9.1 SUMMARY

How does the brain develop as a complex communication network? During undisturbed healthy development, the brain grows an immense network consisting of thousands of millions of neurons and each neuron can connect to thousands of other neurons. The organization or topology of connections between neurons (at the micro scale) and *between* brain regions (at a larger scale) appear crucial for efficient communication in the brain. During normal, undisturbed development, major changes occur anatomically and functionally within brain regions which leads to development of a specific function of that brain region. Concurrently with these region specific changes, signal transduction between more distant brain regions is gradually starting to improve, leading to integrated, more complex functions of the brain. Previous studies mostly studied these local and global developmental changes independently and the focus was on selections of brain areas and connections instead of on the whole brain network as an integrated entity with an optimal balance between local specialization and global integration. It is not well understood yet if young children already present balanced local and global communication and if this balance will change during normal development. The main aim of the studies in this thesis was to gain new insight in normal and abnormal brain development by investigating the effects of normal and abnormal development on the organization of large scale functional brain networks.

Chapter 1 started with an introduction on the methods for characterization of large scale brain functional networks. The neurophysiological principles of the brain were outlined and the electroencephalogram (EEG), the magnetoencephalogram (MEG) and functional magnetic resonance imaging (fMRI) were introduced as neuroimaging techniques for measuring brain activity non-invasively from outside the brain. Synchronization was proposed as the concept for measuring functional connectivity between brain regions. Then, different approaches for constructing networks were outlined followed by an introduction on graph analysis for characterizing brain network organization. For instance, measures of local connectivity (clustering) and global integration (path length) were introduced. Subsequently, a short overview was given on the neurobiological and neurophysiological

changes during healthy and abnormal development. On the one hand, the focus was on SGA+ children (who showed spontaneous catch-up growth) as these children are born with a developmental delay which might have consequences for brain development later in life. On the other hand, disordered neurodevelopment was studied in toddlers with autism to investigate whether aberrant connectivity is present already at the very early developmental stages in this disorder. This chapter ended with the aims and outline of the thesis: to map brain functional network organization in young children and explore possible changes in functional network organization during typical and atypical brain development.

Typical development of functional brain networks

The first three studies (**Chapter 2-4**) examined organizational changes of functional brain networks during healthy development based on resting-state EEG recordings. **Chapter 2** presented a study examining the effects of typical development on functional brain network topology in a large group of young children at 5 and at 7 years of age. Whole brain connectivity strength decreased over time in all frequency bands, signifying that functional networks become weaker connected possibly due to weakening or loss of physical connections in children at school age. Importantly, both normalized clustering and path length increased, indicating that brain functional networks evolve towards a more ordered small-world organization in young children. Interestingly, girls showed stronger whole brain connectivity and a higher mean clustering than boys suggesting a gender difference in connectivity topology possibly influenced by sex hormones. Moreover, these findings suggest that girls are ahead in brain development or that functional networks evolve differently in boys and girls. Taken together, the findings in this chapter suggest that normal brain maturation leads to an efficiently organized brain network and that this development is influenced by gender.

Chapter 3 provided supporting evidence and extended the hypothesis of a developmental shift of functional network organization. The minimum spanning tree method was introduced as a solution to normalization problems and problems that arise when networks of different groups and / or conditions, possibly with different size or density, have to be compared. Significantly increased diameter and eccentricity, coinciding with reduced leaf number were found with development in young children. This was indicative of a shift toward more line-like or path-like and decentralized organization which was related to the previously found shift towards more ordered small-world networks. In girls, longer diameter and less leafs were found suggesting less centralized trees than boys. Taken together, the findings of chapter 2 and 3 suggest that networks shift towards more ordered small-world configurations with a larger span during childhood development and additionally, girls might present more mature configurations than boys and that they start with- and maintain more densely connected networks.

The main aim of **Chapter 4** was to investigate the development of functional brain networks over the life span in a large sample aged 5 to 71 years. Whole brain *c*onnectivity strength showed an inverted U shape with large increases from childhood to adolescence to adulthood and peaking at ~ 55 y, and thereafter, during aging, a weakening of connectivity strength was found. Additional to the changes in connectivity strength, strong increases in both clustering and path length were found from childhood to adolescence, indicating a shift toward more ordered small-world networks. Interestingly, a positive correlation was found between connectivity strength and white matter volume and additionally, path length was positively correlated to both white and grey matter volumes. In sum, the findings implicate that EEG connectivity and efficiency of functional brain networks can be used to trace functional and structural brain development and gender differences.

Atypical development of functional brain networks

The second part of this thesis (**Chapter 5-8**) described the possible effects of atypical development on functional brain network organization in young children. To this purpose, two clinical groups were examined: SGA born children at school age and toddlers with autism spectrum disorders.

SGA born children have suffered from intra-uterine growth delay resulting in a birth weight/length that is ‘small for gestational age’. SGA has been associated with decreased cognitive abilities and deviant brain volumes later in life. Postnatal spontaneous catch-up growth occurs in most of these children and is associated with better cognitive and brain developmental outcome (compared to children without catch-up growth). In neonatal periods, SGA children showed increased power in low, and reduced power in high frequency bands compared to controls suggesting delayed development of brain oscillatory activity as normal development of oscillatory activity is characterized by a shift toward increased power in high and decreased power in low frequency bands. However knowledge on development of brain activity later in life in SGA children is limited. In **Chapter 5,** MEG resting-state brain activity was measured in SGA children (only SGA children who showed postnatal spontaneous bodily catch-up growth were included in this study) (mean age 6 years) and in control children (mean age 6 years). Apart from significantly lower absolute power in the gamma band, SGA children did not differ from controls in other frequency bands, suggesting that the presumed developmental delay (i.e. increased low and decreased high frequency power) at neonatal age has largely been compensated at school age in SGA born children who showed catch-up growth. In this study only brain oscillatory activity originating from brain regions underlying MEG sensors were measured and possible deviations in synchronization between different brain regions which might contain important information regarding integration of information in the brain network were not taken into account. The patterns of interactions between brain regions were studied in **Chapter 6** in both SGA+ children and in healthy control children. The aim of this study was to capture the characteristics of MEG based functional brain networks in control children on the one hand, and on the other hand the hypothesis of aberrant connectivity topology in SGA+ was tested. MEG based resting-state networks in control children showed small-world organization and weak modularity indicating that already at school age networks show a balanced local and global information processing in the brain. In SGA+ children a subtle increase in connectivity strength was found in high frequencies (beta band: 12-25 Hz), however network organization parameters were comparable with control children. The pattern of over-connectivity might be interpreted as a compensation mechanism to obtain and maintain balanced local and global information integration. In summary, postnatal spontaneous catch-up growth appears to have a favorable effect on delayed brain maturation in SGA born children. Future longitudinal studies might aim to investigate which factors and which developmental stages are particularly crucial for catching up delay.

In **Chapter 7** resting-state networks were explored in a continuous resting-state functional connectivity MRI study that investigated a mixed group of 5-8 years old control children and an SGA group that showed catch-up growth and normal intelligence. Children were prepared in a mock MRI scanner prior to the scanning session to reduce motion artifacts and stress or anxiety in order to improve data quality. Fourteen independent resting-state networks were identified. Networks consisting of brain areas involved in sensory and motor processing largely resembled adult networks. Networks involved in higher order cognitive processing, such as the attention network and the default mode network (DMN) that is mostly active during awake rest were incomplete or fragmented compared to adults. This indicated that connectivity patterns within and between these networks are still immature in children at school age. Combining multiple imaging techniques in future studies, might lead to new insight in the relation between anatomical and functional networks and how they mutually influence each other during development.

In **Chapter 8**, children with the neurodevelopmental disorder autism were investigated. Functional networks based on EEG recordings during a passive visual task were studied in toddlers with autism. Toddlers with autism showed reduced whole brain connectivity strength suggesting a pattern of under-connectivity compared to controls. Additionally, a reduced clustering coefficient and increased path length was found which suggests a disturbed balance between local and global communication in the brain. Taken together, the findings indicate a reduction of global communication capacity in autism and moreover that aberrant connectivity is manifested already at the very early stage of development.

In Chapter 9, the most important findings were summarized, discussed and interpreted in the context of existing literature and methodological issues were considered. In retrospect, an important conclusion is that studying the child brain with non-invasive techniques (EEG, MEG and fMRI) and constructing and analyzing functional brain networks gives important new insights in brain development. Already at young ages, typically developing children have balanced local and global communication capacity brains and during development, this balance between local and global organization shifts towards a more ordered small-world topology. Delayed brain maturation at neonatal stages of development in SGA children is largely caught up or compensated at school age in SGA children who showed spontaneous bodily catch-up growth. Autistic toddlers showed disordered neurodevelopment which was characterized by an abnormal balance of local and global connectivity patterns leading to reduced global communication capacity already at the very early stages of development.

In future studies, the combined use of EEG, MEG and MRI, might generate important new knowledge on the mutual relation between function and anatomy in developing brain networks. Modeling activity upon anatomy based network models might help testing and generating new hypotheses for new empirical network based research. Applying new methodological approaches, such as the MST might add to our understanding of the complete space of complex brain networks

In conclusion, this thesis gave new insight in normal brain development and mechanisms underlying delayed development and neurodevelopmental disorders. Brain connectivity and graph analysis can be used to map very early stages of development which might be the crucial stages for acquiring more complex cognitive functions and which might be the same periods during which children are most vulnerable to environmental stress or developing disorders.