional measures, evidence from longitudinal studies has shown similar patterns of increased MD and decreased FA with advancing age (Barrick et al., 2010; Bender, Prindle, Brandmaier, & Raz, 2016; Sexton et al., 2014; Storsve et al., 2016). Thus, while rates of age-related changes cannot be inferred from the presented results, the directional pattern of higher MD and lower FA in older adults relative to young adults do correspond to those commonly observed in both longitudinal and cross sectional studies (Barrick et al., 2010; Bender et al., 2016; Bennett et al., 2010; Burgmans et al., 2010; Burzynska et al., 2010; Charlton et al., 2010; Davis et al., 2009; Kennedy & Raz, 2009b; Madden et al., 2009; Marstaller et al., 2015; A. Pfefferbaum et al., 2000; Sexton et al., 2014; Voineskos et al., 2012; Westlye et al., 2010).
Although longitudinal studies are clearly preferred, these designs are not infallible either. For instance, lower cognitive performance among dropouts is commonly observed in longitudinal studies, resulting in a selection bias effect towards higher functioning individuals (Salthouse, 2014). Both young and older individuals who dropped out early in the present study performed lower on IQ relative to the participants retained. To control for possible selection bias, a group of included participants, who matched the participants who dropped out, was compared to the rest of the sample in terms of memory improvement. The results showed that the matched groups did not differ from the rest of the training group in terms of memory improvement. Thus, although the average IQ was lower in the group of participants who dropped out, this does not necessarily imply a selective sample bias towards retaining individuals who would benefit more from the training.

Another issue in longitudinal studies are the practice effects incurred due to repeated cognitive testing (Bartels, Wegrzyn, Wiedl, Ackermann, & Ehrenreich, 2010; Nyberg et al., 2012; Wesnes & Pincock, 2002). Although the control groups in the present study did not improve significantly from baseline to follow up, they did increase their memory performance to some extent. Thus, direct comparisons of improvement in periods of training and no training are necessary in order to establish training effects beyond those caused by repeated testing. One such analysis is reported in paper III, where the memory improvement in the group of participants who started with ten weeks as passive controls was demonstrated to be substantially larger after the subsequent training period.

8.3.2.2 Group allocation

Randomisation of participants in experimental studies is perceived as the golden methodological standard, especially in clinical research (Herbert, 2005; Schulz & Grimes, 2002). Random allocation to groups prevents sample bias and systematic group differences, and enables comparable groups to be generated provided a sufficient sample size (Jadad & Enkin, 2008). In the present study, the participants were assigned to one of three intervention groups at registration. Group assignments based on date do not comply with the suggested criteria for randomisation (Schulz &
Grimes, 2002). However, practical considerations forced a compromise due to the extensive data collection with strict time intervals and assessments locked to specific dates across 40 weeks. Hence, ensuring continuous participation had to take precedence over strict randomisation. However, the data collection was on-going and continuous for the three conditions simultaneously. As such, young and old participants from all three experimental groups were scanned and tested interchangeably during the study, reducing the possibility of group differences with regard to the assessment and scanning conditions.

### 8.3.3 Measuring training gains

One important issue to consider in training studies is how to measure training outcome or training gain. Common measures include difference scores, calculated as the performance at time point two minus baseline performance (Engvig et al., 2010; Lovden, Bodammer, et al., 2010), absolute scores, i.e. performance after training (Draganski et al., 2004) and proportional gain, such as percentage scores (Engvig et al., 2014). Absolute scores and difference scores do not take into account differences in relative improvement across individuals. Thus, difference scores do not account for the influence of baseline variance in analyses. For instance, two individuals, one with a low (5 points) and one with a high baseline score (10 points), may both exhibit the same training gain (2 points). In a difference analysis they are treated equivalently, even though their gains relative to baseline (40% and 20%, respectively) are not equal. Standardised residuals, however, provide a measure of training gain where baseline performance is accounted for. Although difference scores, absolute scores, percentages and residual scores may all be suitable for measuring training gain, knowing the differences they represent is crucial in order to compare findings across studies.

Although beyond the scope of this thesis, another aspect related to training gains is whether the effects of cognitive training can transfer to other domains. Thus, for instance, that memory strategy training may influence performance on tests of processing speed or executive functions. Some training studies have reported effects of working memory training on fluid intelligence tasks in children (Jaeggi, Buschkuehl, Jonides, & Shah, 2011) and attentional control tasks in children with
ADHD (Klingberg et al., 2005; Klingberg, Forssberg, & Westerberg, 2002), and it has thus been suggested that the neural networks engaged during working memory training may be involved in performance on a variety of tasks (Klingberg, 2010). Although evidence for transfer effects in adults has been less consistent (Shipstead, Redick, & Engle, 2012), near transfer effects in terms of improvement on tasks that are somewhat related to the training, have been demonstrated in both young (Erika Dahlin, Neely, Larsson, Bäckman, & Nyberg, 2008) and older adults (S. C. Li et al., 2008; Schmiedek et al., 2010).

Although transfer effects represent the exciting potential of targeted training to improve cognitive function in more general terms, highly variable results are presented across studies (Shipstead et al., 2012). In a literature review by Shipstead et al. (2012), it was concluded that the general evidence of transfer effects was insufficient, and that benefit from training was mainly restricted to tasks that had relevance to the training. The authors also expressed concerns related to the validity of tasks used to measure transfer effects, in addition to a lack of adequate control groups (Shipstead et al., 2012). A meta-analysis by Melby-Lervåg and Hulme (2013) also concluded that working memory training mainly produced short term, specific training effects that did not generalise (Melby-Lervåg & Hulme, 2013). Although transfer effects are beyond scope for the present thesis, the inconclusive results in the current literature should motivate further research on the generalizability of training effects.

Measuring training effects on a test closely related to the training strategy also involves certain challenges. Standardised neuropsychological memory tests such as the California Verbal Learning Test (Dean C Delis, Kramer, Kaplan, & Thompkins, 1987) are susceptible to ceiling effects with repeated administration (Benedict, 2005; Hawkins & Wexler, 1999). Achievement of the highest possible score on such memory tests is even more likely to occur in cases where the training is targeted to verbal recall. In the presented study, memory performance was measured using a supervised word list test that was experimentally developed with the purpose of measuring training gains. The word list consisted of 100 words, with a five minutes time frame for encoding. No ceiling effects were observed at any point during the study. It is however important to note that improvement on this specific memory task
to not imply a general improvement in episodic memory, as performance on a single task cannot account for any cognitive ability in general (Allaire et al., 2014).

Another important issue related to cognitive testing involves standardisation (Golden, Hammke, & Purisch, 1978). Although substantial care was taken in the present study to keep assessment sessions as identical as possible for all participants, testing older individuals within standardised frames can be challenging. For instance, instructions may have to be repeated to make sure the participants understand the task such that the test protocol can be considered a valid reflection of the individual’s abilities. Furthermore, factors such as tiredness or fatigue, stress and nervousness may lead to underperformance, and because of the strict time intervals between test sessions, there was little flexibility to reschedule test sessions freely. As is practically inevitable in large-scale research projects, several test administrators were involved in participant assessment. Test protocols including detailed instructions were used, and frequent meetings were held with the goal to obtain and maintain equivalent routines and procedures across test administrators and assessment sessions.

8.3.4 Diffusion tensor imaging

8.3.4.1 Subject motion

During an MRI scan, the signals acquired from each position in space are attributed to specific locations in the brain, which are known as voxels. Subject motion over the duration of an image acquisition sequence can cause the tissue in a given voxel to migrate into neighbouring voxels, resulting in degradation in image quality. Such motion artefacts are intrinsic to MRI data, and can render scans useless if subject motion occurs beyond the scale of a single voxel (Poldrack, Mumford, & Nichols, 2011). In order to achieve a sufficiently high image resolution such that fine brain structure can be observed, typical MRI voxel sizes range from 0.5 - 2 mm (Allisy-Roberts & Williams, 2007). Thus, subject motion even at the millimetre level can cause serious artefacts in the data. This problem is magnified in ageing studies, as older individuals are known to move more relative to young adults during scanning sessions (Peh, 2014). Although careful correction of motion artefacts was performed using state of the art methods (J. L. R. Andersson & Sotiropoulos, 2016), scanner
motion must be recognised as a general issue in all imaging studies including the present work.

### 8.3.4.2 Tractography

Because the microstructure of the brain manifests at a much smaller scale than the typical resolution of MRI images, multiple fibres can pass through any given voxel. This leads to uncertainty in the measurement of the specific pathways of the tracts. This uncertainty is not accounted for in deterministic methods such as TBSS. More advanced approaches, such as probabilistic tractography (Behrens, Berg, Jbabdi, Rushworth, & Woolrich, 2007), take this uncertainty and additional measurement errors into consideration. In this method, diffusion ellipsoids are randomly reoriented many times, and new pathways measured each time. This provides a distribution of possible pathways, each of which gets assigned a certain probability based on how often it was found. Thus, pathways that are assigned a high probability are those most likely to be genuine and robust against experimental error. Future approaches could apply more advanced tractography methods to the presented data, in order to reduce measurement errors and provide a comparison of results using more than one method.

Tractography can be also confounded by regions of crossing fibres, as only one major fibre direction is represented in each voxel (O'Donnell & Westin, 2011). Thus, the tensor model does not presume the microstructure to be arranged in any particular way within a voxel (Jbabdi, Behrens, & Smith, 2010). Crossing fibre models include parameters that estimate the number of fibre populations within each voxel, enabling the identification of fibre populations with a distinct orientation. Correlations between diffusion metrics and variables of interest can be ambiguous when the effects include regions containing crossing fibres (Carlo Pierpaoli et al., 2001; Tuch et al., 2005; Wheeler-Kingshott & Cercignani, 2009). Hence, additional measures identifying crossing fibre regions would be useful to improve the interpretation of findings. The presented work did not however include any findings that were particularly indicative of issues relating to crossing fibre regions.

To ensure that observed changes in white matter do not just reflect individual variation in brain structure, voxel wise analyses in DTI rely on the precise matching of anatomical locations across individuals. TBSS is suggested to be relatively robust
to individual anatomical differences, as it assesses diffusion indices only in the estimated centres of white matter tracts after non-linear registration of different individuals into a common space (Berlot, Metzler-Baddeley, Jones, & O'Sullivan, 2014; Smith et al., 2006). However, as ageing is associated with white and grey mater loss, diffusion metrics may be influenced by cerebrospinal fluid based partial volume artefacts (Metzler-Baddeley, O'Sullivan, Bells, Pasternak, & Jones, 2012). Thus, it is possible that the present study could benefit from corrections for partial volume effects to improve the certainty of the white matter measures. However, while partial volume effects may influence DTI measures, it has been reported that the commonly observed age-related differences in these metrics cannot be attributed to partial volume effects (Bhagat & Beaulieu, 2004.; Adolf Pfefferbaum & Sullivan, 2003).

As detailed above, WM microstructures appear at much smaller scales than the resolution of MRI data. Thus, the use of MRI, with its finite image resolutions currently limited to the millimetre level, cannot give direct access to cellular level neuronal processes, which occur at the nanometre to micrometre level. As this limits the degree to which accurate inferences can be made, histological studies and animal studies are useful for comparison of fibre architecture (Jbabdi & Johansen-Berg, 2011). Scanners operating at higher magnetic field strengths, such as 7 Tesla, provide promising improvements in accuracy by allowing images to be acquired with higher resolution (Heidemann et al., 2010; Polders et al., 2011). Thus, as smaller voxel size will enable DTI to come closer to WM microstructure identification, the on-going advancement of high field strength technology is a source of great enthusiasm for the future of imaging studies.
9. Conclusions and further implications

Three main conclusions can be drawn on the basis of the presented results:

1) Individual differences in WM microstructural brain characteristics influence the potential to benefit from cognitive training. Thus, conditions that promote preservation of brain integrity throughout the lifespan should represent an important focus for future research. As a number of early and later life factors influence brain and cognition across the lifespan, future studies should aim towards investigating the complex interplay between genetics and environment in relation to plastic potential.

2) Cognitive plasticity can be found in both young and older adults. However, the present study identified WM microstructural changes in relation to cognitive improvement in the older adults only. While these results provide promising evidence of preserved microstructural plasticity in older age, further research is needed to determine the conditions that may evoke microstructural brain changes in different age groups. The occurrence of WM microstructural plasticity may possibly depend on whether the training places sufficient demands on intrinsic processing capacity.

3) Cognitive training has the potential to moderate the magnitude of age-related decline in WM microstructure, but continuous training appears to be a premise for the persistence of such effects. Thus, the evident message may be that one needs to be continuously and consistently immersed in cognitive engagement in order to mitigate age-related neural decline. Furthermore, continuous training on the same tasks may have less effect on WM microstructural plasticity relative to learning new skills. Further investigations are required to determine the nature of training effects in the longer term, and whether these conclusions also apply to other brain characteristics.

The evidence for a lifelong potential for cognitive and brain plasticity provides a promising outlook. However, it should be noted that effect sizes in intervention studies are generally small (Allaire et al., 2014). As the growing commercial industry of ‘brain training games’ promotes their products to allay the public anxiety about brain decline, training effects are often exaggerated and limitations ignored in the
marketing of such games (see Allaire et. al, 2014 for ‘A Consensus on the Brain Training Industry from the Scientific Community’). Hence, although accumulating evidence demonstrates the potential for experience-dependent plasticity throughout the lifespan, the premises for, and limitations of, plasticity is far from fully understood. The magnitude of the relations uncovered in the presented results suggests that they can only explain a portion of the variance. It is thus likely that a multitude of variables need to be mapped in order to gain a fuller understanding of the factors that promote and restrict plasticity at different ages.

In conclusion, the presented results provide important contributions to the understanding of possible premises for WM microstructural plasticity in young and older adults. Establishing core knowledge about factors that contribute to cognitive and brain health throughout the lifespan has implications at both the individual and community level, by promoting mental health and wellbeing, longer lasting function in older age and the reduction of health care cost.
10. References


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11. Papers I-III
12. Appendix I

Assessed for eligibility
N = 217

Excluded N = 3 (older adults)

Allocation

Group 1 ABAB
Young adults N = 43
Older adults N = 57
Drop outs = 25

Group 2 BABA
Young adults N = 30
Older adults N = 50
Drop outs = 3

Group 3 Active control
Young adults N = 18
Older adults N = 19
Drop outs = 6

First follow up

Young adults N = 31
Older adults N = 44

Second follow up

Young adults N = 29
Older adults N = 40

Third follow up

Young adults N = 25
Older adults N = 25

Fourth follow up

Young adults N = 24
Older adults N = 24

Drop outs = 6

Drop outs = 27

Drop outs = 14

Drop outs = 4