Chapter 7

Summary & Discussion

OVERVIEW

Adolescence is a period of major changes in hormone levels, cognition, physical development, and brain development. The aim of this thesis was to study the development of the adolescent brain and how hormone levels and intelligence are related to that development. This was done with data from the BrainSCALE cohort, a longitudinal study with up to 3 assessments in twins and their older siblings from late childhood to late adolescence. The twin design allows for the disentanglement of genetic and environmental influences on the traits of interest. In this chapter, a summary per chapter is provided, followed by a discussion on the main findings. In short, the outcomes of the studies in this thesis can be summarized as follows:

- Reproductive hormone levels increase 2- to 9-fold from late childhood to early adolescence, and these levels are related to secondary sexual characteristics and structural brain development.
    - These traits and their correlations are largely explained by genetic factors, but environmental influences also play a role; the correlations between hormone levels and brain structure are driven by environmental factors.
- From late childhood to early adolescence, changes in the communication capacity of the structural brain network go hand in hand with changes in intelligence.
- During adolescence, the correlation between communication capacity of the structural brain network and intelligence increases.
    - This correlation is influenced by genetic factors; genes explain up to 87% of the observed correlation in late adolescence.
- Adolescent development of the communication capacity of the structural brain network follows a cubic pattern and is related to intelligence.
    - Communication capacity is influenced by genetic factors; heritability up to 66%.

SUMMARY

In this dissertation, I have studied changes in hormone levels, cognition and brain structure in a longitudinal design with 3 assessments in twins and their non-twin siblings to allow for the disentanglement of the influences of genes and environment on these developmental changes. The procedure of data collection for this project and in particular for the third measurement is described in **Chapter 2**. In Chapters 3 and 4, I analyzed data collected at ages 9 and 12 of the twins. In Chapter 5, twins and their siblings were included, resulting in a mean age of 10 and 13. In Chapter 6, all three assessments were included (mean age 10, 13, and 18 years for the twins and their siblings).

Between ages 9 and 12 years, luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol and testosterone levels in boys and girls showed a 2- to 9-fold increase (**Chapter 3**). Hormone levels at age 9 in boys and girls were related to hormone levels and secondary sexual characteristics at age 12. Hormone levels and secondary sexual characteristics were both under influence of genes, as was the correlation between hormone levels at

age 9 and secondary sexual characteristics at age 12. An exception was seen in girls where a common environmental factor influenced the relation between estradiol levels at age 9 and stage of breast development at age 12. When comparing the heritability of hormone levels between the sexes, heritability estimates were higher in boys than in girls. Another difference between boys and girls was that LH was predictive of secondary sexual characteristics in girls whereas FSH was more predictive in boys. Both findings suggest that hormone levels act differently in boys and girls during early puberty.

Next, the relation between hormonal development and brain development was explored. **Chapter 4** demonstrated that in girls, changes in FSH levels between age 9 and 12 were positively related to change in gray matter density, and at age 12, estradiol was negatively related to gray matter density. No significant associations between hormone levels and gray matter density were found in boys. The relations between hormone levels and gray matter density or change in gray matter density were driven by environmental factors. These findings further illustrate the different developmental windows in boy and girls, as both physical development (Mul et al., 2001) and brain maturation (Raznahan et al., 2010) are delayed in boys when compared to girls. Although physical and brain development appear go hand in hand, it remains an open question whether FSH and estradiol cause the changes in gray matter density directly or whether the relation is indirect via an underlying third source that triggers both pubertal development in hormone levels and brain development.

Chapters 5 & 6 focused on the development of the white matter network and reported the influence of intelligence on network development in typical adolescents. **Chapter 5** showed that changes in network efficiency (processing or communication capacity of the brain) were related to changes in IQ (intelligence quotient). Between ages 10 and 13, FA-weighted network efficiency increased on a brain wide level, with the largest increases in the posterior part of the brain. In addition to (developmental) changes in white matter network efficiency, teenagers also showed changes in IQ: one in six participants had a decrease or increase of more than one standard deviation (i.e. more than 15 IQ points) over a period of 3 years (between age 10 and 13 years). Interestingly, those participants with most pronounced increases in IQ also showed higher increases in global and local FA-efficiency. This positive correlation between change in IQ and change in local efficiency was present in frontal and temporal regions. Participants who lost IQ points showed a small decrease or remained stable in network efficiency over the 3-year interval; participants who gained IQ points showed an increase of local efficiency in these regions.

The development of streamline count-weighted network efficiency was also investigated. Streamline count refers to the number of lines that can be constructed between two regions; sometimes also referred to as fiber count. During the 3-year interval, both increases (frontal and occipital areas) and decreases (subcortical, temporal, and parietal regions) in local efficiency took place; resulting in a net-decrease of streamline count-weighted global efficiency. Changes in local streamline count-weighted efficiency were negatively related to changes in IQ. Clearly, the adolescent streamline count-weighted network reflects a different aspect of the white matter network than adolescent FA-weighted network.

Both FA- and streamline count-weighted local and global efficiency were for a large part influenced by genes, and a stable genetic factor influenced global and local efficiency of the network at both measurements. The correlation between IQ and local FA-efficiency was for a

large part driven by common genes. Factors influencing the changes in global or local FA network efficiency or changes in IQ could not be disentangled. No significant correlations between streamline-weighted network efficiency and IQ were found. In several regions, changes in local streamline count-weighted network efficiency were driven by genetic factors.

Chapter 6 illustrated that during puberty, development of FA-weighted network efficiency could be characterized as a cubic function with age, with an increase in local and global efficiency from age 10 to 13, followed by a decrease until around age 20, after which efficiency increased again. Moreover, the development of the teenage network was related to IQ. On a group level, network efficiency decreased between ages 13-18. However, participants with a high IQ showed stable network efficiency in this age span. This was seen on a global as well as a local level. The correlation between network efficiency and IQ seems to be a process of adolescent development: whereas there was no correlation between local or global efficiency and IQ at age 10 and only a small correlation in a few regions at age 13, there was a brain wide, FDR-corrected significant correlation between local efficiency and IQ at age 18.

Local and global FA-network efficiency at age 18 were partly influenced by genes. The correlation between IQ and local FA-efficiency was to a large extent driven by genes that influence both FA-efficiency and IQ. This genetic association was also present at age 13, but interestingly, at age 13, a unique environmental factor had a negative influence on the correlation between network efficiency and IQ, resulting in small phenotypic correlations. This environmental factor was not present at 10 or 18 years.

DISCUSSION

Cognitive development: changes in IQ?

The considerable change in IQ observed between ages 10 and 13 in some teenagers may seem surprising. After all, the assessment of IQ takes age into account and IQ is assumed to be stable throughout life (Deary et al., 2000). Indeed, the majority of the teenagers in our cohort had a stable IQ over the 3 measurements, but 12% showed a decrease or increase of more than one standard deviation (15 IQ points) after three years, and 13% showed change of more than one standard deviation after 8 years. It has to be noted that a decrease in IQ does not necessarily mean that a participant performed worse on the task itself. In fact, often they performed the task better than 3 years before, however, relative to their peers task performance was not as good as 3 years ago. In addition, at all three assessments, a different set of IQ subtests was used (full scale WISC at assessment 1, six WISC subtasks at assessment 2, four WAIS subtasks at assessments 3), which could also account for individual longitudinal variance.

Several studies report changes in IQ during adolescence (Burgaleta et al., 2014; Ramsden et al., 2011; Waber et al., 2012). Because these studies also found a relation between changes in IQ and changes in the brain (Burgaleta et al., 2014; Ramsden et al., 2011), it seems unlikely that these findings are simply due to measurement errors. As discussed by Waber et al. (2012) and already considered in mid-twentieth century by Bayley (1949), it is likely that the underlying cause of these changes lies in individual differences of developmental pace. On a group level IQ was stable over the three measurements. The 95% confidence interval

of the change in IQ was -2.97 to -0.46 between age 10 and 13, and 0.71-3.55 between age 13 and 18. The correlations between the IQ measurements were 0.72 (between age 10 and 13) and 0.77 (between age 13 and 18), which is comparable to test-retest correlations over intervals of similar length in other cohorts (Truscott et al., 1994; Watkins and Smith, 2013).

Changes in IQ in the typical teenage population are not only an interesting finding in light of normal teenage cognitive development and teenage cognitive development in relation with brain development, but also in the light of the onset of psychiatric disorders. Several disorders have their onset during puberty (Kessler et al., 2007; Paus et al., 2008) and for some psychiatric illnesses, a drop in IQ can serve as a marker for disease onset or liability (Hedman et al., 2013; Khandaker et al., 2003; Mesholam-Gately et al., 2009). Hence, it is important to know that fluctuations in IQ also occur in the typical population.

Brain development

The main aim of this thesis was to get information on the development of the white matter network of the teenage brain. In addition, possible factors like pubertal hormones and cognitive functioning that could influence brain development were examined. Concerning white matter development, a profound increase of FA-weighted efficiency was found in early puberty, followed by a plateau or decrease in efficiency in mid puberty. While literature shows that white matter volume and FA increases until adulthood (Kochunov et al., 2012; Lebel et al., 2012; Lebel and Beaulieu, 2011), a stable or decrease in FA in mid puberty is also in agreement with the literature (Achterberg et al., 2016; Miller et al., 2012; Simmonds et al., 2014). The small decrease in FA-weighted efficiency is probably overseen by studies that explore a broad age range, and may reflect a period within adolescence entailing specific reorganization of the brain. This notion is supported by higher genetic influences on FA in left inferior and middle frontal gyri in teenagers compared to adults, suggesting local and temporary developmental mechanisms that influence FA (Chiang et al., 2011b). In our study (Chapter 6), heritability of FA-weighted network efficiency was lower at age 13 compared to age 10 and 18.

A change in efficiency can be caused by a change in edge weights (in our case FA or streamline count) and/or a change in distribution of weights and/or a change in binary topology. A decrease in local efficiency means that the direct neighbors of that node are less strongly interconnected (see Figure 1.1 on page 8; one has to keep in mind that a direct neighbor is not per se spatially close, but 'one edge apart'). A change in local efficiency thus refers to an increase or decrease in the interconnection of a local brain area with other brain areas via about several white matter bundles; it is a summary statistic. A decrease in efficiency can also co-occur with an increase in FA: it depends on which edges increase and which do not, leading to a change in weight distribution. Future studies are needed to determine which bundles had the strongest influence on the change in network efficiency. Since changes in network characteristics are directly or indirectly due to changes in edge weights, in the following paragraph mechanisms underlying changes in FA are considered. Factors influencing streamline counts are discussed in the next section.

Mechanisms underlying changes in FA

FA of the white matter is considered to reflect myelination, axon diameter, axon alignment,

and axon density (how many axons cross one voxel, thus also reflecting how much water is in between the axons); visualized in Figure 7.1 (Beaulieu, 2002). During puberty, myelin thickness as well as axon diameter are thought to increase (Paus, 2010a). It seems unlikely that at this age new axonal pathways are formed on a scale that can be measured, but it is possible that axons lie closer together. Another possible contributing factor to a decrease in FA is the occurrence of crossing fibers. The majority of the white matter voxels contain crossing fibers (estimates range from 63 to 90%; Jeurissen et al., 2013). When bundles in primary directions do not change while the secondary direction becomes more myelinated, FA of the voxel containing these crossing fibers will decrease. In our analyses, FA of a bundle was determined by mean FA over the entire length of the bundle. Localized changes due to crossing fibers thus influence FA of the whole bundle.

On a synaptic scale, it has been shown that new dendritic spines can evolve within one hour after onset of motor training in rats (Xu et al., 2009). In humans, changes in the white matter can be detected with MRI scans after six or more weeks of learning a new task or skill. Skill learning (reviews by (Taubert et al., 2012; Valkanova et al., 2014; Zatorre et al., 2012)) and physical exercise (Svatkova et al., 2015) are mostly associated with an increase in FA. However, there are also studies in rats (Blumenfeld-Katzir et al., 2011) and humans (Taubert et al., 2010) that report a localized decrease in FA after a training paradigm. The decreases in FA were local and accompanied by increases in FA in other regions. In rats, it was found that changes in FA after 5 days of training were related to an increased immunoreactivity and a number of astrocytic processes (Blumenfeld-Katzir et al., 2011). This indicates that learning can be accompanied by decreases in FA and that neuronal factors besides myelin influence observed changes in FA. On the other hand, the neuronal factors that influenced the change in FA in rats may be related to only a short-term change FA, which leads us to the question what governs long term changes in FA. (Tuch et al., 2005) did not investigate training-in-

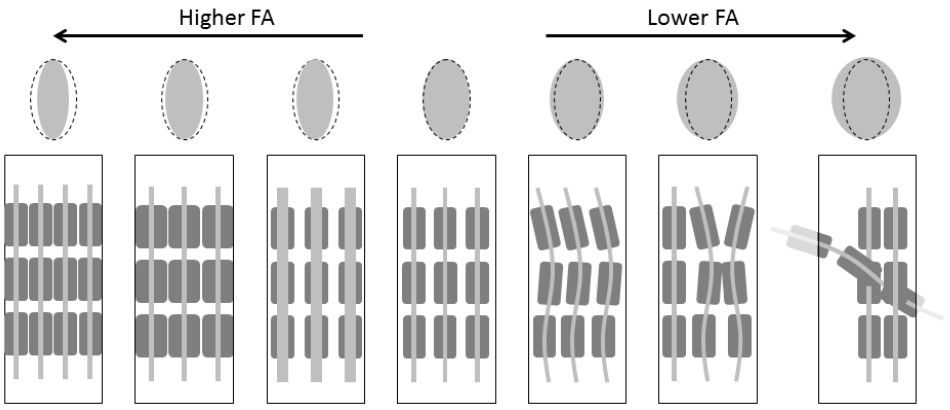

Figure 7.1 – FA is influenced by packing density of fiber tracts, alignment of the fibers, axonal diameter, and myelination. Axons are reflected by light gray lines, myelin by dark gray rectangles surrounding the axons. When taking the middle example as a reference (dashed black oval), FA will be higher (thus a more elongated ellipsoid) with increased packing density, myelination and axonal diameter (left hand side). FA will be lower (more sphere like) with less-smooth alignment and crossing fibers (right side).

duced effects, but whether variance in reaction times could be explained by variance in FA of projection and association pathways supporting visuospatial attention. They report both negative and positive correlations between FA with reaction times. Similarly, Schmithorst and Wilke (2002) found lower FA of the corona radiata and internal capsule in musicians compared to non-musicians, and a higher FA in the genu of the corpus callosum. These relations between lower FA and 'better' skill are likely attributable to local rearrangements and/or crossing fibers.

It can be concluded from the above two paragraphs that the interpretation of changes in FA is not straightforward, and, that higher FA is not always for the better. Likewise, network efficiency is not always positively associated with functioning. For example, although lower efficiency has been related to several diseases (Bassett and Bullmore, 2009), a higher level of local efficiency has been related to autism (Courchesne et al., 2005) and ADHD (Cao et al., 2014; Konrad and Eickhoff, 2010) – two typical developmental disorders.

Developmental changes of structural network efficiency over the lifespan have mainly been investigated using streamline count as edge weight. One study examined the FA-weighted network efficiency in old age (Wen et al., 2011). Although streamline count is based on FA and thus related to FA, it is not the same (see next section). Previous literature reports that both increases and decreases in local streamline-weighted efficiency occur between 12 and 30 years (Dennis et al., 2013), and in adulthood (19-85yr) (Gong et al., 2009). Other studies found an increase in (weighted with inverse apparent diffusion coefficient (ADC); the magnitude of diffusion) global efficiency over the ages 2–18 (Hagmann et al., 2010) and a decrease in local and global streamline-weighted efficiency between the ages 4 and 40 (Lim et al., 2015). Our findings add to these reports that during early puberty (between 10 and 13 years), streamline count weighted efficiency increased in frontal and occipital areas, and decreased in subcortical, temporal, and parietal regions (Chapter 5). These changes were accompanied by a strong increase in white matter integrity and FA-weighted network efficiency. During mid-puberty, FA-weighted network efficiency decreased a little.

Additionally, our findings also provide evidence that developmental pace and shape, and the amount of genetic influences on FA-weighted local efficiency differ across brain regions. Future longitudinal follow-up studies of this cohort are needed to learn how FA-weighted efficiency develops after age 18. The white matter network is suspected to change at least until mid-30s because FA still increases until the third decade of life (Lebel et al., 2012; Yap et al., 2013) and white matter volume has been found to increase till age 45 (Hedman et al., 2012). As suggested, longitudinal studies are more likely to pick up fast changing developmental patterns that occur over a few years than cross-sectional studies spanning a decade or more.

Streamline-weighted networks; how are they different from FA-weighted networks?

Results presented in Chapter 5 indicate that during early adolescence, streamline-count weighted networks show a different aspect of the white matter network than FA-weighted networks. Streamline-count refers the number of lines that can be constructed between two regions. Although it may appear as if the number of streamlines is a proxy for the number of axonal connections, it cannot be interpreted as such: voxel size and number of seed points per voxel play a role in the detected number of streamlines. Because FA is used as a guide for the reconstruction process of streamlines, streamline-count reflects density of fiber tracts,

alignment of the fibers, axonal diameter, and myelination as well. Thus, factors influencing changes in streamline count during puberty can be the same factors as mentioned above that influence FA. However, streamline count is more vulnerable to length, curvature and branching of the bundle (Jones et al., 2013). FA of a bundle is computed as the average over the entire bundle. As such, a patch of low FA will have a small influence on the FA of the bundle. A lower number of streamlines due to e.g. branching of the bundle will be a weakest link or bottleneck of the number of streamlines that go from region A to B, and thus influence the entire bundle.

Two studies employed streamline and FA based analyses on data from adult cohorts. They found both types of analyses to be in agreement for associations with age (Stadlbauer et al., 2012) and IQ (Li et al., 2009), thus contrasting our findings. It is possible, however, that the relation between FA and streamline count – and their associations with IQ – changes with brain maturation. Indeed, a recent longitudinal study showed that during late adolescence both streamline and FA weights locally increase and decrease throughout brain. However, there seemed to be a bias towards FA-increases in hub-to-hub connections whereas streamline-count showed both increases and decreases in these connections (Baker et al., 2015). Moreover, the authors report that streamline-count increased in frontal regions and decreased in subcortical regions, which is in agreement with our finding that streamline-weighted local efficiency increased in frontal regions and decreased in subcortical regions. More studies are needed to see if streamline-count and FA become more similar in terms of associations with IQ when participants grow older.

Associations between IQ and brain development

Our finding of an association between changes in IQ and changes in white matter network efficiency (Chapter 5) suggests a possible causal relation between the white matter network and IQ. After all, the brain is the seat of intelligence; the development of one feature may be related to the other. Of course, the relation will probably not be a simple causal relation. The brain and IQ are likely interconnected through a feedback loop where small changes in the brain (that cannot (yet) be picked up with MRI scans) could lead to enhanced learning, new synaptic connections, a higher IQ, which in turn could lead to specific environment seeking behavior, stronger connections, and so forth.

We also found that the white matter network of the brain and IQ become more strongly correlated during adolescence, and that this association is in part explained by genes common to both brain network efficiency and intelligence (Chapter 6). Several mechanisms could explain the increase of a (genetic) correlation between white matter network efficiency and IQ in mid adolescence. First, it can be hypothesized that gene expression triggered by education may depend on IQ and thus diverge structural brain network development to more efficient networks in smarter children. For example, rats showed upregulated mRNA expression of brain-derived neurotrophic factor (BDNF) and basic fibroblast growth factor (bFGF) in the hippocampus a few days after learning a spatial memory task (Gómez-Pinilla et al., 1998; Kesslak et al., 1998). This shows that training or activating the brain could lead to (a cascade of) activation of genes that regulate long-term plasticity. A second hypothesis is that people with a higher IQ may have different genetic variants that directly of indirectly cause genes related to adolescent brain development to be more efficiently translated. For

instance, heritability of FA in the thalamus, the genu and posterior limbs of the internal capsule, and the superior corona radiate was higher in people with high IQ compared to people with lower IQ (Chiang et al., 2011b). In another paper by these same authors, it was shown that the association between FA and object assembly performance was positive in BDNF-Val carriers but around zero or negative in BDNF-Met carriers (Chiang et al., 2011a). A third hypothesis is that teenagers may follow their 'genetic drive' (based on their IQ) to an environment that will trigger specific developmental changes. For example, genes that are involved in (higher) IQ may also make a person more curious and interested in school-related work. This may allow for a longer sensitive period in individuals with a higher IQ as reflected by a higher common environmental influence on IQ during adolescence in teenagers with a higher IQ compared to teenagers with a lower IQ (Brant et al., 2013).

Our results show that although a genetic correlation between IQ and network efficiency was present at age 13, a negative unique environmental correlation seemed to counteract this genetic correlation between IQ and global and local efficiency, leading to a lower phenotypic correlation. We do not know what specific environment may have caused the negative correlation. In the Netherlands, children change from primary to secondary education around the age of 12, and thus change schools and peers. Possibly, the higher unique environmental variation of global and local efficiency at 13 reflects the effects of this new unique environment on individual variation of 'stage of brain development'. As a result, the genetic programmed stage of development (and thus the correlation with IQ) may be overruled by environmental effects. This environmental influence may also increase the variation in the pace of individual brain development, and thus partly account for the correlation between change in IQ and change in network efficiency between the ages 10 and 13 (Chapter 5) that was not observed between ages 13 and 18.

Environmental factors may also positively influence the relation between brain structure and intelligence as both IQ and brain structure can be influenced by environmental factors. IQ in childhood is highly correlated with IQ in old age suggesting a high level of stability throughout life (Deary et al., 2000). However, despite being overall stable, IQ can change in relation to the environment. Intellectual abilities of children adopted from a deprived (institutional) background often increase in their new, relatively enriched, environment (van IJzendoorn et al., 2005). This illustrates that childhood IQ has been shown to have a higher influence of the common environment than adult IQ (Plomin and Deary, 2015). Similarly, heritability of regional brain structure depends on both age and relative developmental timing of that brain region (Lenroot et al., 2009): regions that are not fully developed show a higher influence of environmental factors, allowing for environmental influences during adolescence. Together with our findings, this shows that both IQ and the brain are a work in progress and that they may influence each other.

Future research is needed to investigate the correlation between IQ and the white matter network – as well as other brain features, e.g. cortical thickness (Brouwer et al., 2014; Schnack et al., 2015) – after age 18 to determine if the correlation reaches a stable adult-like level around age 18, or if it decreases or continues to fluctuate throughout life. A study by Tamnes et al. (2011) found that the correlations between IQ and brain structure (cortical thickness, white matter volume, FA, MD) were stronger in teens (8-20 yr) compared to young adults (21-31yr). In addition, it would be of value to know how development of white

matter is related to development of gray matter in relation to IQ (cf. Bohlken et al., 2016b).

Influence of hormones on typical brain development

Because of our interest in the relation between brain development and hormonal development, I investigated the presence of an association between hormone levels and white matter network characteristics. Associations between testosterone levels and white matter integrity have often been reported in pubertal boys and young adults (Herting et al., 2012; Menzies et al., 2015; Peper et al., 2013; Peper et al., 2015; Perrin et al., 2008). Therefore, we expected to find at least a correlation with testosterone. However, no significant associations were found (unpublished data). Possibly, network analyses are too large scale to find significant correlations: brain wide network characteristics take all bundles in consideration, and local efficiency also comprises several connections. On the contrary, many previous studies report small regions on the white matter skeleton, or study specific regions or bundles. The next paragraph discusses existing literature on testosterone and white matter integrity or volume in pubertal boys and girls.

Although quite a few studies have reported a correlation between pubertal testosterone levels and white matter volume or integrity in boys (Herting et al., 2012; Menzies et al., 2015; Paus et al., 2010; Peper et al., 2015; Perrin et al., 2008), there are some discrepancies between these studies. Some report a positive association between testosterone and white matter integrity (Herting et al., 2012; Paus et al., 2010; Perrin et al., 2008), whereas others report a negative association (Menzies et al., 2015; Peper et al., 2015). In addition, some find a stronger association with MD than FA (Menzies et al., 2015) or the other way around (Herting et al., 2012). Literature is also not conclusive on the relation between testosterone and white matter integrity in girls. Some studies find a correlation with white matter integrity (Peper et al., 2015) whereas other do not (Herting et al., 2012; Peper et al., 2013). These inconsistencies could be due to selection of specific regions or bundles, and whole brain vs. regional analyses. In addition, the measurement of steroids differs between studies; saliva and serum testosterone levels may be highly correlated but interpretation and comparison between the two is complicated (Peper et al., 2011a). A final influencing factor could be minor differences in pubertal status: when participants are in different developmental windows, different associations may be present at that specific developmental period and in that region of the brain.

Chapter 4 reports that in girls, higher estradiol levels were related to a more mature brain (i.e., lower cortical gray matter density). Additionally, girls that increased the most in FSH levels also showed local increases in (sub-) cortical gray matter. These associations were partly driven by a (common or unique) environmental factor whereas no genetic influences on these associations could be found. We think an early pubertal marker, which triggers both these hormonal and brain changes, is the underlying cause of the correlations. The early pubertal marker could in turn be influenced by environmental factors like alcohol use (Davis et al., 2015; Dees et al., 2009; Dees et al., 2015; Peck et al., 2011; Richards and Oinonen, 2011), tobacco use (Davis et al., 2015; Peck et al., 2011), diet (Mervish et al., 2013; Sowers et al., 2006; Wolff et al., 2015), and father absence (Deardorff et al., 2002). Examples of early pubertal markers include gonadotropin-releasing hormone (GnRH), kiss-peptins that stimulate GnRH (Smith and Clarke, 2007) or other factors triggering the growth spurt

such as growth hormone, or insulin-like growth factors (Styne, 2003). Another promising candidate is the steroid hormone dehydroepiandosterone (DHEA) which has been shown to associate with cortical thickness, especially in the age range between 4 and 13 years (Nguyen et al., 2013b).

In our study, including measurements of 9 and 12 years old twins, significant associations between hormone levels and structural brain parameters were found only in girls, only for FSH and estradiol, and only in gray matter (Chapter 4). Probably, the boys in our cohort were too young to show enough variation in their hormone levels to find an association between hormones and the brain; results regarding testosterone levels at age 17 years await further analysis. Associations between testosterone and cortical thickness (Nguyen et al., 2013a; Nguyen et al., 2013b; Raznahan et al., 2010) or white matter (Herting et al., 2012; Menzies et al., 2015; Paus et al., 2010; Peper et al., 2015; Perrin et al., 2008) in boys have been found in other studies. Reports of correlations between LH and brain development are scarce. We found a correlation between LH and white matter volume in 9 year old boys and girls analyzed together (Peper et al., 2008), but not separately at age 9 or 12 (Chapter 4). No other studies have reported an association between LH and white matter during puberty. In young adults, a negative correlation between LH and MD of the fornix has been reported (De Bondt et al., 2013), suggesting the possibility of a relation between LH and white matter during pubertal development. However, the group size was small and participants with a natural cycle were combined with participants that used oral contraceptives, making interpretations tentative.

Future studies could look at the development of specific white matter bundles in relation to developmental increases in testosterone or other hormone levels. An interesting point of research are the cortico-subcortical connections. There is evidence from electro-encephalo-graphic (EEG) data and functional connectivity MRI data that testosterone plays a role in decreasing subcortical-cortical connectivity, and increasing connectivity between subcortical areas (Miskovic and Schmidt, 2009; Schutter and van Honk, 2004; Volman et al., 2011; van Wingen et al., 2010). In addition, recent work by Peper and colleagues suggest a role for testosterone in frontostriatal (Peper et al., 2013) and fronto-temporal-subcortical (Peper et al., 2015) white matter bundles mediating aggression in boys and girls (Peper et al., 2015) and impulsivity in boys (Peper et al., 2013). With our longitudinal data, future studies can look at the influence of testosterone on development of specific white matter bundles in relation to behavioral traits during mid and late adolescence. This can yield important new insights on the development of problem behavior in adolescents.

Sex differences

Although several studies report sex differences in FA during childhood and adolescence (Lebel and Beaulieu, 2011; Schmithorst et al., 2008; see also review by Ladouceur et al., 2012), no significant sex differences in FA-weighted efficiency were found in our studies. Boys had a larger streamline-weighted efficiency, which is most likely due to larger brain size of boys: they have thus more voxels, more starting points and more streamlines. One possibility for our null finding concerning FA-weighted efficiency is that the differences between boys and girls (at this age) are on a small or regional scale that is not picked up in our network approach. Here I give a short overview of sex differences in the brain during

puberty and adulthood.

Studies in adolescents often report that boys have higher FA, or that boys have a steeper developmental increase than girls do (Ladouceur et al., 2012). We found this pattern in the developmental trajectory of local FA network efficiency in only a few regions: boys tend to start with lower FA-weighted efficiency at age 10, show a steeper increase and end somewhat higher than girls do around age 18. Because the brains of girls develop earlier than boys (Raznahan et al., 2010; Raznahan et al., 2011), boys and girls of the same age can have different brain characteristics. As such, sex differences of brain development are difficult to interpret: they could either reflect increased sex differentiation or a temporary pubertal-timing induced difference (as discussed by Ladouceur et al., 2012). Already at birth, the male brain is larger than the female brain (also after co-varying body length or weight, Ankney, 1992; Witelson et al., 2006) and this continues into adulthood. Additionally, men tend to have a (relatively) larger white matter volume and higher FA than women (Menzler et al., 2011, review by Gong et al., 2011), although this may be related to brain size (Westerhausen et al., 2011). Thus, it seems reasonable that the sex differences in the brain that are observed during adolescence are not only due to a difference in the timing of brain development, but also reflect the start of increased sex differentiation of the brain (Paus, 2010b). In addition, studies by Perrin and Paus and colleagues suggest that white matter development may be governed by different mechanisms in boys and girls: white matter growth during adolescence in males is mainly influenced by increases of axonal caliber, whereas increased myelination plays a larger role in females (Paus and Toro, 2009; Perrin et al., 2009). Mechanisms by which sex differences in brain structure could be governed may also be the result of different behavior of genes in the sexes due to interaction of genes with sex-linked genes, and/or hormonal influences on gene expression and regulation (Weiss et al., 2006).

Interestingly, the correlation between IQ and FA-weighted local efficiency at age 18 was mainly driven by the girls in our cohort. A likely explanation is that girls start their pubertal development earlier than boys do – with regard to both secondary sexual characteristics and brain development. Possibly, the sex difference in manifestation of IQ in the brain at age 18 is the result of developmental timing. A fourth follow-up measurement in this cohort would be able to elucidate this question. An alternative explanation for our results is that men and women have different neuronal pathways to IQ (see review by Deary et al., 2010). It has been suggested that there is a higher association between IQ and white matter in women than in men (Gur et al., 1999; Haier et al., 2005), although there are also studies that find the opposite (Dunst et al., 2014). A study by Schmithorst (2009) reports a positive correlation between IQ and FA in girls, and a negative correlation in boys. Moreover, these correlations were present in the older children (12-18yr) and not in the younger children (5-11yr). In adults, positive correlations between cognitive functioning and FA were found in women, and negative correlations in men (Tang et al., 2010). This suggests that sex dependent correlations between IQ and white matter during adolescence could reflect the beginning of a differentiation between male and female 'intelligence paths in the brain'.

Implications

The studies described in this thesis cover different aspects of adolescent development. Future studies in health and psychiatric disorders can build on our findings. The finding of

different developmental trajectories depending on IQ implies that studying (aberrant) developmental trajectories of teenagers who are at high risk for psychiatric disorders, such as schizophrenia or depression, may be more informative and predictive than a brain scan at a single time point (Giedd et al., 2008; Moran et al., 2013; Rapoport and Gogtay, 2008; Shaw et al., 2010).

Another approach for studies that want to find markers for disease liability could be the combination of hormone levels and brain structure. Hormones influence behavior and the brain (Peper and Dahl, 2013) and hormone levels may be different in adolescents at low vs. high-risk for psychiatric disorders (Martel et al., 2009; van Rijn et al., 2011). As a result, different hormone levels could lead to different developmental trajectories or influence development of the healthy brain differently than the predisposed brain (cf. Peper et al., 2011b). Because we did not find a relation between hormone levels and white matter network topology, future studies may want to use a bundle- or voxelwise approach.

Heritability of network efficiency in adolescents was moderate to high. Structural and functional network efficiency is affected in several (heritable/genetic) psychiatric diseases (Bassett and Bullmore, 2009; Bohlken et al., 2016a; van den Heuvel and Fornito, 2014; van den Heuvel and Hulshoff Pol, 2010). Genetic mechanisms that influence network topology in the healthy population may also be involved in diseases that show aberrant network characteristics. The genetic link between brain structure and IQ may be involved in the onset of psychiatric disease. Recently, it was found that IQ, FA, and cortical thickness attribute to schizophrenia partly through a common genetic factor, as well as independently (Bohlken et al., 2016b). Understanding the environmental and genetic factors that influence hormone levels, white matter efficiency, intellectual functioning, and their relations is fundamental when looking for ways to optimize developmental trajectories for every individual.

Methodological considerations

Although the studies presented in this thesis were not specifically focused on the methodological aspects of studying brain development, the following findings deserve some consideration.

First, a broad age range during a period of rapid development like puberty may include a considerable variance. It is important to be aware of developmental or pubertal stages in addition to age that may increase the variance of the variable of interest.

A second point of consideration is the edge-weight of the structural brain networks: in early adolescence, different results were found when using streamline-weighted or FA-weighted networks (Chapter 5). This emphasizes the difference between two closely related white matter features. Hence, careful consideration is required when selecting edge weights, interpreting (developmental) changes in edge weights (see also Jones, 2010; Jones et al., 2013), and comparing studies that differ in network construction. Future studies are encouraged to examine both features in order to learn more about the developmental differences.

CONCLUDING REMARKS

The aims of the studies in this thesis were to gain insight into seemingly very different processes of typical adolescent development. We conclude that changes in hormone levels, cog-

nition, and brain structure co-occur and are influenced by the same genetic and environmental factors. Adolescent-specific brain development is linked to puberty, and intellectual functioning is related to development of the white matter network. Genes play an important role in hormonal, physical, and brain development. This thesis provides new and important information for future studies to build on. It also invites new research to study the mechanisms that underlie the relation between IQ or hormones and brain development. Another question is how the brain continues its development after age 18. A fourth inclusion of the BrainSCALE participants would be most suitable to answer that question.

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