Chapter 12

Summary and discussion
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Summary of main findings

This thesis aimed to 1) increase insight into the nature of the problems in motor, cognitive, and behavioral functioning of very preterm children at school age, 2) elucidate alterations in brain development underpinning these problems, and 3) investigate the potential of a neonatal nutritional intervention, directed at reducing the incidence of serious neonatal infections and associated altered brain development, to improve long term motor, cognitive, and behavioral functioning of very preterm children. The main outcomes are summarized in Table 1.

In the first part, we addressed the nature of the problems in motor, cognitive, and behavioral functioning of very preterm children at school age. Using meta-analytic methods, we found evidence for substantial motor impairment in very preterm children from infancy up to adolescence (chapter 2). Our findings indicated that very preterm children were on average $-0.57$ to $-0.88$ SD behind their term-born peers in terms of their motor development, as measured by three psychometrically sound and widely used motor tests: the Bayley Scales of Infant Development - second edition, the Movement Assessment Battery for Children, and the Bruininks-Oserethsky Test of Motor Proficiency. The presence of perinatal complications, including bronchopulmonary dysplasia, sepsis, necrotizing enterocolitis, and/or intraventricular hemorrhage, further increased the degree of motor impairment in very preterm children. Interestingly, motor problems were evident on all aspects of motor behavior, including balance skills, ball skills, manual dexterity, as well as fine and gross motor development. Birth weight and gestational age were strongly and positively related to motor abilities in the first five years of life. These meta-analytic outcomes underline the negative impact of very preterm birth on motor development throughout childhood and adolescence. The nature of these motor problems was further addressed using a newly developed visuomotor task (chapter 3). Using this task, we showed that poor visuomotor performance in very preterm children was solely present in an unpredictable, non-structured condition. In contrast, visuomotor performance was not different between very preterm children and term controls in a predictable, structured
condition. Although less prominent, differences in visuomotor performance between very preterm children and term controls in the unpredictable condition remained present after excluding children who fulfilled research diagnostic criteria of developmental coordination disorder (DCD). Furthermore, we found no evidence for differences in capacity of the visuomotor system between both groups, as visuomotor performance of very preterm children was not more sensitive to a systematic increase in workload than visuomotor performance of term controls. Together, these findings show that unpredictability is an important factor underpinning poorer visuomotor performance of very preterm children compared to term controls. In addition to motor problems, we investigated the nature of problems in cognitive and behavioral functioning of very preterm children (chapter 4). Besides cognitive impairment, we confirmed a medium-sized increase in attention problems in very preterm children at school age, as both parents and teacher reported more behavioral symptoms of inattention. In order to elucidate the brain functions underlying the attention problems, very preterm children were compared to term controls on a range of neurocognitive abilities involved in the multifaceted construct of attention. Compared to term controls, very preterm children showed poorer visuospatial working memory abilities and higher rates of attentional lapses (extremely slow responses during a task indicating short moments of attention loss). Importantly, both the poorer visuospatial working memory skills as well as the increase in lapses of attention were significant mediators of the behavioral symptoms of inattention of very preterm children as observed by parents and teacher.

Taken together, the outcomes of the first part of this thesis confirm the presence of substantial impairments in multiple aspects of motor, cognitive, and behavioral functioning of very preterm children. Our findings suggest that the predictability of a situation may play an important role in (visuo)motor performance differences between very preterm children and term controls. Altered brain functions, including visuospatial working memory and lapses of attention, are important factors mediating the attention problems of very preterm children at school age, both at home and in a classroom situation.
Table 1. Overview of findings in this thesis

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<th>Chapter</th>
<th>Participants</th>
<th>Measures</th>
<th>Description of main outcomes</th>
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| 2       | Meta-analytic sample of 9653 very preterm/VLBW children                      | Meta-analytic results of 41 studies on motor functioning assessed with the BSID-II, MABC, and/or BOTMP                 | - Very preterm/VLBW children obtained significantly lower scores on all three motor tests: BSID-II: $d=-0.88$, $p<.001$, MABC: $d=-0.65$, $p<.001$, and BOTMP: $d=-0.57$, $p<.001$.  
- BSID-II outcomes show a catch-up effect in the first years of development ($r=0.50$, $p=0.01$), MABC outcomes demonstrate a non-significantly greater deficit with increasing age during elementary school and early adolescence ($r=-0.59$, $p=0.07$). |
| 3       | Very preterm children derived from the GEEF-cohort; term controls recruited from same classrooms or from school located in the same area | MABC; newly developed visuomotor task                                                                                  | - Forty-six percent of very preterm children had a research diagnosis of DCD, compared to 16 percent of term controls ($p<.001$).  
- No group differences in visuomotor performance were present for the structured condition of the novel visuomotor task.  
- Both very preterm children with a research diagnosis of DCD ($p<.001$) and very preterm children free of motor impairment ($p<.009$) had poorer visuomotor performance compared to term controls in the non-structured condition of the visuomotor task, suggesting that predictability of the required motor response plays a crucial role in visuomotor deficits of very preterm children. |
| 4       | Very preterm children derived from the GEEF-cohort; term controls recruited from same classrooms or from school located in the same area | ANT; CBCL; Klingberg’s visuospatial working memory task; TRF; WISC-III                                                   | - Very preterm children had impaired visuospatial working memory abilities as measured using Klingberg’s visuospatial working memory task ($d=0.87$, $p<.001$), and increased inconsistency in information processing speed, indicated by a higher tau ($d=0.55$, $p=0.02$), as measured using the reaction time distribution derived from the ANT.  
- Very preterm children had higher parent and teacher ratings of inattention ($R^2=0.40-0.56$) as measured using the CBCL and TRF, respectively.  
- Tau and visuospatial working memory were significant predictors of parent ($R^2=0.161$, $p<0.001$ and $R^2=0.71$, $p<0.001$; respectively) and teacher ($R^2=0.152$, $p<0.001$ and $R^2=0.64$, $p<0.002$; respectively) ratings of inattention, and completely explained the effects of very preterm birth on attention problems. |
| 5       | Meta-analytic sample of 818 very preterm/VLBW children and 450 term controls | Meta-analytic results of 15 studies on brain structure volumes as measured by sMRI                                       | - Very preterm/VLBW children had significantly smaller total brain volume than the comparison group ($d=0.58$, $p<0.001$), as well as smaller volumes of white matter ($d=0.53$, $p<0.001$), grey matter ($d=0.62$, $p<0.001$), cerebellum ($d=0.74$, $p<0.001$), hippocampus ($d=0.47$, $p<0.001$), and corpus callosum ($d=0.71$, $p<0.001$).  
- Brain volume reductions were associated with decreased general cognitive functioning, but no significant relations with age at assessment were found. |
| 6       | Very preterm placebo control children derived from the GEEF-cohort; term controls recruited from same classrooms or from school located in the same area | Brain structure volumes as measured by sMRI; FA values of 18 major white matter tracts as measured using DTI; MABC; WISC-III | - Very preterm children had reductions in FA of the cingulum hippocampal tract right ($d=0.75$, $p=.003$) and left ($d=0.76$, $p=.001$), corticospinal tract right ($d=0.56$, $p=.02$) and left ($d=0.65$, $p=.009$), forceps major ($d=1.04$, $p<.001$) and minor ($d=0.54$, $p=.02$), as measured using DTI.  
- Reduced FA values were moderately to strongly related to motor impairment measured using the MABC (range $r=0.40-0.67$), and particularly present in very preterm children with a research diagnosis of DCD.  
- A ROC curve discriminating between very preterm children with and without a research diagnosis of DCD, using average FA (as calculated from those tracts that significantly discriminated between both groups), had an area under curve of 0.87 (95% CI 0.74-1.00, $p=.001$). |
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<td>7</td>
<td>Very preterm placebo control children derived from the GEEF-cohort; term controls recruited from same classrooms or from school located in the same area</td>
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<td>- Very preterm children had slower reaction times than term controls when interfering stimuli were presented in the Eriksen Flanker task ($d=0.67$, $p=.005$), indicating poor interference control.</td>
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<td>- No evidence was found for differences in the activation pattern of the cortical regions involved in interference control between very preterm children and term controls. Mean FA values of specifically those fiber tracts that innervate the cortical regions involved in interference control were significantly lower in very preterm children ($d=0.61$, $p=.01$).</td>
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<td>- A relation between lower white matter tract integrity and poorer interference control was only found for very preterm children ($r=-.46$, $p=.02$).</td>
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<td>8</td>
<td>Meta-analytic sample of 13,755 very preterm/VLBW children with perinatal infections</td>
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<td>- Very preterm/VLBW infants with perinatal infections have poorer mental ($d=0.25$, $p&lt;.001$) and motor development ($d=0.37$, $p&lt;.001$) compared to very preterm/VLBW infants without infections.</td>
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<td>- Mental development is most impaired by NEC ($d=0.40$, $p&lt;.001$) and meningitis ($d=0.37$, $p&lt;.001$), and motor development is most impaired by NEC ($d=0.61$, $p&lt;.001$).</td>
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<td>- Chorioamnionitis did not affect mental ($d=0.05$, $p=.37$) or motor development ($d=0.19$, $p=.08$).</td>
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<td>- The overall effect sizes for MDI and PDI scores were significantly different ($p=.003$), indicating that perinatal infections have a greater impact on PDI than on MDI scores in VLBW/very preterm infants.</td>
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<td>9</td>
<td>Very preterm children derived from the GEEF-cohort</td>
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<td>Intelligence quotient, processing speed, attentional functioning, working memory and parent- and teacher-rated behavioral outcomes were not different between children treated with glutamine or placebo (all $d$'s&lt;0.49, all $p$'s&gt;.05).</td>
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<td>- Visuomotor abilities as measured by the Ball Skills subscale of the MABC were poorer in the glutamine group ($d=0.67$, $p=.002$); this group difference persisted after taking into account the beneficial effects of lower serious neonatal infections rates in children treated with glutamine ($p=.005$).</td>
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<td>10</td>
<td>Very preterm children derived from the GEEF-cohort</td>
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<td>Glutamine supplementation in the first month of life was associated with increased volumes of white matter ($d=0.54$, $p=.03$), hippocampus ($d=0.47$, $p=.02$), and brain stem ($d=0.54$, $p=.04$) at school age.</td>
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<td>- Exploratory analyses using an uncorrected p-value indicated higher FA values of the bilateral cingulum hippocampal tract in the glutamine group compared to the placebo group.</td>
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<td>- All differences were either strongly associated (hippocampus volume, brain stem volume, and FA values of cingulum hippocampal tract) or completely mediated (white matter volume) by the lower number of serious neonatal infections in the glutamine group.</td>
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<td>11</td>
<td>Very preterm children derived from the GEEF-cohort</td>
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<td>- Neonatal glutamine supplementation increased head circumference growth ($p=.008$) in the first year of life, but not with increased growth in weight ($p=.44$) and length ($p=.73$).</td>
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<td>- Larger average head circumference was significantly associated with larger white matter volume and grey matter volume (range $r=.55-.81$, all $p$'s&lt;.002) at school age.</td>
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The second part of this thesis addressed the alterations in brain development underpinning the motor, cognitive, and behavioral problems of very preterm children at school age. We investigated the impact of very preterm birth on brain structure volumes throughout childhood and adolescence using meta-analytic methods, and found a consistent reduction in total brain volume of 0.58 SD in very preterm children compared to term controls (chapter 5). Importantly, these reductions were present to a similar extend for grey matter, white matter, corpus callosum, cerebellum, as well as the hippocampus. Furthermore, by reviewing all studies that included functional outcomes, we showed that volume reductions of all analyzed brain structures were associated with impaired brain functions of very preterm children, including language, motor skills, memory, IQ, and executive functioning. Using state of the art imaging methods, we further investigated the relation between altered brain development and problems in motor, cognitive, and behavioral functioning of very preterm children. First, a whole-brain approach confirmed the medium to large-sized reductions in brain structure volumes in very preterm children (chapter 6), which were equal to reductions found in the meta-analysis of chapter 5. Second, we found medium to large-sized reductions of white matter integrity in the majority of white matter tracts in very preterm children compared to term controls. Whereas reduced grey matter volumes were related with cognitive impairment, reductions of white matter integrity in major white matter tracts were primarily related to motor impairment of very preterm children. Reduced white matter integrity was most prominent for very preterm children with a research diagnosis of developmental coordination disorder (DCD), as compared to both very preterm children free of motor impairment and term controls. Importantly, the average white matter integrity of all affected major white matter tracts had a high sensitivity and specificity for the detection of DCD in very preterm children. The method of fMRI-guided probabilistic diffusion tensor tractography was used to investigate the role of abnormalities in white matter and grey matter development in the brain function of interference control, a core aspect of attention (chapter 7). Very preterm children had significantly more difficulty to inhibit irrelevant information than their term born peers on the Eriksen Flanker task, indicative of impaired interference control abilities. We found prominent lower white matter integrity of specifically those fiber tracts innervating the activated cortical region involved in interference control in very preterm children as compared to term controls. Interestingly, a relation between lower white matter integrity
Growing into a different brain

and poorer interference control was only present in very preterm children, further underlining the importance of white matter alterations in the attention problems of very preterm children.

Taken together, the findings from the second part of this thesis illustrate that, besides reductions in brain structure volumes, white matter integrity alterations are an important factor in the motor, cognitive and behavioral problems of very preterm children at school age. Promisingly, the role of white matter alterations in the functional problems of very premature children signifies the potential of measurement of white matter integrity in the detection and prediction of very preterm children at risk for adverse (motor) outcomes.

In the third part of this thesis, we investigated the potential of a nutritional intervention in the first period after birth, to improve long term functioning of very preterm children by reducing the incidence of serious neonatal infections and associated alterations in brain development. This section started with a meta-analysis aimed at clarifying the impact of perinatal infections in very preterm children on long term development, as measured by the Bayley Scales of Infant Development - second edition (chapter 8). We found evidence that very preterm children with perinatal infections have poorer mental and motor development compared to very preterm children without perinatal infections. In addition, perinatal infections had a significantly greater impact on motor development than on mental development in very preterm children. In the GEEF (Glutamine Enriched Enteral Feeding) study, enteral glutamine supplementation in the first month after birth reduced the incidence of serious neonatal infections in very preterm children. We investigated potential beneficial effects of glutamine supplementation on long term development, and found that the majority of long-term motor, cognitive, and behavioral outcome measures were not different between children in the glutamine and placebo group (chapter 9). If any, negative effects were obtained for ball skills in the glutamine group, although the isolation of this effect does not suggest the presence of adverse neurological effects caused by neurotoxicity of glutamine supplementation. Interestingly, we found that glutamine supplementation in very preterm children was associated with medium sized increases in white matter volume, hippocampus volume, and brain stem volume at school age (chapter 10). Furthermore, we showed that the increase in brain structure volumes of the glutamine group was mediated by the lower number of serious neonatal infections in this group. By investigating growth
trajectories of head circumference, weight, and length, we demonstrate that differences in brain structure volumes between the glutamine and placebo group were not part of general enhanced growth, rather enhanced growth was limited to the brain (chapter 11). Very preterm children from the glutamine group had a significantly larger increase in head circumference and brain growth than children from the placebo group, whereas no differences in growth trajectories of body length and body weight were found between groups.

In summary, our meta-analysis has now convincingly demonstrated the negative impact of serious neonatal infections on motor and mental development of very preterm children. Furthermore, the outcomes of the third part of this thesis illustrate that glutamine supplementation had beneficial effects on early brain development of very preterm children, which persisted up till school age. Larger brain volumes in the glutamine group were mediated by the lower incidence of serious neonatal infections in these children. Interestingly, increased brain volumes were not (yet) associated with improvements in motor, cognitive, or behavioral functioning at school age, at least as measured in our studies.

**General discussion**

**Motor, cognitive, and behavioral functioning**

In accordance with the results of previous meta-analyses on cognitive problems, our meta-analytic findings on motor impairment (chapter 2) as well as the results based on our own data (chapters 3 to 7) emphasize the presence of adverse developmental outcomes in very preterm children at school age. In addition to impaired motor skills and intellectual abilities, we showed that very preterm children have considerable difficulty in other neurocognitive functions, including visuospatial working memory skills and aspects of attentional functioning (lapses of attention, difficulty to inhibit irrelevant information). Interestingly, visuospatial working memory skills and lapses of attention were found to mediate the behavioral symptoms of inattention observed by parents and teacher, at home and in the classroom, respectively (chapter 4).

Higher rates of attentional lapses, short moments of attention loss, are associated with fluctuations in the brain’s default state network. The default state network in the brain is a collection of brain regions that support internally directed mental activities and is active
when an individual is not focused on the outside world. Recently, it has been found that functioning of the default state network in the brain is essential for sustaining attention.\textsuperscript{5-7} Furthermore, there is emerging evidence showing that functioning of the default state network, as measured by resting state connectivity, is altered in very preterm children at term age as well as in childhood.\textsuperscript{8-10} By now, there are several studies indicating that altered functioning of the default state network in the brain is related with abnormal white matter connectivity, for instance in children with traumatic brain injury.\textsuperscript{5} The differences in lapses of attention between very preterm children and term peers in this thesis (chapter 4), may reflect the presence of poor white matter connectivity in very preterm children. Interestingly, a similar relation has been described between altered visuospatial working memory abilities and abnormal white matter connectivity.\textsuperscript{11,12}

In this thesis, we showed a specific vulnerability for visuomotor performance of very preterm children to unpredictable circumstances in which the brain has to generate and utilize ‘internal models’ of motor planning and online motor control (chapter 3). Interestingly, similar differences in the functioning of ‘internal models’ of motor planning and online motor control have been described in children with DCD.\textsuperscript{13,14} In the brain, the cerebellum and posterior parietal cortex are thought to be critically associated with ‘internal models’ used for motor planning and online motor control,\textsuperscript{13,15,16} as both increase their activity in situations in which ‘internal models’ are utilized. In very preterm children, we found significantly decreased brain volumes, including the cerebellum, which may relate to this poor use of ‘internal models’ in motor planning and online motor control. In addition, the subtle differences in white matter integrity between very preterm children and term controls may result in poor transmission of signals from the cerebellum to the posterior parietal cortex and vice versa. Interestingly, there are some preliminary findings showing white matter alterations children with DCD.\textsuperscript{17} Furthermore, we found that very preterm children with a research diagnosis of DCD had significantly larger reductions white matter integrity (chapter 6). Together, these findings suggest that subtle impairments in white matter development may underpin the poor use of ‘internal models’ in motor planning and online motor control in children, although future preferably longitudinal studies directly investigating the possible causal relation between measures of white matter integrity and the usage of ‘internal models’ of motor planning and online motor control are needed.
Brain development

We confirmed the widespread differences in white matter integrity of very preterm children compared to term peers, which are strongly related with problems in motor, cognitive, and behavioral functioning (chapter 6 and 7). Recent imaging studies indicate that at least 50% of all very preterm infants show findings consistent with encephalopathy of prematurity, an umbrella term comprising all white matter injury in very preterm children due to an amalgam of destructive and developmental disturbances. The finding that differences in white matter integrity are related with global measures of motor and cognitive functioning (chapter 6) as well as with more isolated brain functions such as interference control abilities (chapter 7), is in concert with the view that altered connectivity of the brain is an important factor related with poor developmental outcomes following a very preterm birth. Eventually, the alterations in white matter development of very preterm children may lead to a substantial loss in efficiency of brain connectivity, with associated suboptimal processing and integration of information, underpinning the problems in motor, cognitive, and behavioral functioning of very preterm children at school age. Interestingly, our results indicate that very preterm children, of whom the alterations in white matter integrity were most prominent, are characterized by a relatively lower birth weight, shorter gestation, and smaller head circumference at birth (chapter 6). These findings suggest a particular vulnerability of this subgroup of very preterm children for the presence of encephalopathy of prematurity. Indeed, negative associations between, on the one hand, lower birth weight, shorter gestation, and smaller head circumference, and on the other hand, motor development in the first years of life, are frequently found and confirmed by the results of our meta-analysis (chapter 2). Notably, very premature children with no overt motor impairment at school age, still show altered white matter integrity in several white matter tracts compared to term peers. We found that this degree of white matter differences in very preterm children appears to be highly predictive for the presence of motor impairments and a research diagnosis of DCD, implying that the degree of white matter differences can be clinically used for an early diagnosis of very preterm children at risk for adverse motor development (chapter 6). Although the interpretation of our finding was limited by the fact that imaging data were collected at approximately the same age as developmental outcomes, previous studies confirm the potential predictive value of white matter integrity at term age for later development.
In addition to the crucial role of white matter integrity, we confirmed the presence of widespread reductions in all brain structure volumes of very preterm children (chapter 5). Volume reductions were relatively equally sized for each of the investigated brain structures instead of showing a pattern of differential reductions of specific brain areas. In other words, very preterm children have relatively smaller brains than their term peers. The presence of reduced brain structure volumes have been associated with a lower cognitive reserve, which may be unfavorable for academic achievement or indicate an increased vulnerability for neurodegenerative diseases later in life. Furthermore, our findings demonstrated some evidence for differential effects between white matter and grey matter differences on functional outcomes (chapter 6). Whereas white matter integrity differences were mostly associated with motor impairment, reduced grey matter volume was associated with poorer cognitive functioning of very preterm children. It is well-established that reduced grey matter volume is related to poorer cognitive functioning at various ages. We speculate that children being born small for gestational age (SGA), who have an additional decrease in brain volume as compared to children being born appropriate for gestational age (AGA) despite the presence of a so-called brain sparing effect, have increased impairment of their cognitive abilities. In these SGA children, the negative effects of additional reductions in brain volume may add up to the negative effects of prematurity on white matter development. Indeed, there are several studies showing greater cognitive problems or lower academic achievement in very preterm SGA children, as compared to very preterm AGA children.

Glutamine intervention

We found evidence that perinatal infections are related with poorer neurodevelopmental outcomes in very preterm children (chapter 8). Furthermore, we found that the reduction in serious neonatal infections following glutamine supplementation was related with increased brain growth in the first year of life (chapter 11), and eventually led to larger brain structure volumes and enhanced integrity of some white matter tracts at school age (chapter 10). It has been hypothesized that excitotoxicity and free-radical accumulation events, co-occurring with serious neonatal infections, lead to damage of late-developing granular cells (interneurons) by reducing the population of neurons that will later differentiate into specific brain structures. As a consequence, reducing the incidence of
serious neonatal infections may have increased various brain structure volumes in very preterm children. In contrast to the beneficial effects of glutamine supplementation in the first weeks after birth on volumes of various brain structures, we did not find any beneficial effects on motor, cognitive, and behavioral outcomes at school age (chapter 9). However, it would be interesting to explore the cognitive reserve of very preterm children who received glutamine supplementation in a future study, as an increase in brain capacity has been associated with a larger cognitive reserve, which may eventually be beneficial for academic achievement or be protective for neurodegenerative diseases later in life. The observed increase in brain structure volumes, as opposed to a limited increase in white matter integrity of children who have received glutamine supplementation compared to those children who have not received glutamine supplementation, may be related to the timing of the intervention. Whereas there is a striking four-fold increase in cortex volume between week 28 and week 40 of gestation, axon development of the underlying white matter tracts is concentrated before week 28 of gestation and myelination proceeds from 40 weeks onwards. As glutamine is supplemented for a period of four weeks starting between 25 and 32 weeks of gestation, it is not surprising that beneficial effects on the number of serious neonatal infections may have particularly acted upon cortex growth. Nevertheless, one would expect that an increase in brain volume is accompanied with enhanced cognitive functioning. Given that SGA children have an additional decrease in relative brain volume as compared to AGA children, increased cognitive functioning would especially be expected in SGA children receiving glutamine supplementation. In a future study, we will further investigate if the beneficial effects of glutamine treatment on the incidence of serious neonatal infections and brain volumes have led to additional increase in cognitive abilities in SGA children, and may be advantageous for the long term functional outcomes for this particular subgroup of very preterm children.

**Strength and limitations**

The studies described in this thesis also have their limitations. One of the main concerns is that all empirical studies on very preterm children are performed in children derived from the GEEF cohort, thereby capitalizing on the same group of children. Choosing one group of children may bring along the risk of restrictions in the ability to generalize findings, as the included children can always differ on one or more characteristics from the
whole population of very preterm children. Although the GEEF cohort was derived from a representative population of infants at a neonatal intensive care unit, future cross-validation studies are warranted to signify the ability to generalize the current findings to the larger population of very preterm children. Another limitation of this thesis is that not all children of the GEEF cohort participated nearly eight years after enrolment in the original study. Of the 102 infants included in the original study, 89 infants were alive at one year of age. Although the majority of these children still participated at eight years of follow-up (76.4%), this may have limited the representativeness of the study population. Nevertheless, drop-out is inevitably related with this type of long term follow-up study, and the drop-out rate of the study population described in this thesis was relatively low. Furthermore, we found no evidence for differences between participating and non-participating very preterm children on measures of socio economic status, gender, birth weight, gestational age, and the mental and psychomotor subscales of the Bayley Scales of Infant Development test assessed at two years of follow-up, suggesting no selective drop-out. A final limitation of this thesis is the one year interval between, on the one hand, the neurocognitive and behavioral follow-up, and on the other hand, the imaging study. As brain maturation processes continue during childhood, changes in motor, cognitive, and behavioral functioning may have taken place during this year interval, introducing some additional variation in our outcomes. Nevertheless, despite this potential additional source of variation, we still demonstrated clear associations between measures of brain development and outcomes of neurocognitive and behavioral functioning of very preterm children.

The strength of this thesis was that developmental outcomes of very preterm children were investigated at multiple levels of measurement, including neurocognitive outcomes (motor and cognitive functioning), adaptive functioning (behavioral functioning both in the classroom and at home) and brain development (structural MRI, functional MRI, and DTI). In combination with the use of newly developed neurocognitive instruments, advanced imaging techniques, and sophisticated statistical approaches, the current thesis enabled the unique opportunity to study interrelations between the different levels of measurement. Furthermore, the longitudinal design incorporated in the randomized controlled trial on the effect of glutamine supplementation, allowed us to disentangle the long term effects of intervention in the neonatal period on developmental outcomes at school age.
Research agenda

While our findings have added a piece to the puzzle of understanding the complex interaction between altered brain development and problems in motor, cognitive and behavioral functioning of very preterm children, they have also raised a number of important and interesting new themes for future research.

The first research theme concerns the early identification of very preterm children at risk for adverse motor, cognitive, and behavioral functioning, as early identification would enable and facilitate early treatment and reduction in costs of unnecessary medical follow-ups of typically developing very preterm children. Based on the findings of this thesis, the crucial relation between, on the one hand, altered brain development and functioning, and, on the other hand, adverse functional outcomes at school age, indicate the potential for using early differences in brain development and functioning as predictors for later functional problems in very preterm children (chapters 4 to 7). Currently, major functional disabilities in early childhood can usually be adequately predicted, but prediction is extremely poor for mild or moderate functional disabilities in long-term outcomes. In order to progress towards a ‘clinical toolbox’ for early identification of adverse development in very preterm children, data on brain development, neurocognitive functioning, maternal and child related medical risk factors, academic outcomes, behavioral and emotional functioning should be longitudinally collected in a representative cohort at crucial stages in development. In such data collection, more emphasis should be placed on genetic diversity as explanatory factor for the large variability in outcome. In addition, the influence of the socio economic aspects should be taken into account, as it becomes increasingly clear that these factors play a significant role in the development of very preterm children. For instance, genetic variation in pro- and anti-inflammatory cytokine genes and their respective receptors may significantly interact with environmental influences, and contribute to developmental differences. Longitudinal data collection at crucial stages of development should ideally include the prenatal period, as growing evidence supports the notion that an adverse intrauterine environment (including placenta underperfusion with associated intrauterine growth restriction and hypoxic insults of the fetal brain) is an important factor associated with adverse brain development and functional outcomes that manifest later in development. More insight into the effects of timing and severity of prenatal insults may provide clinicians with important information that enables early prediction and identification.
of adverse outcome. The availability of new techniques and analyses, such as white matter connectivity importance maps or personalized connectomic analysis providing measures of altered neural connectivity for each individual, are promising, and are currently explored in the field of predicting long term outcomes following traumatic brain injury in children. Preliminary outcomes of one study showed that personalized connectomics analysis can be successfully used for estimating differences in neuronal development and neurodevelopment outcomes in very preterm SGA infants, although more studies are needed to validate these findings. Using a longitudinal prospective design, such data collection and analyses will contribute to a better understanding of the etiology and pathophysiology underlying adverse development in very preterm children, besides the obvious clinical advantages of determining the best predictors and risk factors at multiple age stages for later development of very preterm children.

The second research theme concerns the development and implementation of primary prevention strategies. Although we are still far away from complete prevention of adverse brain development and functional problems in very preterm children, there are some promising pathways along which future research could progress. In this thesis, we found evidence for long lasting effects on brain structure volumes by reducing the incidence of serious neonatal infections in very preterm children (chapter 9). In addition, as we speculate about a crucial mediating role of abnormalities in white matter integrity and neural connectivity on the functional impairments of very preterm children, future interventions should in particular target the reduction of white matter injury. A promising pathway would be to limit the presence of cytokines in the brain in the period that white matter development and injury predominantly occurs, i.e. before week 28 of gestation. Besides a direct reduction of serious neonatal infections, future interventions may benefit from improving the integrity of endothelial cells of the blood-brain barrier, limiting the crossing of cytokines from the blood into the brain. Some nutritional agents have been postulated to positively interact with blood-brain barrier functioning, including fish oils, garlic, specific fruits, soy, vitamin C and vitamin E. Furthermore, long-chain polyunsaturated fatty acids have been found important for synaptogenesis, membrane function, and, potentially, myelination. Interestingly, a large body of studies have indicated an important role of early-life nutrition on brain development and long term functional outcomes. Using meta-analytic methods, one study showed a positive effect of breast
feeding on cognitive development of (very preterm) children throughout childhood and adolescence, suggesting that nutrients present in breast milk may have a significant effect on neurologic development. In the developing brain, nutrients may be vital for the proliferation of neurons as well as supporting glial cells. Importantly, babies born during the Dutch famine in the second world war showed an increased risk of obesity later in life, suggesting an ‘early programming’ effect of malnutrition in the first period before and after birth on later development. Indeed, postnatal dietary malnutrition and a slower rate of postnatal growth have been associated with adverse brain development and impaired neurodevelopment in very preterm children. Promisingly, some studies showed that supplementation of long-chain polyunsaturated fatty docosahexaenoic acid or arachidonic acid in the neonatal period may improve problem solving abilities or cognitive development of very preterm children. Early programming effects of early-life nutrition suggest the presence of epigenetic modulation of gene transcription, that is, an adjustment at the level of expression of genes that are being transcribed, driven by environmental differences in nutrients availability. Although the precise mechanism of epigenetic early programming by early-life nutrition of the very preterm neonate remains to be clarified in future studies, the perinatal period appears to be a crucial time window in which very preterm infants are vulnerable for the effects of nutritional interventions.

The third research theme concerns the development and implementation of secondary prevention strategies, which aim to improve the current motor, cognitive, and behavioral problems of very preterm children using interventions and treatments. Thus far, little success of interventions directed at improving motor, cognitive, and behavioral functioning of very preterm children has been reached. A recent meta-analysis indicated that current interventions showed no improvement of motor skills, and only small improvement of cognitive abilities, of very preterm children in childhood. Therefore, the development of cost-effective interventions and treatments that have the potential to improve problems in cognitive, motor, and behavioral functioning of very preterm children is of utmost importance. Given the widespread reductions in white matter integrity that play an important role in the functional problems of very preterm children, some findings of recent studies are hopeful, as they indicate that practice and training are able to improve white matter integrity of specific white matter tracts in the brain. For instance, one study showed that white matter integrity of the corticospinal tract substantially increased in young
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children and adolescents who learned to play the piano. Another study showed that adults that practiced on their meditation skills improved white matter integrity of the tract that connects the anterior cingulated cortex with other parts of the brain. Interestingly, this is the same tract as we found to be associated with interference control (chapter 7), a core aspect of the attention, suggesting the possibility that meditation could potentially benefit the very preterm children with attention problems. Furthermore, the specific susceptibility of very preterm children for the predictability of a situation, similar to children with DCD, may indicate that interventions developed for children with DCD may be useful for treating visuomotor impairment of very preterm children as well. It might be worthwhile to encourage a therapeutic approach based on cognitive strategies, mental imagery, and training to accommodate to unpredictable circumstances, which appears as one of the most beneficial therapeutic approaches in children with DCD. Promisingly, some preliminary positive results have been described for motor interventions in children using interactive computer technologies such as computer games or virtual reality. The use of these technologies can be recommended in future studies, as they give the unique opportunity to create fun and engaging environments that motivate the child to exercise, combined with the potential to reduce the costs of long periods of hospitalization and traveling long distances. Interestingly, one study showed that children with DCD improved their motor skills after ten weeks of table tennis exercise intervention, and additionally improved their interference control abilities. Indeed, there is an increasing body of literature indicating crucial interrelations between motor abilities and cognitive abilities, suggesting that other problems in cognitive and behavioral functioning may eventually also benefit from motor interventions and physical training. Based on the findings from this thesis, problems in behavioral functioning in very preterm children are mediated by differences in neurocognitive functions, including visuospatial working memory and lapses of attention (chapter 4). In children with attention deficit hyperactivity disorder (ADHD), similar differences in visuospatial working memory and lapses of attention have been described, and several interventions have been developed to specifically address and improve these underlying neurocognitive dysfunctions. These interventions include neurofeedback, working memory training, and methylphenidate treatment, which have all been shown able to effectively reduce the behavior problems in children with ADHD. Interestingly, neurofeedback may have a positive effect on default state network functioning.
addition, findings from recent studies suggested that default state network functioning and associated working memory abilities can be successfully improved using pharmacological interventions,\textsuperscript{80,81} indicating another promising area of future research.

Finally, for all future (intervention) studies, selecting the appropriate outcomes measures is extremely important. Based on the findings of this thesis, the potential effects of interventions can be powerfully quantified using neurocognitive task outcomes and measures from brain imaging (structural MRI, functional MRI, DTI), providing objective measures of brain functioning and brain development, respectively. Therefore, the findings from this thesis illustrate that implementing these techniques in the evaluation of future interventions directed at improving development of very preterm children should be highly encouraged.
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