Brain development - Hippocampal subfields, microstructural white matter and relation to working memory

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2015
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1 Acknowledgments

First and foremost, I would like to thank my supervisors and LCBC group leaders, Professor Kristine B. Walhovd and Professor Anders M. Fjell for their initiative to pursue groundbreaking research and making this project possible. Their passion for research is highly contagious and their determination and hard work have been a valuable inspiration throughout this process. Kristine and Anders’ accomplishments are admirable, and I feel lucky to be part of the LCBC group that has grown to be an international team of researchers with great competence, collaboration and loyalty. I would also especially like to thank my co-supervisor, Christian K. Tamnes for his patience and intellectual guidance throughout my candidacy.

The large data collection for this thesis would not be possible without competent and patient assistance from Håkon Grydeland, Unni Sulutvedt, Espen Langnes, Lia Mork, Knut Øverbye and Hilde W. Aasland.

I am indebted to Professor Heidi Johansen-Berg for inviting me to work at The Oxford Centre for Functional MRI of the Brain (FMRIB), University of Oxford, and also to Cassandra Sampaio-Baptista and Jesper Andersson for making the four months at FMRIB very fruitful and inspiring.

The Department of Psychology, University of Oslo and the Norwegian Research Council funded the work presented in this thesis. All the participants and the children’s parents also deserve a particular praise for their contribution to research and collaboration during hours of testing.

Thank you to all my friends and family for always being there for me. Finally, special thanks to my fiancé, Tom Jurin, for being my biggest supporter and the rock in my life. I cannot wait to spend more time with you.
2 List of papers


3 General summary

In this thesis, the developing brain and development of working memory capacity are investigated. The aims of the three studies presented here are to explore age-related developmental trajectories of brain structure and working memory, and regional brain differences in developmental trajectories during childhood and adolescence. First, the different developmental trajectories of hippocampal subfields and total hippocampal volume are described. Then, the development of white matter microstructure change is explored. Change of white matter microstructure is investigated both globally and regionally, and in relation to change in visuospatial and verbal working memory. Childhood is a period of ongoing brain development and improvements in many cognitive functions. Critically, the majority of developmental MRI studies available do not include children from as young as four years of age and several unresolved issues still remain. Among these are the controversy regarding hippocampus being a heterogeneous brain structure and the development of its different subregions. White matter microstructure has been found to change rapidly in infancy, but the specific developmental course of different white matter characteristics remains elusive through the preschool and early school years. And to date, no longitudinal studies have demonstrated relationships between change in white matter microstructure and change in working memory from the age of four. While the field of developmental cognitive neuroscience is starting to get a general understanding of structural brain development, less is known about the brain-cognition relationships. Thus, although the current thesis can only cover aspects of the broad memory function, this approach constitutes a step towards an understanding of the relations between the biological and cognitive accounts of memory development.

In the first study, we explored hippocampal development both globally and regionally within subfields in 244 healthy participants during childhood and adolescence aged 4 to 22 years. Volumetric analyses showed nonlinear developmental pattern of hippocampal subfields where volume increased until 13-15 years, followed by little age-related changes during adolescence. Also, small regional differences were detected. For the second study, a longitudinal diffusion tensor imaging (DTI) study, we measured white matter microstructure change in 159 children aged 4 to 11 years to characterize the global and regional pattern of change in fractional anisotropy (FA), mean (MD), radial (RD) and axial diffusivity (AD). White matter microstructure change was investigated in fifteen major white matter tracts and along medial-
to-lateral, posterior-to-anterior and inferior-to-superior gradients in the brain for all DTI metrics. The main findings from the study showed global white matter changes to be of equivalent magnitude at different ages during childhood, with increase in FA, and decrease in MD and RD over time. For mean AD, little change was observed. For white matter tracts, regional differences were found where eight tracts showed nonlinear development patterns for one or several DTI metrics, with a deceleration in change with age. Spatially, there was a posterior-to-anterior gradient of change with more change in frontal regions for all metrics, indicating that change can be described across gross spatial gradients, in addition to specific tracts, to characterize the regional pattern of white matter microstructure development across pre-adolescence childhood. In the third study we explored change in ten white matter tracts in relation to change in visuospatial and verbal memory in 148 children 4 to 11 years of age. The results showed that improvement in visuospatial working memory capacity was associated with increase in FA in two tracts and decrease in MD in four tracts. These relationships were driven by negative relationship between visuospatial working memory change in both RD and AD change. These findings yield new knowledge about brain development and corresponding working memory improvements in childhood.

Taken together, the three studies contribute to the description and understanding of specific aspects of structural brain development and how this relates to, and likely is important for the development of selected higher-order cognitive functions. Considerations of methodological constrains include, among other, the limitations of using a cross-sectional design for the first study, limitations related to discerning hippocampal subfield boundaries in vivo, and issues concerning MRI-derived motion artifacts. Also, the theoretical dilemma of assigning a causal direction to the relationship between brain variables and memory performance is discussed in this thesis.
4 Introduction

4.1 Why study the developing brain?

Knowledge about the developing brain is of importance for all aspects of child development. In the field of psychology, brain development has been of interest through centuries to better utilize the understanding of child development, neurobiological growth and mental processes (Baldwin, 1911, Piaget, 1996). Studies comparing the normal and atypical developmental trajectories in individuals with and without psychopathology have the potential to aid our understanding of the developmental origins of disturbances in brain maturation during childhood and adolescence (Marsh et al., 2008). The neural foundations for multiple developmental disorders makes the study of normal individual brain differences in development even more important to understand (Walhovd et al., 2014b). The need to understand normal brain development as a foundation for cognition and behavioral adjustment is also becoming increasingly clear. This thesis aims to understand how specific brain structures develop and change during healthy childhood and adolescence, and how such changes relate to cognitive function.

It is reasonable to assume that cognitive development is supported by the ongoing structural maturation of the brain (see e.g Sowell et al., 2004, Østby et al., 2011, Fjell et al., 2012, Tamnes et al., 2013b, Ullman et al., 2014). Function is inherently linked with brain structure, so a detailed knowledge of healthy brain development is crucial for a better understanding of cognitive and behavioral changes that occur as one develops. Brain maturation is a complex process and it overlaps with the timing of normal development of cognitive functions. Although these processes at least to a certain degree mirror each other in time, relatively little is known about the specific relations between them. One of the major challenges of cognitive neuroscience is to understand functional anatomy based on these structural maturations. The relationships between brain and cognitive variance at any given age are moderate, and appear to be of a complex and dynamic nature. Interestingly, there is great individual variability within the normal range of development (Scholz et al., 2009, Qin et al., 2015).
4.1.1 What is the origin of individual differences in normal development?

Within normal development, there is a large range of variance in both structural properties of the brain and cognitive performance (Ullman et al., 2014). The range of individual differences observed in normal development might be caused by the different degree of maturation and experience (Demerens et al., 1996, Ishibashi et al., 2006, Sampaio-Baptista et al., 2013). Within the field of neuroscience, maturation and development are highly intertwined processes. However, in an attempt to distinguish between the terms for the purpose of this thesis, Todd (1937) defined maturity as indicators that ought to be present in every individual of both sexes and are universal. Cameron (2015) highlighted that maturity indicators are not related to the passage of chronological time, but to the progression of the individual from an immature to a mature state and is irreversible. Brain maturation might consist of biological unfolding, physical growth and is influenced by genetics (Morishita and Hensch, 2008, Tau and Peterson, 2009, Chen et al., 2011, Chen et al., 2013). It has been suggested that the maturational process is independent of the environment but that its timing can be influenced by environmental factors (Sultan, 2003). Because genes can be "turned off" and "turned on" the individual's initial genotype may change in function over time, giving rise to further developmental change (for review see Kolb, 2009). Brain development has been defined as the combined work of gens and environment (Berardi et al., 2015). Both pre- and postnatal environmental factors such as diet and disease exposure, as well as social, emotional, and cognitive experiences could affect development (Meck and Williams, 2003, Håberg et al., 2007, Wurtman, 2008, Lupien et al., 2009, Walhovd et al., 2010). Developing brains are also known to be highly plastic, where the direction of development is guided by environmental factors as well as initiated by genetic factors (Huttenlocher, 1984, Hochberg et al., 2011). When an aspect of development is strongly affected by experience, it is said to show a high degree of plasticity. This neural plasticity yields potential for positive and negative impact with huge individual and societal consequences. The complex interaction between maturation and environment might be what causes individual variance in the developing brain and cognitive performance. Striking variance even among high functioning children and adolescents leads one to question how well normal ranges can be defined. This thesis aimed to study the continuity of individual differences in specific brain structures and cognition across the normal range of development.
4.1.2 What is age and how should it be treated in developmental samples?

Brain maturation has been found to happen gradually in time, and the neuroanatomical individual variance increase with age from the first two decades of postnatal life until twenty years of age (Brown et al., 2012). To what extent do children of the same age differ among each other in their degree of biological maturity? By using multimodal MRI, Brown et al. (2012) demonstrated that developmental brain phase can be assessed with great precision, accounting for more than 92% of the variance in age. Age is easily quantified with high reliability and validity and therefore allows easy comparison across studies and investigative techniques. Furthermore, it provides a linear scale throughout the human life cycle, allowing studies to compare the absolute and relative growth at different stages of life (Tamnes et al., 2013a). Recent studies have also pointed to prolonged development of brain and cognitive function throughout adolescence (Brain Development Cooperative Group, 2012, Fjell et al., 2012, Squeglio et al., 2013). Several cross-sectional brain imaging studies have related brain structure to age, and it allows easy comparison across developmental studies (Nagy et al., 2004, Østby et al., 2009, Tamnes et al., 2010b). Also, longitudinal studies model brain development during childhood and adolescence against age, and it remains the most popular measure of biological development (Gogtay et al., 2004, Sowell et al., 2004, Lebel and Beaulieu, 2011). When studying the effect of age in development, this gives us insight about age trajectories and developmental pattern such as deceleration or accelerations of change with increasing age. In this thesis, age trajectories were of interest for all three papers. We have studied cross-sectional age-related differences in global hippocampal volume and hippocampal subfields (paper I), we have longitudinally tested how white matter microstructure changes with age both globally and regionally (paper II), and we have explored how working memory performance change with increasing age (paper III). Common for all three papers, we estimated age trajectories using methods not assuming a linear relationship, allowing us to detect potential decelerations or accelerations of various trajectories with increasing age during childhood and adolescence.

Although developmental change runs parallel with age, age itself cannot cause development, so how to deal with age when studying the developing brain? Using age as a measure against which to judge brain development provides little information on the underlying physiological mechanisms. To test for more specific association between imaging indices of neuroanatomy and measures of cognitive functions in developmental samples, the simplest way is to
statistically control for the effect of age. Interestingly, age does not explain the entire proportion of the variance in brain development (Brown et al., 2012, Ullman et al., 2014). When controlling for the effect of age, significant individual variability might be detected. Therefore, in addition to study age trajectories in hippocampal subfields, white matter microstructure and working memory, we also statistically controlled for the effect of age in all three papers. In paper I, age-related differences in hippocampal subfield volumes were separated into two age-groups to explore differences in hippocampal development between children and adolescence. Both hemisphere differences and sex were then investigated independent of the two age-groups. In paper II, longitudinal changes in global white matter microstructure and potential sex differences were investigated when controlling for age. In paper III, we corrected for age when exploring the relationship between change in white matter microstructure and change in working memory capacity. For the latter, the key reason to correct for age would be to remove possible nonspecific effects of development, and to better estimate the developmental aspect of working memory and white matter microstructure change. Relationships between brain and cognitive measures are typically moderate and may fluctuate with age (Salthouse, 2011).

4.1.3 The relationship between brain development and cognition

Brain development is closely linked to cognitive development and we cannot understand the foundation of cognitive development without knowing the brain. Function is inherently linked with brain structure, so a detailed knowledge of healthy brain development is crucial for better understanding cognitive and behavioral changes that occur as one develops. Understanding the neural foundations for cognitive behavior in normal development is fundamental to understand the mechanisms of both neurodevelopmental disorders and normal adaptation. MRI studies exploring development of structural brain foundations might give us knowledge about the normal cognitive behavioral development. Further, development of neurocognitive systems in the brain might have predictable behavioral correlates (see e.g Sowell et al., 2004, Østby et al., 2011, Fjell et al., 2012, Tamnes et al., 2013b, Ullman et al., 2014). However, characterization of brain-cognition relationships in developmental populations poses severe challenges related to analyses and interpretations. The beautiful graphics neuroimaging produce, and the excitement about what it implies, often mask the immense complexity of the physical, biophysical and engineering procedures generating them (Logothetis, 2008). In addition, there are also constrains related to the validity of tasks used to
index cognitive functions. Therefore, a straightforward relationship between brain and cognition is challenging to establish. An observed brain-cognition association could likely be explained by the common influence of age, but the normal individual variation at any given age is substantial (Ullman et al., 2014). To study the relationship between brain and cognition, and not age-related changes, age is often statistically controlled for (Vestergaard et al., 2010, Tamnes et al., 2012, Østby et al., 2012). Can we expect to find a relationship between brain and cognition that is age-independent? Age-independent brain–cognition correlations are often moderate (Salthouse, 2011), and rest on the principle that there is much variance in brain and cognitive development at any given age. Due to ethical and practical constraints, experimental designs involving direct manipulation of these variables are not viable, and we are therefore left with a correlational approach. Although correlations between cognitive and structural brain variables do not necessarily imply that there is a causal relationship between the two, one could benefit from the advantage of longitudinal over cross-sectional designs (discussed in section 4.3) (Salthouse, 2011). When studying brain development and behavioral correlates, the approach is not sufficient to infer causal relations between brain development and cognitive development, but correlation-based procedures can be informative. In the study of paper III, we investigated the relationship between change in white matter microstructure and change in working memory capacity longitudinally. Based on previous findings in the literature, we expected to observe moderate relationships between brain and cognition. When studying brain and cognition independent of age we might exclude much of the individual variance in brain development (Brown et al., 2012). What causes the remaining individual differences observed is not yet fully understood. Genetic and pre- and postnatal environmental factors interact in brain development, and environmental effects influencing brain and cognitive development may occur even before the child is born (Håberg et al., 2007, Wurtman, 2008, Walhovd et al., 2010). Still, research is needed to fully understand the interactions between brain maturation and environment.

4.1.4 Information processing - Visuospatial and verbal working memory

Why study development of working memory capacity? Our ability as human beings to retain and manipulate information over short periods of time is fundamental to an enormous number of everyday tasks. Cognitive psychology has put considerable effort into making sense of the means by which we can accomplish this feat, and the notion of a working memory has made a substantial contribution in providing a framework for advancing the area (Baddeley, 1986,
Adams and Hitch, 1997, Gathercole et al., 2004). Development of working memory has been found to be important for several abilities that are considered hallmarks of mature, higher level cognitive functions (Baddeley, 1986, Adams and Hitch, 1997, Nelson et al., 2000). Tests of working memory demonstrate practical limits that vary, depending on whether the test circumstances allow processes such as grouping or rehearsal, focusing of attention on just the material relevant to the task, and the use of modality- or material-specific stores to supplement a central store (Cowan, 2001). Working memory capacity develops throughout childhood and early adolescence, and with standardized tests we are able to study central capacity limits and capture individual differences in cognitive development (Gathercole et al., 2004, Klingberg, 2006). Working memory capacity can be measured by increasing the amount of information that can be retained in various types of working memory tasks, such as those included in Wechsler Memory Scale – Third Edition (WMS-III) (Wechsler, 1997). In paper III, Spatial Span Backward and Digit Span Backward were selected to measure visuospatial and verbal working memory, respectively. Functioning in concert, these components provide a flexible mental workspace that can be used to maintain and transform information in the course of demanding cognitive activities, and that acts as a temporary bridge between externally and internally generated mental representations (Alloway et al., 2006). Storage of information is mediated by two domain-specific slave systems: the phonological loop, which provides temporary storage of verbal information, and the visuospatial sketchpad, specialized for the maintenance and manipulation of visual and spatial representations (Baddeley and Hitch, 1974). By studying two different working memory functions we were able to demonstrate some specificity in the neuroanatomical basis of working memory development. To ensure that the tasks required manipulation of the retained information and active rehearsal of the visuospatial/verbal sequence, measuring working memory, the current study focused on backward sequences for both tests, and the number of items in the longest correctly recalled trial was used as each participants’ score. Although several other neuropsychological tests could be assessed and used to study working memory development, one advantage of using Spatial Span and Digit Span tasks is that we could administer the same tests to all participants ranging from 4 to 11 years of age with no ceiling effect for the older children. The span procedure enables the same basic test structure to be used over a wide age range, with comparable sensitivity at different ages. The tasks were easy enough for the youngest children to understand the instructions, and because the tasks’ difficulty level increases we were able to capture individual differences in capacity among also the oldest children.
4.2 What is known about structural brain development?

The human brain undergoes tremendous development from birth (Mukherjee et al., 2001, Hermoye et al., 2006, Dubois et al., 2008, Geng et al., 2012) and through childhood and adolescence (Lebel et al., 2008, Shaw et al., 2008, Schmithorst and Yuan, 2010, Tamnes et al., 2010a, Peters et al., 2012). Childhood is a period of rapid structural and functional brain development, and at age six, the brain volume reaches about 90% of its adult size (Reiss et al., 1996, Lenroot et al., 2007). Today, in vivo neuroimaging research has gained new insight into aspects of brain anatomy and function. MRI scanning is a safe procedure that does not use ionizing radiation. This is a non-invasive and painless technique, allowing us to scan healthy children several times. The amount of developmental studies has increased rapidly over the last ten years. The next section of this thesis presents an overview of the studies available today giving us insight about how the brain develops. However, the majority of the developmental MRI studies available do not include children from the age of four.

4.2.1 Volumetric brain development

Brain development generally involves increase in white matter volumes, and concomitant gray matter volume increases in the first years of life and decreases during childhood and adolescence (Tamnes et al., 2010a, Westlye et al., 2010b, Lebel and Beaulieu, 2011, Gilmore et al., 2012, Aubert-Broche et al., 2013, Amlien et al., 2014). Cortical thickness has been found to decrease continuously through childhood and adolescence, and surface area is known to increase until early adolescence before leveling off (Østby et al., 2009, Amlien et al., 2014). Area and thickness are genetically unrelated (Panizzon et al., 2009), but often confounded in measures of cortical volume, which is the product of the two. Results from MRI studies indicate that subcortical gray matter structures show differential developmental trajectories (Giedd et al., 1996, Sowell et al., 2002, Toga et al., 2006, Østby et al., 2009). Specifically, development of hippocampal volume during childhood and adolescence is of interest in this thesis, and age-related differences in hippocampal volume have been investigated by several MRI studies. It is clear that the hippocampus undergoes growth in childhood (Brown et al., 2012, Uematsu et al., 2012, Hu et al., 2013), but studies have given varying results concerning the second decade of life: the majority have not found age-related differences in adolescents (Østby et al., 2009, Mattai et al., 2011, Sullivan et al., 2011, Uematsu et al., 2012, Hu et al., 2013), while others have found volume decreases (Tamnes et al., 2013a) or increases (Dennison et al., 2013).
Importantly, the hippocampus is anatomically and functionally heterogeneous (Yassa and Stark, 2011). Anatomically, the hippocampus is a unique structure consisting of distinct regions including the cornu ammonis (CA) sectors and the dentate gyrus (DG) (Amaral and Lavenex, 2007). Gogtay et al. (2004) found no changes in total hippocampal volumes but found heterogeneous changes in different subareas of hippocampus. These findings indicate that the hippocampus should not be treated as a single functional entity (Strange et al., 1999, Kesner, 2007). Understanding normal hippocampal development at a subregional level may utilize the understanding of the neurobiological basis of hippocampal involvement in several neuropsychiatric illnesses (Gogtay et al., 2006). The aim of paper I was to study hippocampal development, thereby shedding light on the dynamic development within hippocampus during childhood and adolescence.

4.2.2 White matter microstructure development

In paper II, the focus was white matter microstructure development in pre-adolescence childhood. The preschool years constitute a period of “blossoming” within the brain, during which anatomical and physiological substrates show some of their most dynamic and elaborative developmental changes (Jernigan et al., 2011). To study change in white matter microstructure, we used diffusion tensor imaging (DTI), a neuroimaging technique which provides a more sensitive measure of white matter development than overall volume. DTI measures the degree and direction of water molecule permeability related to white matter microstructure (Beaulieu, 2002). Fractional anisotropy (FA), an intra-voxel metric characterizing degree of diffusion directionality, and mean diffusivity (MD), the average magnitude of water diffusion, are frequently used metrics. In addition, diffusivity across [radial diffusivity (RD): mean of λ2 and λ3] and diffusivity along [axial diffusivity (AD): λ1] the main axis of the diffusion tensor can be measured (Concha, 2014). It is known that white matter microstructure changes rapidly in infancy (Mukherjee et al., 2001, Hermoye et al., 2006, Dubois et al., 2008, Geng et al., 2012), and that changes continue into early adulthood (Tamnes et al., 2010a, Lebel and Beaulieu, 2011, Peters et al., 2012). A few longitudinal studies are now also confirming widespread white matter FA increases, and MD and RD decreases through late childhood and adolescence, while the results for AD are less consistent (Bava et al., 2010, Giorgio et al., 2010, Lebel and Beaulieu, 2011, Brouwer et al., 2012). Importantly, white matter development varies regionally in the brain (Lebel et al., 2012). For instance, major white matter tracts with fronto-temporal connections have been found to
develop more slowly than other white matter tracts (Tamnes et al., 2010a, Colby et al., 2011, Lebel and Beaulieu, 2011). Cingulum, known as one of the major fiber bundles, has been shown to have a particularly prolonged development (Westlye et al., 2010b, Lebel et al., 2012), and corpus callosum has been found to develop sharply after birth, while association fibers develop later (Hermoye et al., 2006, Uda et al., 2015).

While white matter change may be both global as well as tract-specific, a consideration of regional age-related changes regardless of the often long-ranging specific tracts may be of interest. There is evidence to suggest possibly broad regional differences in brain maturation. For instance, for cortical gray matter, a posterior-anterior sequence of maturation has repeatedly been identified (Gogtay et al., 2004, Tzarouchi et al., 2009, Tamnes et al., 2010a), and white matter development varies regionally in the brain (Lebel et al., 2012). White matter maturation, including myelination, starts prenatally and appears to progress in an orderly manner during infancy from posterior-to-anterior, inferior-to-superior, and central-to-peripheral regions (Barkovich et al., 1988, Bendersky et al., 2006, de Graaf-Peters and Hadders-Algra, 2006). Later systematic regional age-related white matter differences have been investigated in vivo using DTI (Tamnes et al., 2010a, Westlye et al., 2010b, Colby et al., 2011, Lebel and Beaulieu, 2011, Lebel et al., 2012). In a cross sectional study, Colby et al. (2011) demonstrated gradients in the developmental timing of white matter maturation, as measured by FA along inferior-to-superior and posterior-to-anterior directions from 5 to 28 years. Westlye et al. (2010a) showed that intra-cortical T1 signal intensity followed a posterior-to-anterior gradient from childhood to adulthood. In adults, age-related changes have been found to increase gradually by posterior-anterior and inferior-superior gradients (Sexton et al., 2014). Prefrontal white matter has shown reduced FA in aging (Salat et al., 2005), and an anterior to posterior gradient of degeneration has been suggested with support from several studies (Pfefferbaum et al., 2000, Head et al., 2004, Bennett et al., 2010).

4.2.3 Brian development and cognitive abilities

If cognitive development is supported by the ongoing structural development of the brain, can we expect to detect a relationship between change in MRI indices of neuroanatomy and change in neuropsychological test scores? Especially childhood represent a time of great cognitive and behavioral change, with the emergence in early form of many essentially human psychological abilities. Coincidentally, the brain undergoes rapid developmental changes. As
discussed above, the brain-cognition relationships are expected to be moderate (Salthouse, 2011). However, there is evidence in the literature that developmental patterns in the brain might be associated with development of higher-level cognitive functions. Generally it is thought that brain regions associated with more basic cognitive functions mature first, followed by areas involved in more complex higher-level functions of cognition and behavior (Johansen-Berg, 2010). In a longitudinal study, Sowell et al. (2004) demonstrated a relationship between cortical development and improvement in verbal intelligence. Trajectory of change in cortical thickness, rather than cortical thickness itself, has also been related to level of intelligence (Shaw et al., 2006). Since a large number of brain areas are involved in even simple cognitive tasks, integrity of nerve fibers is necessary to ensure efficient transfer of information. This makes development of white matter very important for cognitive development. Of much interest, there is evidence that observed regional differences in developmental patterns for white matter tracts might be associated with development of higher level cognitive functions (Nagy et al., 2004, Walsh et al., 2011, Østby et al., 2011, Yeatman et al., 2012, Klarborg et al., 2013, Treit et al., 2013, Gautam et al., 2014, Peters et al., 2014, Ullman et al., 2014).

In paper I we studied developmental trajectories in hippocampal subfield. This was of interest due to its functional importance for the encoding, storage, and retrieval of recollection memory. A growing number of studies suggest that diverse behavioural functions such as memory and emotion processing are selectively associated with distinct hippocampal subregions (Eldridge et al., 2005, Kesner, 2007, Fanselow and Dong, 2010). A first step towards understanding the complex developmental process of memory, we aimed to explore the regional developmental differences within hippocampus. The process of memory consolidation is of great importance to developing minds, given the enormous amount of information that is new to us in the first two decades of life. Yet, little is known about the development of this process, and how it relates to hippocampal development. For instance, better memory has been found to be associated with larger posterior and smaller anterior segments, where overall hippocampal volume does not predict memory (Poppenk and Moscovitch, 2011). However, in this thesis, working memory was studied in relation to white matter microstructure. A relationship between white matter microstructure and working memory has been suggested by cross-sectional studies in adolescence (Nagy et al., 2004, Vestergaard et al., 2011, Walsh et al., 2011, Østby et al., 2011, Peters et al., 2012, Nomura et
al., 2013, Peters et al., 2014). However, in order to establish the impact of development of these brain substrates on the development of working memory, longitudinal investigation is critical. Most studies on the relationships between white matter tract microstructure and working memory in developmental samples have been cross-sectional, but Ullman et al. (2014) demonstrated relationships between FA and development of spatial working memory from the age of six.

In addition to structure–behavior associations during development, there is also evidence for a connection between functional brain activation and white matter microstructure in the developing brain measured by functional magnetic resonance imaging (fMRI). It has been argued that white matter maturation might increase functional brain activation in connected gray matter regions by enhancing the effectiveness of communication between regions (Olesen et al., 2003). In children and adolescence from eight years of age, FA values in fronto-parietal white matter correlated with functional activity in closely located gray matter regions in the superior frontal sulcus and inferior parietal lobe known to be involved in visuospatial working memory. This might suggest that the white matter tracts together with correlated gray matter regions could constitute a developing functional network underlying working memory performance in children. However, since correlation does not imply causality there is the possibility that both FA and functional activity could be explained by another underlying biological factor. Also, this correlation disappeared when age was statistically controlled for.

4.2.4 Neurodevelopment of working memory

The hippocampus is characterized by a plasticity of connections throughout life, which is what may make its contribution to memory consolidation possible (Leuner and Gould, 2010). Volume increase could also be explained by processes such as myelination (Benes et al., 1994) and neurogenesis, found in hippocampal tissue, especially in DG (Toni et al., 2008, Cayre et al., 2009, Leuner and Gould, 2010). These differences point to the importance of continued research within the field. A clarification of the developmental patterns within hippocampus may have profound impacts upon the understanding of how memory development is related to hippocampal subregions. The importance of white matter pathways for efficient working memory performance is thought to reflect the need for speeded and robust long-distance communication between distant brain regions. One of the underlying biological processes in
white matter development is myelination, whereby axons get insulated and able to conduct action potentials at greater speeds (Benes, 1989, Lebel et al., 2008). Animal studies indicate that RD is related to myelination and axonal packing (Beaulieu, 2002, Song et al., 2002), and FA and MD reflect a variety of microstructural features, including the relative alignment of individual axons, their diameter and thickness of the myelin sheath, as well as axonal density (Beaulieu, 2002). Other processes, such as axonal alignment, axonal density and axon circumference (Concha et al., 2010) must be kept in mind as well.

4.3 Longitudinal vs cross-sectional designs in developmental studies

In paper I we used a cross-sectional design including 244 participants. Cross-sectional studies require many more participants because comparative differences are affected by both measurement precision and natural variation in brain sizes – a proportion of which will not likely be relevant. For example, a sample size of at least 146 participants is necessary to have adequate power to detect a 5% difference in whole brain volume between groups in a cross-sectional design, whereas only 4 participants are required to detect changes of similar magnitude in a longitudinal design (Steen et al., 2007). For the current study, we deemed the current sample size to be large enough to detect individual variance in hippocampal subfield development. A limitation of the design is the possibility of cohort effects. This could cause a false developmental effect when characteristics typical of one cohort or time period also cause changes in brain and cognition. When factors such as nutrition, education practices and intellectual stimulation are different in different age cohorts, they may give a false impression of developmental effects. This could influence results and might explain the inconsistent findings in the research literature from cross-sectional studies (Kraemer et al., 2000). For this thesis, one of the main aims was to explore age-related differences in specific brain structures. Because paper I was based on a cross-sectional design, we could not describe individual trajectories with the same sensitivity as with a longitudinal design. Nor could we explore differences in change across hippocampal subfields. On the other hand, developmental effects may be harder to detect in a cross-sectional design, due to the great variability in brain size and brain function between individuals.

The studies in paper II and III were based on longitudinal designs, benefitting from an increase in statistical power relative to cross-sectional designs. The reason for this is the reduction in between-subject variability that occurs as a result of using each subject as his or
her own control (Reuter and Fischl, 2011). Another statistical advantage of longitudinal designs relates to inferences of causality regarding relationships between age, brain structure and cognition. When exploring relationships between brain cognition, correlations are often used. The correlation approach does not imply causality, although a longitudinal design could benefit from being temporally restricted compared to a cross-sectional design. In particular, confidence in the hypothesized brain-cognition linkage would be strengthened if there were evidence that the relations occur across time within the same individuals in longitudinal data, and not just across individuals of different ages at a single point in time, as in cross-sectional data (Salthouse, 2011). That is, in the case of correlations between change variables we can be certain that inter-individual variation in change scores were manifested within the temporal constraints of the study (i.e., they occurred together between measurements). Longitudinal data also benefits of the possibility of estimating individual change in both neuroanatomy and cognitive performance (Sowell et al., 2004). This might increase sensitivity to developmental processes, as well as specific associations between brain development and cognitive improvement. Even though longitudinal studies themselves are subject to limitations, including selective dropout and test-retest effects, the benefits of longitudinal designs far outweigh the potential pitfalls in most cases (Schaie, 2005).

To date, the majority of studies investigating the relationship between white matter microstructure and working memory in pre-adolescence childhood are cross-sectional, making it impossible to quantify the degree to which the different measures change in concert over time. Differences between cross-sectional and longitudinal estimates of memory change, for example, have been shown previously (Rönnlund et al., 2005, Salthouse, 2010, 2011). Specifically, cross-sectional data tend to overestimate memory change over short intervals as compared to longitudinal data and predict an earlier onset of memory decline in aging (Rönnlund et al., 2005). Given that cross-sectional and longitudinal estimates of brain atrophy have also been shown to differ (Raz, 2005), it is likely that cross-sectional studies provide inaccurate estimates of brain-cognition relationships. The study in paper III therefore employed a longitudinal developmental design, directly relating changes in white matter microstructure of specific tracts to changes in well validated tests of visuospatial and verbal working memory (Wechsler, 1997). Tracts of interest (TOIs) were selected from well-documented association tracts and major white matter bundles and based on available empirical findings: longitudinal fasciculus (ILF), inferior fronto-occipital fasciculus (IFOF), superior longitudinal fasciculus (SLF), uncinate fasciculus (UF), forceps minor (FMin) and
forceps major (FMaj) (Nagy et al., 2004, Vestergaard et al., 2011, Walsh et al., 2011, Østby et al., 2011, Peters et al., 2012, Nomura et al., 2013, Peters et al., 2014).
5  **Main research objectives**

Three broad questions were investigated in three separate but somewhat related research papers. The first two research questions were related to all three papers, while the third question was related to the third paper only:

1) *Are there age-related differences in developmental trajectories of brain structures and working memory during childhood and adolescence?*

Age-related differences were explored both cross-sectionally and longitudinally. The aim of Paper I was to explore age-related differences in hippocampal volume across childhood and adolescence. Paper II sought to study longitudinal changes in white matter microstructure during pre-adolescence childhood, and paper III investigated age-related changes in visuospatial and verbal working memory capacity longitudinally during pre-adolescence childhood.

2) *Do specific structural brain regions follow different developmental trajectories?*

In addition to global developmental patterns, regional differences were studied in all three papers: In paper I, hippocampal subfield volumes were explored. In paper II, change in white matter microstructure on a voxel-wise level, within tracts and along medial-to-lateral, posterior-to-anterior and inferior-to-superior gradients in the brain was studied. In paper III, regional differences in white matter tracts were further studied in relation to working memory capacity. In addition, hemisphere differences were explored in all three papers.

3) *To what extent are changes in white matter microstructure in specific tracts related to changes in working memory?*

In answering this question, the Wechsler Memory Scale – Third Edition (WMS-III) Spatial Span Backwards and Digit Span Backwards (Wechsler, 1997) was chosen to measure visuospatial and verbal working memory, respectively. In paper III, the relationship between developmental changes in white matter microstructure in ten tracts and change in visuospatial and verbal working memory capacity were studied longitudinally during pre-adolescence childhood.
6 Methods

6.1 Design

In paper I, a cross-sectional design was employed to describe brain development. Children and adolescents were compared at one time-point, at different ages. We strived towards including an equal number of participants in each age cohort, with an equal number of males and females. The cross-sectional design is valuable in that it gives us an understanding of development within the obtained time frame. However, the design has its limitations because it prevents depiction of individual trajectories and change (discussed in section 4.3). The studies in paper II and paper III are accelerated longitudinal designs where each subject is his or her own control. Here, individual change within each individual at two time points can be estimated. The main advantage of an accelerated longitudinal design is its ability to span the age range of interest in a shorter period of time than would be possible with a single cohort longitudinal design. Especially for paper III, the observed relationship between brain and cognition occur across time within the same individuals and not just across individuals of different ages at a single point in time. Paper III uses correlation analyses to describe the relationship between brain variables and memory function in the brain. This design is based on the assumption that there are individual differences in brain microstructure and in memory scores, and that these individual differences are partially coherent with each other. However, causality is not possible to ascertain in the present design.

6.2 Participants

Recruitment

For the current thesis, participants were recruited by the Norwegian Medical Birth Registry through the national Norwegian Mother and Child Cohort Study (MoBa) (Magnus et al., 2006) to participate in our project run by the Research Group for Lifespan Changes in Brain and Cognition (LCBC) at the Department of Psychology, University of Oslo, Norway. MoBa is a population based study undertaken by the Norwegian Institute of Public Health and have identified 100,000 children during the first half of pregnancy, and questionnaire and biological data from them and their parents are available, yielding a unique source of information. From 1999 to 2006, about 70% of all pregnant women (from at least gestational week 16) in Norway were invited to participate. However, during the recruitment period more
than 55% of those invited to participate did not give their consent. In light of this, there is concern as to what extent the MoBa database is valid for the total pregnant population (Nilsen et al., 2009). For the current thesis, all participants in the cohort study living in the greater Oslo area were invited to participate. Before this project, no brain imaging had been conducted in MoBa.

In paper I cross-sectional data from 244 participants were included in the study, and participants were recruited from two different projects run by the LCBC. Out of the 244 participants, 167 were older children and adolescents between 8 and 22 years of age, who participated in Neurocognitive Development (NCD) (Østby et al., 2009, Tamnes et al., 2010a). For the NCD project, participants were recruited through newspaper advertisement, mass e-mails to University staff at various departments, students, contact with schools within Oslo, and through snowball sampling, i.e. recommendation from other participants. This kind of convenience sampling increases the risk of a sample that is not representative of the general population.

In paper II 159 pre-adolescent children participated in the study, and 148 of these participated in the study of paper III, all recruited from the MoBa study. The reasons for the varying number of participants in the three studies are that data collection was not completed when starting paper I. Longitudinal data collection is time consuming and paper I is therefore based on a cross-sectional design including the first children recruited for the study. Secondly, paper I is based on two samples and because the adolescents originally took part in a different study, the DTI sequence, measuring white matter microstructure in paper II and III, was not directly comparable to the sequence used in the study including pre-adolescence children. Therefore the adolescents were not included in paper II and III. The third reason for the varying number of participants is that eleven participants from paper II were excluded from paper III based on handedness (see Inclusion).

Inclusion

All participants were screened before entry into the study where a parent of each participant completed a structured interview to ascertain participant eligibility at both time points. Included participants were required to be fluent Norwegian speakers and have normal or
corrected-to normal vision and normal hearing. Exclusion criteria were history of injury or disease known to affect central nervous system (CNS) function, including neurological or psychiatric illness, serious head trauma such as having been unconscious, being under psychiatric treatment, use of psychoactive drugs known to affect CNS functioning, low birth weight (< 2500 g), and MRI contraindications. All participants’ scans were examined by a neuroradiologist and required to be deemed free of significant injuries or pathological conditions at both time points. One participant did not meet this inclusion criterion at time point one and was not invited to participate at time point two. One participant was also excluded on this basis from the NCD study.

Participants from the NCD study were all recruited based on the same criteria as participants for the MoBa study, except from participants for the NCD study were recruited to be right handed. Participants recruited for the MoBa study were not excluded based on handedness. In paper I, left handed participants (n = 4, age M = 6.3) were excluded from all analysis where volumes were not averaged across hemispheres. In paper II, left handed participants were not excluded (N = 11, mean age = 6.5, SD = 1.2). The sample for paper II and paper III was identical except the eleven left handed participants being excluded from all analyses in paper III. Left handed participants were excluded in paper III because we hypothesized to find lateralization effects when exploring change in white matter microstructure and change in visuospatial and verbal working memory. Previous literature have shown greater relationships for verbal working memory in the left hemisphere, and visuospatial working memory to be more right hemisphere laterialized (Smith and Jonides, 1997, Henson et al., 2000, Thomason et al., 2008, Tsujii, 2009).

**Characteristics of sample**

Paper I participants: 244 (128 females) were included in this cross-sectional study. The age range was from 4.1 to 21.8 years of age (M = 12.3, SD = 4.8), and subjects were drawn from two different projects run by LCBC at the Department of Psychology, University of Oslo, Norway. The youngest children (N = 77, with age M = 6.7, SD = 1.4, range = 4.1- 9.3, 41 females) were recruited from the MoBa study. Older children and adolescents (N = 167, with age M = 14.8, SD = 3.4, range = 8.2-21.6, 87 females) were included from the NCD study.
Paper II participants: 159 participants (90 females) had longitudinal data and were included in this study. At time point one (tp1) the age range was from 4.2 to 9.3 (Mean = 6.2, SD = 1.1), and at time point two (tp2) the age ranged from 5.8 to 11.0 (Mean = 7.8, SD = 1.1). Mean interval between scans was 1.6 years (SD = 0.1), ranging from 1.3 – 2.2 years. Interval between scans was not significantly correlated with age at tp1 (r = .12, p = .131), but was at tp2 (r = .24, p = .002), and was not different for females and males (t = -1.88, p=.063).

Paper III participants: 148 (82 females) had longitudinal data and were included in this study. At tp1 the age range was from 4.2 to 9.3 (M = 6.2, SD = 1.1), and at tp2 the age ranged from 5.8 to 11.0 (M = 7.8, SD = 1.1). Mean interval between scans was identical to that of paper II. For an overview of the participants characteristics from all three papers, please see Table 1. Briefly, all participants > 6.5 years of age tested within normal range on estimated IQ (Wechsler, 1999) at both time points, and participants ≤ 6.5 years of age tested within normal range on scaled general cognitive measures (Wechsler, 2002) at both time points.
Table 1. Characteristics of the three samples

<table>
<thead>
<tr>
<th></th>
<th>Paper I participants (n = 244)</th>
<th>Paper II participants (n = 159)</th>
<th>Paper III participants (n = 148)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Age</td>
<td>12.3</td>
<td>4.8</td>
<td>6.2</td>
</tr>
<tr>
<td>Age tp2</td>
<td>7.8</td>
<td>1.1</td>
<td>7.8</td>
</tr>
<tr>
<td>Interval years</td>
<td>1.6</td>
<td>0.1</td>
<td>1.6</td>
</tr>
<tr>
<td>Mean scaled score WPPSI</td>
<td>11.8</td>
<td>2.0</td>
<td>11.6</td>
</tr>
<tr>
<td>Mean scaled score WPPSI</td>
<td>12.3</td>
<td>1.3</td>
<td>12.3</td>
</tr>
<tr>
<td>Estimated IQ WASI tp1</td>
<td>109.0</td>
<td>10.6</td>
<td>109.9</td>
</tr>
<tr>
<td>Estimated IQ WASI tp2</td>
<td>108.8</td>
<td>12.1</td>
<td>108.8</td>
</tr>
</tbody>
</table>

Participants ≤ 6.5 years of age completed the vocabulary, similarities, block-design and matrix subtests of the Wechsler Preschool and Primary Scale of Intelligence (WPPSI-III) (Wechsler, 2002). Participants > 6.5 years of age were tested using the vocabulary, similarities, block-design and matrix subtests of the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999). Mean scaled score WPPSI = Mean of available scaled scores form 4 WPPSI subtests.
6.3 Magnetic resonance imaging

All MRI data were collected using a 12-channel head coil on the same 1.5T Siemens Avanto scanner (Siemens Medical Solutions). The pulse sequence used for morphometric analyses (paper I) were two 3D T1-weighted magnetization prepared rapid acquisition gradient echo (MPRAGE) scans with the following parameters: repetition time (TR), 2400 ms; echo time (TE), 3.61 ms; inversion time (TI), 1000 ms; flip angle, 8°; acquisition duration of 7 min 42 s. Each volume consisted of 160 sagittal slices with voxel sizes of 1.25 x 1.25 x 1.20 mm. The total scan time was on average 50 min. For the children recruited for the MoBa study we used a parallel imaging technique (iPAT), using the same scan parameters, acquiring multiple T1-scans within a short scan time (acquisition duration of 4 min 18 s.), enabling us to discard scans with residual movement and average the scans with sufficient quality (see section 6.8). Here, the total scan time was on average 30 min. For both projects, the T1-scans were acquired first in the scanning protocol. The two MPRAGE volumes were averaged to increase signal-to-noise ratio and brain volume estimation reliability in both samples. In our experience, artifacts in smaller children due to movement can be greatly reduced by running several shorter sequences with iPAT. This is important when scanning children down to the age of four, as in the present study.

In addition, seven children (5 males), from 6 to 10 years of age (M = 8.4) were also scanned on a 3T Siemens Skyra scanner, and the same iPAT technique was used for both scanners. This was done to test for the effect of differences in image resolution on hippocampal segmentation results. On the 3T Siemens Skyra scanner a 16-channel head coil was used and the pulse sequence for the morphometric analysis was a 3D T1-weighted MP-RAGE scan with the following parameters: TR, 2300 ms; TE, 2.98 ms; TI, 850 ms; flip angle, 8°; acquisition duration of 5 min 30 s. Each volume consisted of 176 sagittal slices with voxel sizes of 1 x 1 x 1 mm.

For the diffusion weighted imaging (paper II and III), the same scanner (1.5T Siemens Avanto scanner), head coil and sequences were used at both time-points, though with software upgrades from B17 to B19 for most participants at tp2 (n = 136). DTI was performed with the following parameters: repetition time (TR) = 8200 ms; echo time (TE) = 81 ms; voxel size = 2.0 mm isotropic; number of slices = 64; FOV = 128; matrix size = 128 x 128 x 64; b value = 700 s/mm; number of diffusion weighted directions = 32; number of b0 images = 5 (the first
33 participants were scanned with $b_0 = 1$; A GeneRalized Autocalibrating Partially Parallel Acquisition (GRAPPA) factor of 2 was used. Acquisition time was 5 min 30 s.

6.4 Morphometric MRI analysis

In paper I, all brain volumes were estimated using FreeSurfer 5.1 (http://surfer.nmr.mgh.harvard.edu/). First, the whole hippocampal formation was segmented using the standard segmentation procedure (Fischl et al., 2002). The hippocampal segmentation procedure from our analysis was manually inspected for accuracy for each participant before automated segmentation of hippocampal subfields was performed using a technique in FreeSurfer 5.1. This procedure used Bayesian inference and a probabilistic atlas of the hippocampal formation based on manual delineations of subfields in ultra-high T1-weighted MRI scans from a number of different subjects (Van Leemput et al., 2009). Seven hippocampal subfield volumes were calculated: cornu ammonis (CA) 1, CA2/3, CA4/DG, presubiculum, subiculum, fimbria and the hippocampal fissure. The segmentation of the larger subfields (e.g. CA2/3 and subiculum, presubiculum and CA1, respectively) has been shown to correlate well with manual volume estimates (Dice coefficients ranging from 0.74-0.62), while segmentation of the smallest subfields (e.g. fimbria and the hippocampal fissure) is not as accurate (Dice coefficients of 0.51 and 0.53) (discussed in section 8.6) (Van Leemput et al., 2009). All seven subfields generated from FreeSurfer were included in the current study, although fimbria and the hippocampal fissure must be interpreted with great caution due to reliability issues. Additionally, total intracranial volume (ICV) was estimated by use of an atlas-based normalization procedure, where the atlas scaling factor is used as a proxy for ICV, shown to correlate highly with manually derived ICV ($r = .93$) (Buckner et al., 2004).

All analyses were performed at the Neuroimaging Analysis Laboratory, LCBC, University of Oslo.

6.5 Diffusion weighted MRI analysis

DTI was used to obtain information about tissue microstructure by utilizing random diffusion of water molecules in the brain. DTI analyses (paper II and III) were performed at the Neuroimaging Analysis Laboratory, LCBC, University of Oslo and at the Oxford Centre for Functional Magnetic Resonance Imaging of the Brain (FMRIB), University of Oxford.
Analysis of DTI data was carried out using Tract-Based Spatial Statistics (TBSS; Smith et al., 2006), part of FSL (Smith et al., 2004). All DTI images were corrected for eddy-current-induced distortions and head motion by means of an affine registration to the reference (b0) volume (see section 6.8) (Andersson and Sotiropoulos, 2014), and brain-extracted using BET (Smith, 2002). Then, the FA and eigenvalue maps were computed by fitting a tensor model to the diffusion data. All participants’ FA data were then aligned into a common space using the nonlinear registration tool FNIRT in a process where every FA image was aligned to every other one (Andersson et al., 2007a, b), using a b-spline representation of the registration warp field (Rueckert et al., 1999). Next, the mean FA across participants and time points was created based on the FA image that had the smallest amount of average warping when used as a target. The target was affine-aligned into MNI152 standard space and this target-to-MNI152 affine transform was combined with each participant’s nonlinear transform to the target. This single transform was then applied to each subject’s FA image bringing each image into standard space in one transformation. The resulting standard space FA images were then averaged 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 0.25 to reduce the likelihood of partial voluming in the borders between tissue classes, yielding a mask of 152284 white matter voxels. Each participant’s aligned FA data was then projected onto this skeleton by searching perpendicular from the skeleton for maximum FA values. We calculated maps of change between tp2 and tp1 (tp2 - tp1), and the resulting data was fed into voxelwise cross-subject statistics. The FA-derived nonlinear warps were applied to the MD, RD, and AD change maps and values were projected onto the skeleton from the same voxels as in the FA analysis (i.e. the voxel with highest FA perpendicular to each point on the skeleton). MD was defined as the mean of all three eigenvalues ($\lambda_1 + \lambda_2 + \lambda_3 / 3$), RD as the mean of the second and third eigenvalues ($\lambda_2 + \lambda_3 / 2$), and AD as the principal diffusion eigenvalue ($\lambda_1$).

For paper II, two probabilistic white matter tractography atlases (the Johns Hopkins University (JHU) and JHU ICBM DTI White Matter Labels) (Mori et al., 2005) provided with FSL were used to extract diffusivity tract values with a probability threshold of 5%. The relatively liberal threshold was chosen to accommodate inter-subject variation in gross white matter fiber architecture, and for the skeleton voxels to intersect the correct tract appropriately (Smith et al., 2006). DTI indices from the overlap between the FA skeleton and the following
tracts were extracted: left and right anterior thalamic radiation (ATR), left and right cingulum-cingulate gyrus (CCG), left and right cingulum-hippocampus gyrus (CHG), corpus callosum (CC body, CC genu and CC splenium), left and right corticospinal tract (CST), forceps major (FMaj), forceps minor (FMin), fornix, left and right inferior fronto-occipital fasciculus (IFOF), left and right inferior longitudinal fasciculus (ILF), left and right superior longitudinal fasciculus (SLF), left and right superior fronto-occipital fasciculus (SFOF), left and right uncinate fasciculus (UF) (Figure 3). The fit of the atlas white matter tracts were manually checked, and deemed satisfactory with only a minimal/negligible amount of non-tract of interest voxels included.

Further, voxelwise statistics were performed on change maps using “randomise” with 5000 permutations to control the family-wise error rate (Nichols and Holmes, 2002). General linear model (GLM) analyses were run with age, sex, motion at both time points and interval as covariates to investigate change throughout the skeleton for FA, MD, RD and AD, respectively. We extracted the number of significant voxels and equivalent percentages (p < 0.05, after correction for multiple comparisons across space) in the FA skeleton for FA, MD, RD and AD when controlling for age, sex, motion at both time points and interval. Next, we ran the same GLM testing the effect of age on change with sex, motion at both time points and interval as covariates. All covariates were demeaned. Global hemisphere differences were also assessed, testing both left > right hemisphere and left < right hemisphere.

In paper III, tracts expected to be involved in working memory were selected: The probabilistic white matter tractography atlas (the Johns Hopkins University (JHU) (Mori et al., 2005) was used to extract diffusivity tract values with a probability threshold of 5%. DTI indices from the overlap between the FA skeleton and the following tracts were extracted: left and right ILF, left and right IFOF, left and right SLF, left and right UF, FMaj and FMin.

6.6 Cognitive neuropsychological testing

As part of the project, we assessed several cognitive functions, including working memory. Visuospatial and verbal working memory were assessed with the Wechsler Memory Scale - Third Edition (WMS-III) Spatial Span Backward and Digit Span Backward, respectively (Wechsler, 1997). For the Spatial Span Backward participants retain information about the order and position of blocks pointed at by the examiner and points to the same blocks in the
reversed order, while for the Digit Span Backward participants retain information about the order of a sequence of numbers being read out loud and repeat the same digits but in reverse order. To ensure that the tasks required manipulation of the retained information and active rehearsal of the spatial/verbal sequence, measuring working memory, the current study focused on backward sequences for both tests, where the number of items in the longest correctly recalled trial is scored. 148 participants performed Spatial Span Backward at tp1 (M = 3.3, SD = 1.4, range 0 – 7) and 147 participants at tp2 (M = 4.4, SD = 1.0, range 2 – 7), and 145 participants performed Digit Span Backward at tp1 (M = 2.7, SD = 1.1, range 0 – 5) and 146 participants at tp2 (M score = 3.4, SD = 0.9, range 2 – 6).

6.7 Statistical analyses

Paper I

In paper I, a smoothing spline approach implemented in Matlab (Fjell et al., 2010) was used for estimation of age trajectories for hippocampal subfield volumes and total hippocampal volume. Left and right raw hippocampal subfield volumes were summarized, making total volume for each subfield, using PASW Statistics 18.0 (SPSS Inc., Chicago, Ill). Total hippocampal volume was calculated by adding all seven subfields and the remainder of the hippocampus as segmented in FreeSurfer. The remainder is the tail of the hippocampus where the delineation no longer discerns between the different subfields (Van Leemput et al., 2009). To test for nonlinear age-functions, we compared the Aikake’s Information Criterion (Fair et al.) between the linear and smoothing spline models for each subfield. To alleviate the need for arbitrary choosing an appropriate smoothing level, we used an algorithm that optimizes smoothing level based on a version of AIC, i.e. the smoothing level that minimizes AIC for each analysis was chosen. AIC offers a relative measure of amount of information lost when a model is used to describe a set of data, and can be said to describe the tradeoff between bias and variance in the construction of statistical models. AIC rewards goodness of fit, but also includes a penalty that is an increasing function of the number of estimated parameters. Thus, AIC attempts to find the model that best explains the data with a minimum of free parameters, in this case, with greatest possible smoothing level. With no smoothing, the smoothing spline will yield an extremely good apparent fit to the data, but the model would be predictively inaccurate. AIC takes this into account by penalizing for degrees of freedom (Fjell et al., 2010). To ease comparison of AIC between ordinary least squares (OLS) linear models and
smoothing spline models, we used $\Delta_I$, which is the difference between AIC for the model and the lowest AIC - in this case, the difference between the smoothing spline model and the linear OLS model. As a rule of thumb, $\Delta_I \leq 2$ would indicate that the two models are essentially indistinguishable with regard to goodness of fit, $\Delta_I \geq 4$ would indicate considerable differences between the models, and $\Delta_I \geq 10$ would indicate that the model has essentially no support. These criteria were based on (Burnham and Anderson, 2002), justified from likelihood-ration theory, from which can be shown that these offers protection from overfitting that aligns with the conventional alpha level of 0.05 for significance.

Further, we ran partial correlations between age and each subfield volume (CA1, CA2/3, CA4/DG, presubiculum, subiculum, fimbria and hippocampal fissure) as well as raw total volume, controlling for sex. The subfield analyses were Bonferroni-corrected by a factor of 7 (reflecting the seven subfields). These analyses were repeated additionally controlling for ICV and in a separate analysis controlling for total hippocampal volume. The break point of the smoothing spline curves were inspected to identify an age that distinguish early and later hippocampal subfield development. Based on visual inspection of the soothing spline curve for total hippocampal volume, the hippocampal volume increase leveled off around the age of 13 years. The same partial correlation analyses were run for age and each subfield raw total volume for each of the two age groups separated by this point (<13 years vs. ≥13 years), controlling for sex. In order to test effects of hemisphere, sex, age group and their interactions, we conducted general linear model (GLM) analyses with left and right hemisphere (left, right) × age group (<13 years, ≥13 years) × sex (female, male). Here, the left and right hemisphere refers to left total hippocampal volume and right total hippocampal volume, and the seven left and right hippocampal subfield volumes (CA1, CA2/3, CA4/DG, presubiculum, subiculum, fimbria and hippocampal fissure).

**Paper II**

In paper II several spaghetti plots were created to illustrate change within individuals to explore age trajectories. This was made for mean FA, MD, RD and AD and for each specific tract for all DTI metrics. As global fits, such as linear and quadratic models, may be affected by irrelevant factors, such as the sampled age range (Fjell et al., 2010), an assumption-free longitudinal nonparametric general additive mixed model (GAMM) for each measure as a function of age was fitted to accurately describe developmental trajectories across the studied
age range. GAMM obtains a fit line combining longitudinal and cross-sectional information and does not assume a linear relationship. Curve fitting was performed using functions freely available through the statistical environment R, version 3.0.1 (http://www.r-project.org/).

To further explore age-related differences in white matter microstructure, we ran partial correlations between global FA, MD, RD and AD at both time points and age, controlling for motion at each time point and sex, using PASW Statistics 22 (SPSS, Chicago, IL). Additionally, we wanted to test the relationship between mean change for FA, MD, RD and AD (time point 2 – time point 1) and age, partial correlations were run with motion at both time points, sex and interval as covariates. Possible effects of the different predictors on change were also tested by running a GLM to test effects of age, sex, interval, motion at both time points and age x sex interactions on change for FA, MD, RD and AD, respectively.

Annual percentage change (APC) was calculated for global FA, MD, RD, AD, and regional differences were studied by calculating APC for all white matter tracts. To compare differences between global APC and APC in white matter tracts, paired t-tests for all DTI metrics were performed for each tract separately. Paired-samples t-tests were also performed to compare change in all bilateral tracts (left hemisphere-right hemisphere) in FA, MD, RD and AD, and were Bonferroni-corrected by a factor of 9 (reflecting the nine bilateral white matter tracts). To test effects of sex for change in white matter tracts, the GLM was repeated, controlling for age, motion at both time points and interval on change. The significant threshold was Bonferroni-corrected by a factor of 15 (reflecting the fifteen extracted white matter tracts).

Further, to illustrate how developmental change rates varied along medial-to-lateral, posterior-to-anterior or inferior-to-superior gradients, mean change (tp2-tp1) for FA, MD, RD and AD were extracted across all skeleton voxels for each coronal, sagittal and axial slice, excluding the most distal slices with < 500 voxels. The aim was not to test whether white matter microstructure change primarily along major white matter tracts versus along major spatial gradients. We then plotted the z-transformed change values across x, y and z coordinates in MNI space using robust LOESS (rLOESS) fitting in Matlab (Mathworks, Inc.) with span of 30%. We tested whether change was significantly different along gradients by creating a set of ROIs. We tested the medial-to-lateral gradient by averaging the most distal 25 x-coordinates in both left and right hemisphere (lateral), and contrasted this ROI with the
remaining x-coordinates (medial). The posterior ROI and anterior ROI was split at \( y = 90 \) and averaged, and the inferior ROI and superior ROI was split at \( z = 73 \) and averaged. Paired t-tests were run to test for differences in change between ROIs. In addition, mean change for each gradient was calculated and t-tests were run to test for differences between mean change and change along each gradient.

Paper III

In paper III, age trajectories were explored using the same curve fitting as in paper II. Here, spaghetti plots were created to illustrate change within individuals for Spatial Span Backward and Digit Span Backward scores, for FA and MD in all TOIs, and for RD and AD in specific tracts. We also tested whether significant change was observed in all measures of interest.

To investigate how white matter microstructure changes relate to working memory changes, partial correlations were run between change in FA and MD in TOIs, and change in Spatial Span Backward and Digit Span Backward scores, controlling for age, sex, interval and motion at both time points, in PASW Statistics 22 (SPSS, Chicago, IL). The analyses were first performed for FA and MD in TOIs, based on these being the most general DTI metrics. For all analyses including Spatial Span Backward and Digit Span Backward change scores, \( n = 147 \) and \( n = 143 \), respectively. The analyses were corrected for 10 comparisons (reflecting the 10 TOIs) using Bonferroni correction.

Because the Bonferroni procedure assumes independence between the tests, and the DTI metrics are highly correlated, we adjusted the correction threshold to reflect the mean correlation (\( r \)) between tracts within each metric. The partial correlations were repeated with RD and AD for tracts shown to be significant for FA and/or MD and Spatial Span Backward or Digit Span Backward capacity. Further, to illustrate the significant associations between white matter microstructure development and working memory development, change values were z-transformed and FA, MD, RD and AD change were plotted against Spatial Span Backward and Digit Span Backward change, using PASW Statistics 22 (SPSS, Chicago, IL). For each TOI change value, age, sex, interval and motion at both time points were regressed out, and for working memory change values, age, sex and interval were regressed out.

In addition, paired t-tests were run for Spatial Span Backward scores and Digit Span...
Backward scores to test for differences in capacity between tp1 and tp2. Paired t-tests were also run for FA, MD, RD and AD in TOIs to test for differences between tp1 and tp2. In order to investigate to which extent development in the two cognitive measures were related, partial correlations between Spatial Span Backward and Digit Span Backward change scores were run, controlling for sex, age and interval. To test the effects of age and sex on change in working memory capacity, we ran a multivariate GLM with age, sex and interval on Spatial Span Backward and Digit Span Backward change scores separately. Additionally, to test the effects of age and sex on white matter microstructure change, the multivariate GLM was repeated for all DTI change metrics in TOIs with age, sex, interval and motion at both time points. Further, the correlations between change in FA, MD, RD and AD in TOIs and change in Spatial Span Backward and Digit Span Backward scores were tested for left and right hemisphere differences (Lee and Preacher, 2013).

6.8 Head motion

In paper I, each MPRAGE was visually inspected, and rated for movement and artifacts on a scale from 1 to 4 (1: excellent, 2: minor movement/artifacts, 3: some movement/artifacts, 4: major movement/artifacts). Only participants with at least two acquisitions rated excellent or had minor movement were included in the analyses, and if the participants had more than two scans rated excellent, the two best acquisitions were used.

In paper II and III, all DTI sequences were visually inspected, and rated for movement artifacts on the same scale as in paper I. Only participants with DTI scans rated excellent or had minor or some movement at both time points were included in the analyses. Then, all included DTI scans were corrected for eddy current-induced distortions and subject movement as described elsewhere (Andersson et al., 2012, Sotiropoulos et al., 2013). In short, this procedure uses all diffusion weighted volumes to make a prediction (based on a Gaussian Process) what each volume “should look like” and then registers the observed volumes to that prediction using a rigid body model for the movements and assuming a first order eddy current-induce field. In some of these data sets there was signal dropout. This is caused by a rotation (subject movement) coinciding exactly in time with the diffusion encoding and shows itself as multiplicative signal dropout across the entire slice that was affected by the movement. It can also be caused by pulsatile movement leading to a local rotation which will then manifest as a local dropout typically around the brain stem area. The eddy current
correction method described above has been extended to also detecting these dropouts by comparing the observed slice to the predicted and deciding if the difference is large enough to make it an outlier among all such differences (Andersson and Sotiropoulos, 2014). If a slice is determined to constitute an outlier it is removed and the prediction is recalculated without the offending slice and the new prediction is inserted as a replacement for the removed slice. Only scans deemed to have no or minimal movement artifacts were included in the analyses. Based on the eddy outlier report and manual checking, all volumes >10 slices of signal dropout detected by the eddy correction method were deemed bad. For participants (n=85) with 1-6 bad volumes, we excluded the bad volumes and re-corrected for eddy current-induced distortions and subject movement. This was especially done for participants with sudden motion in the scanner. Participants exceeding 6 bad volumes were excluded from the study.

6.9 Quality control

In paper I we took great care to visually inspect every slice of every volume of every subject in the study to ensure that the hippocampal subfield segmentations were accurate. Data from six participants were excluded due to minor segmentation errors identifying by the borders of hippocampal subfields; where either the subfield mistakenly included white matter and/or cerebral cortex, overestimating total hippocampal volume, or the segmentation underestimated total hippocampal volume where cerebral cortex was extended into the hippocampus. In addition, to quantify possible outlier values, Studentized Deleted Residuals (SDR) from hippocampal volume predicted by age were calculated. None of the subjects had SDR values at or exceeding +/-3 (SDR ranged from 2.73 to – 2.52).

Additional analyses for validation purposes were also run. Here, we tested for effects of field strength differences between 1.5T and 3T, and hippocampal subfield segmentation results were correlated across the two imaging resolutions. Seven children (5 males), from 6 to 10 years of age (M = 8.4) were scanned on both scanners. In addition, we tested the correspondence of the hippocampal subfield segmentation across the MPRAGE sequence and the iPAT sequence (see MRI acquisition and processing for scanning parameters) where 24 children (15 males), from 4 to 9 years of age (M = 7.4) were scanned with both sequences.

In paper II and III the DTI data were also manually checked throughout the various steps of
the TBSS analysis. The threshold for the mean FA skeleton was set at 0.25 after manually inspecting various thresholds. The nonlinear alignment at 0.25 was deemed successful where each participant’s major tracts were relatively well aligned to the relevant parts of the skeleton. In addition, all white matter tracts were manually checked and deemed satisfactory at probability threshold of 5%. Possible outlier values were also checked for in paper II and III. In paper II, SDR from mean FA, MD, RD and AD values from both time points predicted by age were calculated. Six participants had SDR values exceeding ±3 (SDR ranged from -4.07 to 4.44) on mean FA, MD, RD or AD values. Partial correlations with age were recalculated after outlier analysis, excluding individuals exceeding SDR ±3 to make sure that these participants did not unduly affect our results. In paper III, SDR for each TOI for FA, MD, RD and AD change shown to be significantly associated with working memory were calculated. Three participants had SDR exceeding ±3 (SDR ranged from -5.56 to 7.41) in some TOIs for FA, MD, RD or AD change. The partial correlations between change in the specific TOIs and visuospatial working memory change were repeated without these three participants to examine potential outlier effects. There were no major alterations in the results when excluding these participants. Additionally, SDR for Spatial Span Backward change scores and Digit Span Backward change scores predicted by age were calculated. No participants had SDR values for Spatial Span Backward or Digit Span Backward change exceeding ±3 (SDR ranged from -2.86 to 2.93). Cognitive tests were scored from 0 to 2 (0: valid, 1: partial valid, 2: not valid) by the person administering the testing. Examples of not valid scores were participants not completing the specific task, participants being shy causing them not to perform well, or participants being tired and not focusing on the task. All participants included in paper III had valid scores for visuospatial and verbal memory at both time points.

6.10 Research ethics

The research projects were approved by the Regional Committee for Medical and Health Research Ethics. Written informed consent was obtained from all participants from 12 years of age and from the parent/guardian for participants <18 years. Oral informed consent was given by participants <12 years of age at both time points. Children must be especially protected in research since they do not have the competence to give informed consent and ensure that their interests are taken proper care of. Parents have both the right and the responsibility to make clinical decisions for their children. In doing so, they are given an important role to make decisions in the best interest of the child.
An ethical challenge in MRI studies stems from the possibility of unexpected findings in normal populations. All participants underwent MRI scans included for neuroradiological evaluation at both time points, and these were examined by a neuroradiologist, where the neuroradiologist made proper referrals if clinical follow-up was needed. Parents were informed of the possibility of unexpected findings of clinical significance, and what would happen in the advent of such, so that they could consider whether they wanted their child to participate given a chance of this.

The MRI scanning is a safe procedure, but the children could risk experiencing discomfort during lengthy test sessions and having to lie still in the scanner for half an hour. The children also ran the risk of becoming claustrophobic. Children must be especially protected in these situations, and extra care was therefore taken to make sure that tests and MRI scanning were stopped if the child exhibited discomfort. In an attempt to discover claustrophobic tendencies and prepare the children for MRI scanning, all children underwent a practice session in a mock scanner to get familiarized with the scan procedure, small space and the sounds of the MRI-scanner. They were also shown an illustration video recorded at Oslo University Hospital with a child going through each step of the MRI session. This was also done at time point two for the children that expressed concern related to the MRI session. For the individual participant, benefits of participating in the study included toys, books or gift certificates and money that they received for spending time and effort in the study, as well as some insight into brain research.
7 Summary of papers

7.1 Paper I

Objectives: The hippocampus supports several important cognitive functions known to undergo substantial development during childhood and adolescence, e.g. encoding and consolidation of vivid personal memories. However, diverging developmental effects on hippocampal volume have been observed across studies. It is possible that the inconsistent findings may attribute to varying developmental processes and functions related to different hippocampal subregions. Most studies to date have measured global hippocampal volume.

Methods: We aimed to explore early hippocampal development both globally and regionally within subfields. Using cross-sectional 1.5T MRI data from 244 healthy participants aged 4-22 years, we performed automated hippocampal segmentation of seven subfield volumes; cornu ammonis (CA) 1, CA2/3, CA4/dentate gyrus (DG), presubiculum, subiculum, fimbria and hippocampal fissure. For validation purposes, seven subjects were scanned at both 1.5T and 3T, and all subfields except fimbria showed strong correlations across field strengths. Effects of age, left and right hemisphere, sex and their interactions were explored. Nonparametric local smoothing models (smoothing spline) were used to depict age-trajectories.

Results: Results suggested nonlinear age functions for most subfields where volume increases until 13-15 years, followed by little age-related changes during adolescence. Further, the results showed greater right than left hippocampal volumes that seemed to be augmenting in older age. Sex differences were also found for subfields; CA2/3, CA4/DG, presubiculum, subiculum and CA1, mainly driven by participants under 13 years.

Conclusion: These results provide a detailed characterization of hippocampal subfield development from early childhood.
7.2 Paper II

Objectives: The purpose of the present study was to detail the childhood developmental course of different white matter characteristics.

Methods: In a longitudinal diffusion tensor imaging (DTI) study of 159 healthy children between 4 and 11 years scanned twice, we used tract-based spatial statistics as well as delineation of 15 major white matter tracts to characterize the regional pattern of change in fractional anisotropy (FA), mean (MD), radial (RD) and axial diffusivity (AD). We tested whether there were decelerations of change with increasing age globally and tract-wise, and also illustrated change along medial-to-lateral, posterior-to-anterior and inferior-to-superior gradients.

Results: We found a significant linear increase in global FA, and decrease in MD and RD over time. For mean AD, a weak decrease was observed. The developmental changes in specific white matter tracts showed regional differences. Eight white matter tracts showed nonlinear development patterns for one or several DTI metrics, with a deceleration in change with age. Sex did not affect change in any DTI metric. Overall, greater rate of change was found in the left hemisphere. Spatially, there was a posterior-to-anterior gradient of change with greater change in frontal regions for all metrics.

Conclusion: The current study provides a comprehensive characterization of the regional patterns of change in white matter microstructure across pre-adolescence childhood.
7.3 Paper III

Objectives: Working memory capacity is pivotal for a broad specter of cognitive tasks and develops throughout childhood. This must in part rely on development of neural connections and white matter microstructure maturation, but there is scarce knowledge of specific relations between this and different aspects of working memory. Diffusion tensor imaging (DTI) enables us to study development of brain white matter microstructure.

Methods: In a longitudinal DTI study of 148 healthy children between 4 and 11 years scanned twice with an on average 1.6 years interval, we characterized change in fractional anisotropy (FA), mean (MD), radial (RD) and axial diffusivity (AD) in 10 major white matter tracts hypothesized to be of importance for working memory.

Results: The results showed relationships between change in several tracts and change in visuospatial working memory. Specifically, improvement in visuospatial working memory capacity was significantly associated with decreased MD, RD and AD in inferior longitudinal fasciculus (ILF), inferior fronto-occipital fasciculus (IFOF) and uncinate fasciculus (UF) in the right hemisphere, as well as forceps major (FMaj), and increased FA in the right IFOF and FMaj. No significant relationships were found between DTI metrics and verbal working memory capacity.

Conclusion: These findings yield new knowledge about brain development and corresponding working memory improvements in childhood.
8 General discussion

This thesis presents three empirical studies that in a broad sense represent a coherent contribution to ongoing research efforts to understand how specific brain structures develop and change during childhood and adolescence, and how such changes relate to working memory function. The following section will briefly discuss the current findings in a broader context and in relation to important work within the neuroimaging field.

8.1 What does age tell us about developmental trajectories in brain and cognition?

Age has substantial impact on both brain structures and cognitive functions (Gathercole et al., 2004, Brown et al., 2012). In this thesis, age-related differences were of interest for all three papers and were studied in global hippocampal volume and across hippocampal subfields (paper I), in white matter microstructure change (paper II), and in visuospatial and verbal working memory performance change (paper III). In paper I, increase in hippocampal volume followed by deceleration in adolescents was expected based on previous literature but it was not clear at what age the deceleration would start. The results gave support for the hypothesis, where a nonlinear increase in global hippocampal volume was observed. This age-related increase gradually decelerated until age 13-15 years, after which little age-related changes were seen. The same developmental pattern was found for six out of seven hippocampal subfields (discussed in section 8.2). The nonlinear developmental pattern is in accordance with the findings on total hippocampal volume from infancy by Uematsu et al. (2012). However, they found hippocampal volume increase until approximately 9 to 11 years of age. Hippocampal volume increase restricted to the right hemisphere only in females in the age range between 4 and 18 years has also been reported (Giedd et al., 1996), while others have demonstrated a significant volume increase in the hippocampus between 13–14 and 18–21 years only in males (Suzuki et al., 2005).

In paper II a shorter age-range was studied compared to the study in paper I, and due to the relative short age-range, we hypothesize to find rather constant changes in white matter development with a slight deceleration of change with increasing age. For change in global white matte microstructure, measured longitudinally by DTI, linear increase in FA and linear decrease in MD and RD was found. The age-related change in global AD was very small. The results showed that global white matter changes seemed to be of equivalent magnitude at
different ages from 4 to 11 years, indicating that this is an age period of rapid and rather constant white matter change. In the literature, nonlinear relationships between different DTI metrics and age have been found in longitudinal data (Lebel and Beaulieu, 2011), and cross-sectional studies have shown nonlinear grown patterns for several white matter tracts in participants 0 to 11 years of age (Mukherjee et al., 2001) and 5 to 30 years of age (Lebel et al., 2008). The present study focused on pre-adolescence childhood, with very dense sampling from 4 to 11 years. Most likely, the global changes in white matter microstructure in this age period are rather stable. With the relatively short age-range, this did not allow reliable detections of deviations from linearity. For instance, inspections of the curves from Lebel and Beaulieu (2011) show that even though highly nonlinear relationships are found for the wider age-range of 5 to 32 years, the curves for the pre-adolescence period seem mainly linear, in accordance with the present findings. Also, the steep nonlinear increase with age from Mukherjee et al. (2001) was mostly observed before the age of four. Thus, the present study indicates that 4-11 years is a period of rapid development of white matter, with the gradual reduction in rate of change expected later in adolescence not yet being observed in global measures. Interestingly, a number of specific white matter tracts showed slight decelerations of change with increasing age, supporting the hypothesis regarding deceleration of change in white matter microstructure with increasing age. Specifically, this was found for FA in forceps minor, left CHG, left IFOF, left ILF, left SFOF, and left and right UF, MD in left UF, RD in left IFOF, left SFOF and left UF, and AD in left CHG, right CST and right SLF (discussed in section 8.2).

In paper III we investigated the relationship between change in white matter microstructure and change in visuospatial and verbal working memory. The sample was approximately the same as in paper II, and age-related differences in white matter were therefore not further explored in the current paper. However, for working memory scores we found nonlinear developmental trajectories, indicating more improvement in working memory capacity for the youngest participants. In the literature, both visuospatial and verbal working memory have shown broadly similar developmental functions, with performance increasing nonlinearily from four years and leveling off around fourteen years (Fry and Hale, 2000, Gathercole et al., 2004). Although differences in strategy contribute to the improved performance in early childhood (Cowan et al., 1994), further working memory development has been described as a quantitative change in capacity, rather than a change in strategy (Fry and Hale, 2000). Taken together, the results indicate that the basic modular structure of working memory is present
from four years of age, with each component undergoing sizable expansion in functional capacity throughout the early and middle school years to adolescence.

8.2 Regional developmental trajectories in the brain

In addition to age-related changes in gross brain structures and microstructure, the thesis also aimed to investigate regional developmental differences in specific brain regions. When exploring age-related differences in hippocampal subfield volumes (paper I), small regional differences were detected. Age-related differences were found for six out of seven hippocampal subfield volumes. The exception was hippocampal fissure where a linear age-related volume decrease was found. However, there are methodological challenges related to segment the smallest subfields, which could explain the linear decrease observed in hippocampal fissure (see section 8.6). In addition, the greatest age-related differences were found for CA2/3, CA4/DG, subiculum, presubiculum and CA1, respectively. The biggest volumes were found for CA2/3, subiculum and CA4/DG, while the smallest subfields are fimbria and hippocampal fissure. Regional differences were also detected across hemispheres. Results showed greater right than left volumes for total hippocampus, CA1, CA2/3 and CA4/DG that all seemed to be augmented in older age. Since the publication of paper I, several studies have explored development of hippocampal subfield volumes. In a longitudinal study based on participants aged 8 to 21, Tamnes et al. (2014) investigated hippocampal subfield volumes using the same segmentation procedure as used for the current study. Extending the results from the current paper with longitudinal measures, these findings showed slight volume increases among the youngest participants in hippocampal subfield volumes followed by decrease in volume throughout adolescence. This was found for CA2/3, CA4/DG, presubiculum, subiculum, hippocampal fissure and the left CA1. Also in accordance with our findings, Lee et al. (2014) found age-related increases in hippocampal subfield volumes into early adolescence and decrease in volume after age 13. However, this was only found for CA3, DG and CA1, not in subiculum, and only in the right hemisphere. The source of the apparent decrease in volume after age 13 is unclear, but is consistent with curvilinear age/volume relations reported for hippocampal subregions along the anterior/posterior axis (Gogtay et al., 2006). The small age-related differences between subfields could be caused by neurobiological processes such as neurogenesis in DG (van Praag et al., 2005, Toni et al., 2008, Jabès et al., 2010, Kalkan et al., 2013, Yu et al., 2013), and myelination in DG, subiculum and presubiculum (Benes et al., 1994, Ábrahám et al.,
Regionally specific developmental patterns are also indicated by a cross-sectional study by DeMaster et al. (2014), where young adults had a larger hippocampal body bilaterally and smaller right hippocampal head and tail compared to older children. The variability in change rates was high, but for several subfields the volume reductions appeared to be greatest in mid-adolescence. Complex interactions among genetic factors, environmental conditions, as well as changes in these factors, strongly contribute to volume changes in subcomponents of the brain (Lenroot and Giedd, 2006). This might result in individual variations within hippocampal development.

For white matter microstructure change (paper II), regional differences were also observed as hypothesised. In contrast to the linear developmental pattern in global white matter change, eight specific white matter tracts showed nonlinear developmental patterns for one or several diffusion metrics. Additionally, there were differences in change rates between white matter tracts. Specifically, small annual percentage change (APC) was found in CC splenium for all DTI metrics. The finding is supported by previous autopsy studies showing early maturation in this specific white matter tract before the age of four, while association tracts are found to develop later (Brody et al., 1987, Kinney et al., 1988). Hemisphere effects were illustrated by APC for bilateral tracts indicating hemisphere differences with an overall larger change in the left hemisphere for most tracts for all DTI metrics. Also, global hemisphere differences were assessed showing greater positive change in left hemisphere compared to right hemisphere for FA, and greater positive change in the right hemisphere compared to left hemisphere for MD, RD and AD. In addition to finding regional differences in white matter between tracts, the results also suggested that white matter development follows major gradients in the brain. As hypothesized, we found more change in anterior than posterior regions for all metrics. This suggests that development of white matter microstructure may follow major gradients in the brain in addition to individual white matter tracts. The developmental pattern observed across posterior-to-anterior gradients is in agreement with a cross sectional DTI finding (Colby et al., 2011), a developmental study examining white matter development (Tzarouchi et al., 2009).

Westlye et al. (2010a) showed that intra-cortical T1 signal intensity also follows a posterior-to-anterior gradient from childhood throughout life. The larger anterior change may indirectly be related to early and primary neurobiological mechanisms such as synapse elimination and maturational myelination at different stages in development (Huttenlocher, 1990, Huttenlocher and Dabholkar, 1997). White matter development, including myelination has been found to start prenatally, during infancy it develops from posterior-to-anterior, inferior-
to-superior, and central-to-peripheral regions (Barkovich et al., 1988, Bendersky et al., 2006, de Graaf-Peters and Hadders-Algra, 2006), and it continues into adulthood (Paus et al., 1999, Bartzokis et al., 2001, Sowell et al., 2002). Basic neurobiological mechanisms such as myelination and axonal development may have brain correlates that can be detected by DTI, and these principles could partly impact the developmental changes in white matter DTI metrics observed in the present study. In an adult longitudinal DTI study, posterior-anterior gradients were found to increase gradually but the gradients were anatomically specific rather than global, and age-related changes appeared to be principally governed by inferior-to-superior gradients (Sexton et al., 2014). The current results do not suggest that white matter development from 4 to 11 years proceeds in a continuous fashion from inferior to superior regions or medial to lateral regions, but indicate greater anterior changes. Although white matter maturation, including myelination, have been found to start prenatally and appears to progress in an orderly manner during infancy from posterior-to-anterior, inferior-to-superior, and central-to-peripheral regions (Barkovich et al., 1988, Bendersky et al., 2006, de Graaf-Peters and Hadders-Algra, 2006), there is not sufficient evidence to enable direct interpretation of the causes of changes along these gradients specifically. In paper III regional differences in white matter tracts were further studied in relation to working memory capacity. The results showed that improving visuospatial working memory was associations with specific white matter tracts for FA, MD, RD and AD.

8.3 The relationship between white matter microstructure development and working memory

Some developmental studies have investigated the relationships between white matter microstructure and cognitive function longitudinally (see e.g Yeatman et al., 2012, Treit et al., 2013, Gautam et al., 2014, Ullman et al., 2014), and to some extent, there is evidence that observed regional difference in developmental patterns for white matter tracts might be associated with development of higher level cognitive functions. Relationships between brain and cognitive variance at any given age are moderate, and appear to be of a complex and dynamic nature. The results in paper III give moderate support for the hypothesis that development of white matter microstructure in specific tracts is related to development of working memory. In paper III improvement in visuospatial working memory capacity was associated with increased FA in right IFOF and FMaj, and decreased MD, RD and AD in right ILF, right IFOF, right UF and FMaj, while no relationships with verbal working memory
capacity were found. Cross-sectional studies have found higher FA and lower RD in IFOF to be associated with higher visuospatial working memory functioning from eight years of age to early adulthood (Peters et al., 2014). IFOF mediates a direct communication between occipital and frontal lobes (Forkel et al., 2014), suggesting a role in visuospatial working memory. In adults object working memory has also been associated with FA in IFOF (Walsh et al., 2011). The presently observed longitudinal developmental relationship between IFOF and visuospatial working memory change fits these previous observations. Left UF has been associated with verbal working memory in tumor patients using three-dimensional fibre tracking (Nomura et al., 2013). UF plays an important role in recurrent maintenance of information, and is connected to the inferior frontal lobe (Kier et al., 2004), but has not been studied in relation to working memory in development. The current results did show relationship between right UF change and visuospatial working memory change. Nagy et al. (2004) found positive relationships for FA in the left SLF, left ILF and genu of corpus callosum with visuospatial working memory capacity between the age of 8 and 18, independent of the effect of age. While FMin has been found to overlap with genu of corpus callosum (Wakana et al., 2004), FMaj projections are interconnected with e.g. temporal, parietal and frontal cortical areas (Vergani et al., 2014) which can explain the observed associations between visuospatial working memory development and increase for FA and decrease for MD, RD and AD in FMaj in the current study (McKenna et al., 2015). The few developmental studies available in the literature exploring the relationships between white matter tract microstructure and working memory show inconclusive results with regard to regional specificity. Østby et al. (2011) found no relationship between FA in SLF and verbal memory, while Peters et al. (2012) found positive associations between verbal working memory performance and FA in bilateral SLF. Also for visuospatial working memory, an association with higher FA and lower RD in left SLF has been found from seven years of age (Vestergaard et al., 2011). The present study did not observe significant relationships between change for FA, MD, RD or AD in SLF and verbal working memory capacity.

Most studies on the relationships between white matter tract microstructure and working memory in developmental samples have been cross-sectional, but Ullman et al. (2014) showed in a longitudinal study that FA at baseline could be used to predict visuospatial working memory two years later in children from six years of age. The latter finding was also confirmed by a study based on the same sample, specifying that this was especially found for change in FA along the fronto-parietal and fronto-striatal white matter pathways (Darki and
Klingberg, 2015). In general, cognitive performance has been associated with higher FA of white matter in cross-sectional studies, but to which extent this association is driven by maturational processes or stable characteristics is not known (Klarborg et al., 2013). In the present study, we find that change over time in visuospatial working memory is related to change in microstructural characteristics of relevant major tracts, suggesting that developmental processes may account for some of the improvement in working memory seen during childhood.

However, white matter microstructure is only one of many brain measures that could be of interest when studying development of working memory capacity. Subregions of the hippocampus have also been found to be involved in memory in children, adults and elderly (Eldridge et al., 2005, Kesner, 2007, Yassa et al., 2010, Hanseeuw et al., 2011, Mueller et al., 2011, Shing et al., 2011, Engvig et al., 2012, DeMaster et al., 2014, Lee et al., 2014, Tamnes et al., 2014). Other structural brain metrics such as cortical thickness have been related to cognitive functioning level (Sowell et al., 2004, Kharitonova et al., 2013, Tamnes et al., 2013b), and combining these measures using multimodal imaging may yield a fuller picture of cognitive foundations. Multimodal neuroimaging have successfully been used to map the structural brain characteristics related to self-regulation and cognitive control in development (Fjell et al., 2012). Gray matter regions together with white matter tracts have been suggested to constitute a developing functional network underlying working memory performance in children (Olesen et al., 2003). Functional connectivity changes measured by functional MRI (fMRI) could be a novel method for investigating the development of large-scale functional brain networks in children (Uddin et al., 2010). Integrating resting state fMRI (rsfMRI) analysis with DTI in a multimodal approach will be important to understand how functional connectivity and structural development of specific fiber tracts shape and constrain cognitive development. Resting state patterns are known to develop with age (Fair et al., 2008, Uddin et al., 2010, Mussolin et al., 2013), and stronger default mode network (DMN) coupling has been linked to greater cognitive skills (Østby et al., 2012, Lee et al., 2013). Large longitudinal DTI and rsfMRI studies on normal brain development including preschool children may yield a fuller picture of cognitive foundations (Supekar and Menon, 2012). Given a moderate relationship between structural and functional connectivity (Olesen et al., 2003), these metrics could possibly explain partly overlapping, but also unique variance in cognitive development.
8.4 Why study visuospatial and verbal working memory in development?

In paper III both visuospatial and verbal working memory were explored in relation to white matter development. Improvement in visuospatial working memory capacity was associated with increase in FA and decrease in MD, RD and AD in specific white matter tracts. Interestingly, no significant relationships were found between DTI metrics of white matter tracts and verbal working memory. The findings suggest that improvement in visuospatial working memory capacity across childhood is associated with development of white matter connections between distributed brain regions. Increasing efficiency of those connections may be a contributing factor to the rate of visuospatial working memory, but the role of white matter development and verbal memory cannot be assumed based on the current findings. However, the latter non-findings are of importance due to the publication bias in the literature where positive relations may be over-represented and negative findings may not be published (Salthouse, 2011). Studying both visuospatial and verbal memory was also of interest based on literature highlighting that a memory system is comprised of separable interacting components (Baddeley and Hitch, 1974, Pickering et al., 1998, Good et al., 2001, Jarvis and Gathercole, 2003). By using two different working memory functions we were able to demonstrate some specificity of development, e.g. that development of a white matter region is more associated with development of spatial working memory and less with verbal working memory. Also, when measuring to which extent development in visuospatial and verbal working memory capacity was related, a rather weak correlation between the two was found. This is consistent with the hypothesis that the phonological loop and the visuospatial sketchpad components of working memory might not depend on a single storage and are somewhat independent of each other (Baddeley and Hitch, 1974, Gathercole et al., 2004, Alloway et al., 2006).

8.5 How to treat sex?

Childhood is a period of rapid structural and functional brain development, and as mentioned previously, at age six, the brain volume reaches about 90% of its adult size in both males and females (Reiss et al., 1996, Lenroot et al., 2007). Across these ages, the average brain size for males is 10% larger than for females. This 10% difference is also found in adults and is often explained as being related to the larger body size of males. However, the boys’ bodies are not larger than girls’ until after puberty (Giedd and Rapoport, 2010). To date, the massive increase in sex hormone levels during adolescence that drives pubertal maturation (Cosgrove
et al., 2007) and the protracted sculpting of neural connectivity during adolescence (Sisk and Zehr, 2005) have not yet been shown to drive the development of sex differences observed in brain images (Marsh et al., 2008). Rodent studies indicate that exposure to pubertal hormones during adolescence produces changes in brain structure that have long-lasting effects on social behavior (Schulz and Sisk, 2006). Thus, pubertal hormones likely contribute to the dramatic changes in behavior and brain structure in human adolescents as well (Angold et al., 1998). Sex differences in brain development have been widely discussed and the literature show mixed findings. However, sex differences were not the focus in this thesis but were briefly explored in all three papers. In paper I we hypothesized to find greater hippocampal volumes in males than females in development, in accordance with previously found sex differences (Giedd et al., 1996, Murphy et al., 1996, Giedd et al., 2012, Uematsu et al., 2012), and we found larger total hippocampal volumes in both left and right hemisphere for males than females. Sex differences were also found for most subfields, especially for CA2/3, CA4/DG, presubiculum, subiculum and CA1, whereas no sex differences were found for fimbria and hippocampal fissure. Our findings, in accordance with other studies, suggest that both sex and laterality might influence the developmental trajectories of hippocampus volume (Uematsu et al., 2012, Hu et al., 2013). Not consistent with previous findings, where Giedd et al. (1999) reporting that the right hippocampus correlated with age only in females, and that the left hippocampus did not increase with age between 4 to 18 years in males. For white matter microstructure development (paper II), we found no significant sex differences on change for global FA, MD, RD, AD or in white matter tracts. In the literature, sex differences have been reported by some longitudinal studies (Lebel and Beaulieu, 2011, Wang et al., 2012, Simmonds et al., 2014), but other studies have reported nonsignificant findings (Bava et al., 2010, Giorgio et al., 2010). Future longitudinal imaging studies should therefore include measures of sex hormones in an attempt to understand the influences that sex hormones and puberty have on adolescent brain maturation and the sex-based prevalence differences in developmental.

8.6 Delineation of hippocampal subregions

In the last decade, the in vivo assessment of hippocampal subfields has received increasing attention. Since the publication of the first paper, a new method in the FreeSurfer 6.0 package is now available limiting a number of limitations related to the FreeSurfer 5.1. Three main issues have been identified: First, the image resolution of the in vivo training data was
insufficient for the human labelers to completely distinguish the subregions, forcing them to heavily rely on geometric criteria to trace boundaries, which affected the accuracy of their annotations. A second issue was that the delineation protocol was designed for the hippocampal body and did not translate well to the hippocampal head or tail. A third problem was that large parts of CA1 were included in the subiculum and CA2/3 (Wisse et al., 2014), and this generated volume estimates that did not agree well with those from histological studies (Šimić et al., 1997, Harding et al., 1998). The volumes derived from the new atlas agree much better with these studies (Iglesias et al., 2015). In addition, the smallest hippocampal subfields such as fimbria and the hippocampal fissure must be interpreted with great caution due to reliability issues (Van Leemput et al., 2009). In general, subfield boundaries are difficult to discern in vivo and part of subfields are counted toward neighboring subfields in all segmentation protocols (Wisse et al., 2014). A substantial number of manual segmentation protocols have been published in the last few years. Indeed, different studies partition the hippocampus into different subregions, with different rules used to define each substructure, and different extents of the region within which the substructures are labeled. Up to now, no common set of rules has been adopted by the research community. A first step towards characterizing the differences between the hippocampal subfield and parahippocampal subregion segmentation protocols used in the in vivo imaging community have been done by comparing the segmentation of the medial temporal lobe from twenty-one research groups (Yushkevich et al., 2015a). Here, the CA1/subiculum border was found as the area of greatest disagreement among the protocols. Now, a strategy for developing a harmonized segmentation protocol has been proposed. This approach aims to produce a subfield segmentation protocol that can be applied reliably and consistently across different research laboratories, different MRI scanners, and different clinical and biomedical applications. The involvement of the large sector of the subfield imaging research community in developing the harmonized protocol would help ensure that the resulting protocol will be adopted by this community. Likewise, since this effort includes all of the groups who have developed automated tools for subfield segmentation (Van Leemput et al., 2009, Pipitone et al., 2014, Yushkevich et al., 2015b), the harmonized protocol will be incorporated into these tools, particularly those made available to the larger research community.
Biological mechanisms underlying brain development

The underlying mechanisms of developmental changes in structural MRI measures are still debated (Paus et al., 2008, Paus, 2013). To date, there are no studies that have directly tested the relationships between developmental changes in morphometric MRI measures and changes in cellular or synaptic anatomy. Overall, gray matter volume has been found to decrease during childhood and adolescence (Tamnes et al., 2010a, Westlye et al., 2010b, Lebel and Beaulieu, 2011, Gilmore et al., 2012, Aubert-Broche et al., 2013, Amlien et al., 2014), and the observed reduction is thought to reflect synaptic pruning (Huttenlocher, 1990). It is also possible that gray matter reductions are attributable to increased myelination, pushing the border between segmented gray matter and white matter in favor of white matter volume (Sowell et al., 2004, Shaw et al., 2008). White matter microstructure developmental changes are mainly thought to relate to processes including increased relative axon caliber and myelin content, as well as changes in fiber packing density (Paus, 2010). In general, a number of factors, including axon caliber, myelin content and fiber density, as mentioned above, as well as brain water content, crossing or diverging fibers and partial voluming, influence DTI indices (Beaulieu, 2002, Johansen-Berg and Behrens, 2009). The relative roles of the various factors in development may likely also be age-dependent. Importantly, MRI findings are not selective markers of specific neurobiological properties. In neurobiology, myelinated axons are characterized at a nanometer scale and can be imaged at a resolution of cubic micrometers, whereas the measurement units in typical in vivo human neuroimaging protocols are on the scale of cubic millimeters. For white matter microstructure, the number of axons has been found to vary greatly across white matter tracts and within a white matter tract. Glial cell numbers have been found to vary less than axonal number, and oligodendrocytes are found to be the most abundant glial cells in the white matter (Walhovd et al., 2014a). Moreover, it is questionable whether any changes in their number or process alignment will have any significant influence on signals measured from a voxel. As noted by Concha (2014), the interpretation of diffusion parameters of white matter rests on knowledge of what is known to drive diffusion anisotropy, namely axonal membranes, density and coherence, as well as myelin sheaths. Such knowledge is starting to accumulate from animal models (Johansen-Berg and Behrens, 2009, Concha et al., 2010), but animal models may not necessarily provide accurate representation of the human condition (Concha, 2014). Precise interpretations of the underlying tissue alterations of DTI changes are thus challenging and should be done with great caution. However, investigating multiple DTI indices, including RD and AD, yields
additional information to better characterize tissue microstructure, and future multimodal imaging studies and studies combining imaging and histology can hopefully be informative in untangling the factors influencing DTI indices and their changes during development.

8.8 Limitations and implication for future research

In research, samples recruited often perform above average on tests of cognitive functioning, and may not be representative of the general population. This was also the case for the current sample. To what extent the participant’s cognitive functioning impact our result, is not known. When interpreting neuropsychological test scores, the question of validity is also of importance. Testing taking place in the afternoon could affect the children’s attention span capacity and the effort they put into the task. Especially DTI measures are highly sensitive to motion artifacts, and only participants deemed to have no or minimal movement artifacts at both time points were included in the analyses. We do not know to what extent excluding participants not able to complete the scan or not being able to lie still in the scanner may have impact on our results.

The study in paper I was based on a cross-sectional design, which prevents depiction of individual trajectories and differences in change and direct estimation of relationships between changes across different variables. Further investigations are needed to confirm the results from paper I in a longitudinal design, as longitudinal studies have the advantage of being more sensitive to individual differences in hippocampal developmental trajectories. Next, change in hippocampal subfields should be investigated in relation with change in working memory in our sample. It would also be of interest to conduct hippocampal segmentation using new automated segmentation techniques available. Longitudinal designs (paper II and III) also have pitfalls. With regard to data acquisition and analysis, the study benefitted from the same scanner and sequence being used at both time points. Although drift in scanner performance over time is possible, and measures could be affected by MRI software upgrades, it is unlikely that such factors could explain the pattern of our findings. In paper III, white matter tracts were chosen based on previous literature shown to be involved in working memory. Also, changes in white matter microstructure in relation to changes in working memory could have been investigated by conducting voxel-based analysis within the skeleton. For the current study, white matter tracts were especially of interest due to the extensive cross-sectional literature available studying adolescents and adults, giving us the
opportunity to study these relations in several tracts longitudinally during pre-adolescent childhood.

This thesis aimed to contribute to the understanding of how specific brain structures develop and change during childhood and adolescence. However, the thesis could only cover a small part of the large field within developmental neuroscience. For instance, to better utilize the understanding of white matter changes in the brain, and their relation to optimal and non-optimal cognitive function, we should rely on integration of cellular and multimodal imaging findings, animal and human data (Walhovd et al., 2014a). This is also increasingly important as imaging markers of white matter are more and more often studied and utilized for understanding the relation of human brain and cognitive changes and identifying negative and positive effects of various influences on development. Longitudinal multimodal approaches may also yield a fuller picture of brain development and cognitive foundations. Change itself must however be sampled in vivo and preferably over prolonged time periods. Then, testing of similarities and differences between patterns of change in healthy development could be undertaken. Studying normal individual brain differences may be important to understand both normal and clinical cognitive behavioral outcomes throughout life.

Further, neuroanatomical variance among individuals tends to increase with age, and factors influencing and detectable in early development are likely to have lifespan consequences (Woodward et al., 2012). This highlights the importance of studies exploring the mechanisms of normal brain development also in the future. Also, to understand the potential and limitations of the developing human brain, we need more knowledge about how genetic and pre- and postnatal environmental factors interact in brain development. Genetic and experiential factors interact at all times throughout life to shape brain and cognition, and studying development as part of a lifespan perspective will yield great new knowledge to the field. Ideally, we would have large longitudinal studies, standardized MRI measures across sites, using multimodal approaches and cover the entire lifespan.
9 Conclusions

Paper I

Paper I demonstrated nonlinear age functions for most hippocampal subfields where volume increased until 13-15 years, followed by little age-related changes during adolescence. Hippocampus undergoes rapid estimated growth in early childhood, before leveling off in adolescence. Regional differences were also found for hippocampal subfield volumes and hemisphere.

Paper II

Paper II points to longitudinal changes in white matter microstructure during pre-adolescence. Interestingly, for the most part, the global changes observed seem to be of equivalent magnitude at different ages from 4 to 11 years, indicating that this is an age period of rapid and rather constant white matter change. However, regional differences were observed in specific white matter tracts showing nonlinear trajectories with a deceleration of change with age. Differences in change rates between white matter tracts were also found. Spatially, there was greater change in the anterior compared to the posterior region, and in the inferior compared to the superior region. The study hence showed both age-invariant global patterns and considerable regional differences in white matter change in the age range 4-11 years.

Paper III

Paper III found relationships between development of white matter microstructure in specific tracts and development of working memory. Relations found for visuospatial, but not verbal working memory, suggest that improvement in visuospatial working memory capacity across childhood is associated with development of white matter connections between distributed brain regions, and that the increasing efficiency of those connections may be a contributing factor to the rate of cognitive development.
References


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Papers I - III