General Discussion

AIMS AND MAIN FINDINGS

In this thesis, the long-term neurodevelopmental sequelae following two common causes of neonatal critical illness were studied: severe respiratory failure in need of neonatal extracorporeal membrane oxygenation (ECMO) treatment and congenital diaphragmatic hernia (CDH). To improve (early) *identification* of patients at risk, we aimed to delineate the specific neuropsychological profile and its underlying neurobiology in these survivors. Our results showed that survivors of neonatal ECMO and/or CDH have specific memory and attention deficits, despite average intelligence (chapters 2, 3, 4). These deficits are associated with alterations in the brain's limbic system, in particular the hippocampus, and global white matter microstructure (chapters 5 & 6). The second aim of our thesis was to evaluate whether Cogmed Working-Memory Training (CWMT) could be an effective *treatment* strategy in school-age survivors of neonatal ECMO and/or CDH with (working)memory deficits. We found that CWMT may be beneficial for patients who have visuospatial memory deficits by showing long-term gains in this domain following CWMT (chapter 7). Furthermore, we found training-induced changes in white matter microstructure immediately following CWMT, demonstrating neuroplasticity in these children (chapter 8). Our findings taken together led to the postulation of a common neurodevelopmental pathway across survivors of neonatal critical illness, where early hippocampal alterations result in memory deficits later in life. This 'growing into deficit' phenomenon seems to exist across survivors of neonatal critical illness, irrespective of underlying diagnosis or gestational age, and may be due to common factors associated with neonatal critical illness such as hypoxia-ischemia, neuroinflammation, stress and exposure to common analgosedatives (chapters 9 & 10).

The work presented in this thesis has shown that, even in absence of major neurological abnormalities such as hemorrhage or periventricular leukomalacia, the brain of critically ill neonates is vulnerable. Our results have increased our understanding of the long-term neurodevelopmental sequelae following neonatal critical illness. Inevitably, our findings have also proven to us that '*the more we know, the more we do not know*' (Aristotle). Two important issues remain:

1) The need for predictors or risk stratification tools to identify patients at risk early, i.e. before the neuropsychological deficits affect school performance and daily life activities.
2) Treatment strategies that prevent or specifically target (all) altered brain regions and/or neuropsychological deficits following neonatal ECMO and/or CDH.

In the following section, our main findings will be placed into a broader perspective. Furthermore, directions of future research will be discussed, aimed at improving early

identification of children at risk as well as prevention or reduction of long-term neurodevelopmental impairment following neonatal critical illness.

IDENTIFICATION OF PATIENTS AT RISK

Neuropsychological assessment

Over the last decade, increasing awareness and knowledge has emerged about the long-term neuropsychological outcome following neonatal ECMO and/or CDH[1-3], which inspired the work presented in this thesis. In initial reports on long-term neuropsychological outcome in school-age survivors of neonatal ECMO and/or CDH, sustained attention deficits and a high incidence of school problems were found, despite normal intelligence.[1-4] However, elaborate assessment of all major neuropsychological domains was lacking. In this thesis, we showed that general intellectual outcome was normal in the majority of survivors of neonatal ECMO and/or CDH from two, five, to eight years of age (chapter 2). Using elaborate neuropsychological assessment, we replicated the findings on sustained attention deficits, but also found specific short- and long-term memory deficits in over half of these children at school-age (chapter 3). Strikingly, using a similar test battery, we demonstrated similar deficits in short- and long-term visuospatial and verbal memory in a group of 17-year-old adolescent survivors of neonatal ECMO, while other neuropsychological domains remained relatively unaffected (chapter 4). Although longitudinal assessment will be needed to increase our understanding of the neurodevelopmental trajectories following neonatal ECMO and/or CDH, this was the first study that performed elaborate neuropsychological assessment in adolescent survivors, indicating that memory deficits following neonatal critical illness are persistent from childhood into adolescence. Confirming earlier findings by our group in different cohorts of neonatal ECMO and CDH survivors[1,2], we found that a significantly higher number of survivors were in need of extra help in school compared to the general population at both 8 and 17 years of age (chapters 2 & 4), We found that these school problems were related to the specific neuropsychological deficits, rather than to general intellectual outcome (chapter 2).

Comparing our findings to neuropsychological outcome in other children who survived a period of neonatal critical illness without serious neurological sequelae shows striking similarities. Although lower IQ has been reported in children born preterm (< 37 weeks of gestation)[5], intelligence has generally been shown to be within the low average to average range across survivors of neonatal critical illness.[5-7] In children growing up after preterm birth and complex cardiac anomalies, impairments have been demonstrated across multiple neuropsychological domains, such as attention, visuospatial processing, executive functioning, and memory and learning.[5-10] Just as in survivors of

neonatal ECMO and/or CDH, memory deficits, both short- and long-term verbal and visuospatial memory are among the most frequently reported neuropsychological sequelae. Following preterm birth, memory deficits have been found to persist from childhood into adolescence and even into young adulthood.[5,11-14] In survivors of cardiac anomalies of differing complexity, short- and long-term verbal and visuospatial memory deficits have been demonstrated as well, becoming increasingly evident with age.[6,8,15] Unsurprisingly, the incidence of school problems is strikingly high across these patient groups[5-7], making long-term neuropsychological sequelae following neonatal critical illness a major concern.

While intelligence is generally in the low-average to average range in these children, the assessment of IQ often remains the main outcome parameter in the few long-term follow-up protocols that are available.[16,17] This is problematic as specific neuropsychological deficits are difficult to pick up using a global outcome measure such as an IQ test.[18] As such, problems in school and/or daily life remain misunderstood and targeted intervention strategies cannot be implemented. Standardized, problem-oriented neuropsychological follow-up that includes all major neuropsychological functions is therefore highly recommended following neonatal critical illness. Important to note is that, as we have shown that IQ at 5 years of age is highly predictive of IQ at 8 years of age, it may be sufficient to conduct a full-scale IQ test at 5 years and a short-form test at 8 years of age to increase efficiency.[19] As higher-order cognitive functions, such as attention and memory, continue to develop throughout childhood and into adolescence[11,20], follow-up assessments should take place both at school-age and into adolescence. In line with this, assessment of general intellectual ability at 24 months (corrected age), e.g. with the commonly used Bayley Scales of Infant and Toddler Development–Third Edition[21], will not identify those patients at risk of neuropsychological deficits. Preferably, we would like to identify children at risk of memory and attention deficits at this time, i.e. well before the neuropsychological deficits have interfered with school performance and activities in daily life. Studies using eye-tracking in infants to assess long-term memory and attention show promising results.[22,23] Future longitudinal studies are needed in survivors of neonatal critical illness that compare memory and attention assessed with eye-tracking in infancy to outcomes from neuropsychological assessment later in childhood to determine the utility of outcomes in infancy as early predictors.

Neuroimaging

Our findings of specific attention and memory deficits emerging in childhood and persisting into adolescence following neonatal ECMO and/or CDH, suggested a 'growing into deficit' phenomenon where subtle brain injuries acquired at a young age become functionally evident over time when demands on cognitive functioning increases.[24] This 'growing into deficit' is nested within different developmental processes that occur in the

brain (e.g. myelination, synaptic pruning and neurogenesis[25]). An important next step in understanding neurodevelopment following neonatal critical illness was therefore to study the underlying neurobiology of long-term neuropsychological impairments.

Using Diffusion Tensor Imaging (DTI) in neonatal ECMO survivors, we demonstrated global as well as specific white matter alterations in the cingulum bundle and parahippocampal part of the cingulum in school-age neonatal ECMO survivors compared to healthy controls (chapter 5). White matter microstructure has previously been found to be particularly vulnerable in the neonatal period, a time when it is undergoing rapid development.[26] In particular the limbic system fibers (i.e. cingulum bundle and parahippocampal part of the cingulum) develop rapidly in the first six months of life, causing fractional anisotropy (FA) to increase and mean diffusivity (MD) to decrease in these tracts.[26] As our subjects were critically ill in the first weeks of life, the development of these specific fibers may therefore be at increased risk.

Since white matter is important for high-speed transmission of neuronal signals between distant brain regions, aberrations in white matter development could affect the orchestration of specific cognitive functions.[26] Indeed, in another cohort of school-age survivors of neonatal ECMO and/or CDH in which we combined neuroimaging with neuropsychological assessment, we found that lower global FA, potentially indicative of reduced coherence of white matter fibers[27], was associated with sustained attention deficits (chapter 6). Global white matter abnormalities have been found in preterm born infants with attention deficits as well.[28] This shared vulnerability in both preterm and term-born neonates may be due to the increased susceptibility of white matter, in particular in the periventricular regions, to hypoxic-ischemic insults – a common complication in critically ill infants. Using animal models, premyelinating oligodendrocytes (pre-OLs) in cerebral white matter have been found to be selectively targeted by oxidative stress. These cells account for approximately 90% of the total oligodendroglial population at 28 weeks of gestation and approximately 50% at term.[29] Increased regional susceptibility of the periventricular white matter is suggested to be due to the distribution of these pre-OLs and relative underdevelopment of distal arterial fields to these areas.[29,30] Neonates exposed to hypoxic-ischemic injuries, both born preterm and at term, may therefore be at increased risk of myelin and axonal disruptions, resulting in white matter abnormalities. As more widespread white matter networks have been found to be underlying attention, this may explain the attention deficits in these children.[31] In addition to the association between global white matter alterations and attention, we found that higher MD in the parahippocampal part of the cingulum, suggestive of decreased integrity in axonal membranes, packing, or myelin[27], was related to long-term visuospatial memory deficits in survivors of neonatal ECMO and/or CDH (chapter 6). This is in line with earlier findings demonstrating that the parahippocampal part of the cingulum, which is bidirectionally connected to the hippocampus, forms

a larger memory circuit together with cortical structures and is thus highly important for intact functioning of various memory types.[32] Taken together, our results suggest that white matter microstructure alterations acquired early in life may have long-lasting implications in survivors of neonatal critical illness.

Given the high incidence of memory deficits (chapters 3 & 4), we also investigated the hippocampus using structural MRI in school-age neonatal ECMO survivors compared to healthy controls. The hippocampus, a gray matter structure within the brain's limbic system, is the critical hub for memory formation and rapidly develops within the first two years of life.[33,34] We found smaller bilateral hippocampal volume in the ECMO survivors compared to healthy controls, which was negatively associated with the ability to recall information from a story that the children had just heard, a measure of episodic memory (chapter 5). In a different cohort of school-age survivors of both neonatal ECMO as well as CDH treated without ECMO, we found these same structure-function relationships (chapter 6). Interestingly, neither the underlying diagnosis (such as meconium aspiration syndrome or CDH) or the type of ECMO-cannulation (venoarterial or venovenous) affected these associations (chapter 6). Although these findings should be interpreted with caution due to the small sample size, they suggest that factors other than diagnosis and treatment determine long-term neurodevelopmental outcomes in these patients.

Placing our findings in a broader perspective, we found that other groups of critically ill infants without overt neurological abnormalities had similar neurodevelopmental outcomes. As described previously, memory deficits are frequently reported across survivors of neonatal critical illness, such as in children following preterm birth and complex cardiac anomalies.[5,6,8,11-15] We therefore wondered whether these memory deficits would be associated with hippocampal alterations in these patients as well (chapter 9). In school-age children who experienced neonatal hypoxia, structural MRI combined with memory assessment demonstrated specific smaller bilateral hippocampal volumes associated with memory deficits in patients compared to healthy controls.[35] These structure-function relationships existed in both children treated with and without ECMO[35], confirming findings in our study population (chapter 6). In this population, the term "developmental amnesia" has been used to describe markedly impaired event, or episodic, memory and relatively preserved fact, or semantic, memory following hypoxic-ischemic insults sustained within the first year of life.[36,37] This has been suggested to be due to relatively selective bilateral hippocampal pathology.[36,38] This pattern of memory deficits and hippocampal pathology, despite relatively intact intellectual abilities, is remarkably similar to the significant impairments in delayed recall and hippocampal volume loss observed following neonatal ECMO and/or CDH (chapter 6), as well as to other groups of critically ill infants. In children born preterm, abnormalities in the hippocampus with impaired long-term memory have been reported as well.[12,39-42] In children with complex congenital heart disease, smaller bilateral hippocampal volumes were demonstrated

in 40% of school-age children who had dextro-Transposition of the Great Arteries and cyanosis compared to healthy controls. These hippocampal reductions were associated with memory deficits.[43] In line with this, 13-year-old children who had undergone cardiopulmonary bypass surgery in infancy had smaller bilateral hippocampal volumes as well as volume loss in other parts of the limbic system's gray matter compared to healthy controls.[44 43,44] The findings reported in these four common causes of neonatal critical illness taken together led to the postulation of a common neurodevelopmental pathway following various types of neonatal critical illness, where early hippocampal alterations result in memory deficits later in life, irrespective of underlying disease or gestational age (chapter 9). As memory problems can greatly affect daily life activities and academic achievement, this is of major concern. Early identification of patients at risk of memory deficits may become possible using hippocampal volume as a neurobiological marker. The hippocampus can be accurately and non-invasively delineated using structural MRI and is the brain's critical hub for long-term memory formation.[33,34] Because of these features, the hippocampus is an important target for future studies aimed at improving long-term outcomes following neonatal critical illness. In preterm infants, studies have shown that MRI can be reliably performed without sedation and that infant hippocampal volumes, measured at term-equivalent age, correlated with memory outcomes later in childhood.[12,45] A critical period of hippocampal development is in the first two years of life when it undergoes a growth spurt.[34] The hippocampus would thus ideally be measured after the first two years of life. However, unless with the use of sedation, it will be difficult to perform a reliable MRI scan at this time, let alone desirable for the patient. Since sedatives may have an additive negative effect on the developing brain (chapter 10), this should be avoided. Furthermore, the rapid development of the hippocampus within the first two years of life likely interacts with the exposure to deleterious factors associated with neonatal critical illness. Therefore, longitudinal assessment may lead to finding the optimal time to assess hippocampal volume and identify specific periods of sensitivity. Such longitudinal data should be coupled with memory assessment later in childhood to evaluate the utility of hippocampal volume as a prediction tool.

Pathophysiological mechanisms

Standardized, problem-oriented neuropsychological assessment can be used to understand why survivors experience difficulties at school or in daily life activities. In its present form, neuropsychological assessment is therefore a diagnostic tool rather than a prediction tool. Furthermore, the use of neurobiological correlates as early predictors, such as smaller hippocampal volume, is promising but will only become feasible once normative hippocampal volumes become available. As we should strive to identify and treat patients at risk well before the neuropsychological deficits have hampered their

school performance, it is imperative that we understand why and how patients develop these deficits.

In chapter 3 of this thesis, we analyzed associations between clinical characteristics at the time of hospitalization and neuropsychological outcome at 8 years of age. We found a specific negative association between the maximum dose of vasoactive medication received during first admission (measured by the Vasoactive Inotropic Score; VIS) and long-term verbal and visuospatial memory following neonatal ECMO and/or CDH. In these analyses, we adjusted for diagnosis and various measures of severity of illness, suggesting that a specific association exists between the VIS and memory later in life (chapter 3). Although currently speculative, receiving high levels of vasoactive medication in the first period of life may be an indirect marker of temporarily (regional) inadequate brain perfusion. As the hippocampus is particularly vulnerable for hypoperfusion and/or hypoxia, the association between the VIS and memory may be the indirect result of this pathophysiological mechanism. In addition, previous findings from both preclinical and clinical studies have shown that the hippocampus shows pronounced vulnerability to hyperoxia.[46-50] Hyperoxia, experienced by critically ill infants in need of oxygen supplementation[51,52], may therefore also play a role in the hippocampal alterations and memory deficits observed following neonatal critical illness. Importantly, the current lack of insight into how hypoxia/hyperoxia may affect hippocampal development in these children has direct clinical implications as the optimum resuscitation strategy in critically ill infants remains a topic of debate.[53,54] Future research that collects dense and detailed data on continuous oxygen saturation and supplemental oxygen supply will allow us to closely monitor oxygen fluctuations in critically ill neonates. Analyzing a combination of absolute values and the area under the curve[55] will provide more detailed information on the exposure to different levels of oxygen throughout the infant's hospital stay. Coupled with outcome parameters such as hippocampal volume and/or memory functioning, this data can provide insight into the extent to which hypoxia, hyperoxia or a combination of both, influences the hippocampus and memory in later life. To further understand the molecular, cellular and behavioral consequences of exposure to hypoxia/hyperoxia on the pathophysiology of the neonatal hippocampus, experimental studies using *in vitro* and *in vivo* animal models are of interest. In these types of experiments, the effect of hypoxia and/or hyperoxia on the hippocampus and memory at different stages of development can also be taken into account by including both a preterm model and a full-term model.[47,56,57] Such translational studies may eventually contribute to earlier identification of critically ill infants at risk of long-term deficits. Moreover, they may also lead to adjustments in the resuscitation strategy and thus the prevention or reduction of damage to the developing brain in critically ill neonates.

Importantly, in addition to hypoxia-ischemia, critically ill infants are exposed to many other potentially deleterious factors (chapter 9). A complex interplay amongst different factors associated with the underlying disease, (pharmacological) treatment and "iatrogenesis" are therefore likely to determine a child's neurodevelopment, further complicated by the child's genetic predisposition[58] and social economic status[59]. Using comprehensive literature reviews, in chapters 9 and 10 of this thesis we explored how these factors may affect the developing brain, and in particular the hippocampus, in critically ill infants. Because of its highly excitable and plastic nature, the hippocampus shows pronounced sensitivity to both internal and external influences and may therefore be particularly vulnerable in critically ill infants.[49] However, the exact pathophysiological mechanisms underlying hippocampal alterations and subsequent memory impairments in these children remain largely unknown. A first step will be to collect dense data on factors associated with neonatal critical illness, such as oxygen fluctuations, analgesic and sedative use, metabolic profiles[60,61], and neuromonitoring data[62] throughout hospital stay. Since children following neonatal critical illness 'grow into their deficits', longitudinal assessment is imperative. The detailed clinical information collected during initial hospital stay should therefore be coupled with neuroimaging and problem-oriented, standardized neuropsychological assessment at later stages of development. Such detailed data collection in combination with longitudinal assessment of neurodevelopment may allow us to identify which specific pathophysiological conditions lead to alterations in hippocampal volume and memory deficits later in life in these children. Furthermore, with a longitudinal design, windows of sensitivity (i.e. when infants are most at risk of impairment, the optimal timing of MRI or neuropsychological assessment) and opportunity (i.e. when it is best to implement therapy or interventions) can be identified. As differences in brain development and the timing of critical illness (e.g. preterm versus full-term brain) are likely to influence how and when the hippocampus is affected, a longitudinal design that starts collecting data in the prenatal period, for instance with the use of prenatal ultrasounds[63], is of interest as well. Favorably, the outcome parameters, such as the neuroimaging data and neuropsychological data, are compared to healthy control data to understand when hippocampal volumes and memory are different from the norm. The Generation R study, a pediatric population study in which over 3000 healthy children have undergone longitudinal neuroimaging, is a good example of such a healthy control cohort[64], which may make the use of normative brain parameters feasible in the future. In our institution, a longitudinal follow-up program in children who have been critically ill in the neonatal period has recently been initiated (Systematic Hospital-based Assessment of Rotterdam's (critically) Ill Infants' Neurodevelopment and Growth: S.H.A.R.I.N.G.). It is important to keep in mind that when comparing neuroimaging data, the image acquisition and type of analyses[65,66] as well as the scanner itself[67] may influence the results and should thus be similar or controlled for

in the analyses. Therefore, large sample sizes are needed. In this respect, multicenter collaborations should be a goal for future studies assessing long-term neurodevelopment following neonatal critical illness as well.

TREATMENT

Neurorehabilitation

Given the complex interplay of factors that are likely to affect the developing brain in critically ill infants (chapters 9 & 10), rehabilitation strategies aimed at improving impaired neuropsychological functions are of great interest as well. Cogmed Working-Memory Training (CWMT) is a widely evaluated cognitive training for both children and adults.[68] In this thesis, we reported the results of a nationwide, single-blind randomized controlled trial on the immediate and long-term effectiveness of CWMT in school-age survivors of neonatal ECMO and/or CDH with (working)memory deficits. Neuropsychological outcome was assessed before, immediately and one year after CWMT, and white matter microstructure was assessed before and immediately after CWMT (chapters 7 & 8).

Immediately after CWMT, we found significant improvements in verbal and visuospatial working-memory in the CWMT group compared to the non-training group (chapter 7). These findings are in line with the effects demonstrated in other clinical and non-clinical groups after CWMT.[69-72] Coupling our data with the neuroimaging findings immediately post-intervention, we found that the improvements in verbal working-memory were associated with an increase in FA in the left superior longitudinal fasciculus (chapter 8). The frontoparietal network has been consistently shown to be affected by working-memory training and is thus a common finding following CWMT.[68,73,74] The specific association between the superior longitudinal fasciculus in the left hemisphere and verbal working-memory may be due to the fact that working-memory is lateralized, i.e. verbal working-memory corresponds with the left hemisphere while visuospatial working-memory corresponds with the right hemisphere.[75,76] Furthermore, as the majority of children in our cohort were right handed (80%), which is generally associated with left hemispheric dominance for language[77], this may also explain this association. Nonetheless, after one year, we found that the improvements in working-memory had disappeared (chapter 7). This is in contrast with two previous studies that have assessed long-term outcome following CWMT in children. Gains in working-memory performance have been found in very low birthweight children seven months post-intervention[69] and in healthy children with working-memory problems one year post-training.[78] However, working-memory was found to be within the average range in our population at baseline (chapter 7). As the children studied in the other two long-term studies did have significant working-memory deficits[69,78], this may explain the incongruent findings. Although speculative,

the benefits of the training – such as an increased capacity to learn and manipulate information – may be more prone to subside after a while in our population because an increase in this particular function was not needed to begin with, i.e. the "use it or lose it" principle. Furthermore, rehabilitation treatment may be disease-dependent, e.g. have different effects in survivors of childhood cancer than in children treated with neonatal ECMO.[79,80]

One year after CWMT, we did find significant and sustained improvements in long-term visuospatial memory in the CWMT group compared to non-trained controls (Chapter 7). These improvements were not associated with microstructural changes immediately after CWMT (chapter 8). At baseline, we found an association between long-term visuospatial memory deficits and higher MD in the parahippocampal part of the cingulum (chapter 5). We therefore expected that improvements in this domain might have been accompanied by training-induced changes in this white matter tract. Since the improvements in long-term visuospatial memory found in our cohort increased from the assessment immediately post-intervention to one year later in the CWMT group compared to the control group, neurobiological changes may have only become detectable one year post-intervention. In line with this, a recent study has shown that a reverse relationship can exist between the brain and behavior, where behavior is actually shaping the brain, rather than the commonly assumed direction of the brain shaping behavior.[81] Such a downstream mechanism may explain why the improvements in long-term visuospatial memory in the CWMT group were not associated with changes in white matter microstructure immediately post-intervention. For instance, an increased ability following CWMT to memorize the location of information within a certain visual context may lead to increased use of this tactic to improve the ability to encode and recall information in various situations, thereby affecting the white matter connections underlying these abilities. However, this remains speculative as the MRI exam was unfortunately not repeated at this time. It is also important to note that neurobiological changes underlying these memory improvements may simply not have been detectable using DTI.[82] Nonetheless, given the fact that over 50% of children following neonatal ECMO and/or CDH has long-term visuospatial and verbal memory deficits at school-age (chapter 3), improving memory in these children is of great importance. We found that larger improvements in long-term visuospatial memory were significantly associated with higher scores on self-rated school functioning and parent-rated attention one year after CWMT (chapter 7). Additionally, the majority of children trained with CWMT and their parents reported to be happy with the results and to see improvements in memory and attention (data not shown). These findings taken together may suggest that the improvements in long-term visuospatial memory in the CWMT group have generalized to daily life activities. If so, intervention before memory problems have interfered with school performance should be strived for. In children with very low birthweight,

memory improvements have been found six months after CWMT at preschool-age[69], suggesting earlier intervention may lead to similar results. However, these results need to be replicated in preschool survivors of neonatal ECMO and/or CDH with long-term visuospatial memory deficits, as well as in other survivors of critical illness such as following complex cardiac anomalies, before any definitive conclusions can be drawn. In future trials, neuropsychological assessment and neuroimaging should be conducted both immediately and one year post-intervention.

Overall, our findings demonstrate that cognition and white matter microstructure are malleable with CWMT in survivors of neonatal ECMO and/or CDH. Working-memory was the primary outcome measure in our trial, which had been based on initial reports of neuropsychological outcome in our study population.[1-3] However, ongoing research, as described in this thesis, led to new insights of primarily short- and long-term memory and sustained attention deficits following neonatal ECMO and/or CDH. Although CWMT may be beneficial for survivors of neonatal ECMO and/or CDH with visuospatial memory deficits, it is not the (complete) answer to the long-term neuropsychological deficits observed in these children. Importantly, our results demonstrated that it is essential to conduct an elaborate neuropsychological assessment before initiating CWMT in survivors of neonatal critical illness to determine its clinical utility. Furthermore, if multiple domains are affected in a child, treatment strategies should ideally affect multiple domains as well. A combination of different intervention programs may therefore be of interest. Findings from both experimental and clinical studies have suggested that multimodal training leads to better results compared to a single training program.[83,84] Exercise training in children has been found to affect memory and learning by targeting the hippocampus.[85] Combining such a physical program with cognitive training aimed at improving attention or memory, may strengthen the results and be beneficial in survivors of neonatal critical illness.

However, these are future perspectives and of little use in today's clinical practice. Currently, survivors of neonatal critical illness with long-term neuropsychological deficits may have to manage with practical tools to improve school performance and daily life activities. To improve long-term outcome in these children, we recommend that survivors of neonatal critical illness receive information on the practical implications of the deficits they may experience (e.g. difficulty remembering homework that is due tomorrow or appointments with friends), as well as learn about compensatory techniques or external (memory) aids that may be used to improve their activities of daily living (e.g. errorless learning, mental imagery to improve recall, writing important things down and using a schedule book[80]). Ideally, this information is personalized to the patient's specific impairments and needs. Personalized information and practical tools can be realized by conducting neuropsychological assessment to evaluate the degree of neuropsychological deficits, as well as by evaluating the degree to which these deficits affect activities of

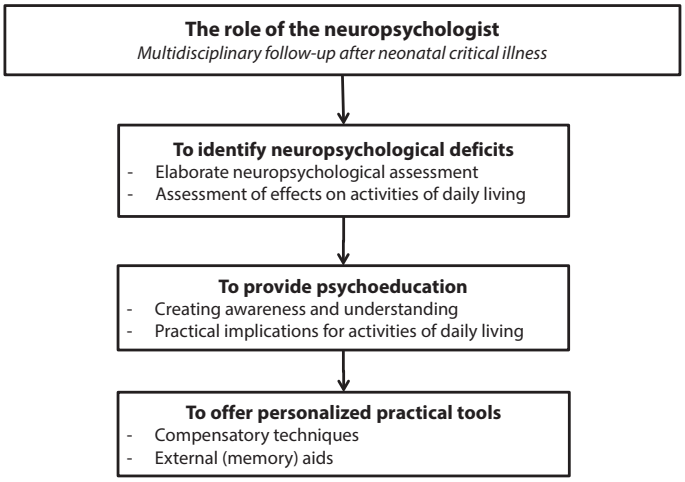

Figure 1. The role of the neuropsychologist in multidisciplinary follow-up after neonatal critical illness

daily living in the patient.[80] The neuropsychologist as such can play an essential role in (the improvement of) long-term outcome following neonatal critical illness (figure 1). A multidisciplinary approach to long-term follow-up after neonatal critical illness should therefore be strived for.

Prevention

Ideally, the deleterious effects of neonatal critical illness on the neonatal brain should be prevented. This may be (partly) accomplished by fine-tuning therapy or treatment strategies, but may also be achieved with the use of neuroprotective agents in the future. While it remains unknown which pathophysiological mechanisms are most detrimental to the developing brain, we do know that the hippocampus is highly vulnerable in critically ill infants (chapters 9 & 10). Besides hypothermia (chapter 9), the use of pharmacological agents that may have neuroprotective effects may therefore be of great value in critically ill infants. For instance, the effect of maternal allopurinol, which may protect the fetus against hypoxic-ischemic brain injury, is currently being investigated.[86] Here, we mention two other agents that are commonly used in the NICU and may have neuroprotective effects.

Dexmedetomidine, used in particular for sedation in the pediatric ICU population, may have neuroprotective effects on the hippocampus, in particular against hypoxic-ischemic damage.[87] These effects have been suggested to result from an activation of α_2-adrenergic receptors by dexmedetomidine, which inhibits inflammation following brain ischemia.[88] As the hippocampus has been found to be vulnerable to both hypoxia-ischemia as well as inflammation (chapter 9), this specific mechanism of action is of interest. However, these findings are mostly based on animal models and studies in adult

populations.[87,88] Future clinical trials that assess the efficacy and safety of dexmedetomidine in critically ill neonates that also include neurobiological outcome parameters, such as hippocampal volume, are therefore needed. Another agent that may be of interest in this respect is erythropoietin. Erythropoietin is produced by various cell types in the developing brain as a growth factor and as an endogenous neuroprotective response to hypoxia.[89] As previously mentioned, in addition to hypoxia, high oxygen concentrations as a result of supplementary oxygen may lead to neonatal brain damage as well.[46-50] A recent study in 6-day-old rat pups showed that a single dose of erythropoietin at the onset of hyperoxia (24 hours 80% oxygen) improved memory impairment and reduced acute oligodendrocyte degeneration up to the adolescent and adult stage.[90] Given the vulnerability of pre-oligodendrocytes in the periventricular white matter during the perinatal period[29], which may potentially be (partly) underlying the attention and memory deficits observed later in life in survivors of neonatal critical illness (chapter 6), reducing microstructural abnormalities in these fibers would have direct clinical benefits. In addition, studies have found that erythropoietin may have neurotrophic effects as well by increasing synaptic plasticity in the hippocampus and improving memory formation.[90,91] The hippocampus shows a uniquely high degree of neuroplasticity, which means it has the ability to adapt and reorganize in response to internal or external stimuli.[49] Although this unfortunately seems to result in more pronounced vulnerability than plasticity – the mechanisms underlying this (im)balance remain largely unknown – its ability to generate new neurons throughout life does make it a promising target in this respect.[49] Trials on potentially neurotrophic agents such as erythropoietin are therefore of interest. In infants with extreme prematurity, hypoxic-ischemic encephalopathy, perinatal stroke, and complex cyanotic heart disease, trials have demonstrated safety, and the potential for efficacy of erytrhopoietin.[92] However, the optimal dose and regimen for neuroprotection in neonates remains largely unknown.[93] Future clinical intervention trials assessing the effects of neuroprotective agents before, during or after exposure to both hypoxia and hyperoxia are needed in critically ill neonates.

CONCLUSION

In this thesis, we have demonstrated that survivors of neonatal critical illness are at risk of sustained attention and verbal and visuospatial memory deficits, despite generally average intelligence. These neuropsychological deficits seem to be associated with specific brain alterations that are mainly located in the brain's limbic system. In particular, we demonstrated that hippocampal alterations and associated memory deficits exist across survivors of neonatal critical illness, irrespective of underlying disease or gestational age. We suggest that this common neurodevelopmental pathway across survivors

of neonatal critical illness is due to factors associated with neonatal critical illness, such as hypoxia-ischemia, inflammation, stress, and analgosedatives, or a complex interplay amongst these factors. Our findings have further demonstrated that CWMT could be considered for school-age survivors of neonatal ECMO and/or CDH who have long-term visuospatial memory deficits. It is therefore important that, in today's practice, neuropsychological assessment is conducted before a child starts an intervention program such as CWMT to establish its clinical utility. Although it is promising that we find neurodevelopmental outcome to be malleable following neonatal critical illness, CWMT is not the optimum solution as multiple neuropsychological domains are affected in these children. To improve outcome following neonatal critical illness in current practice, providing psychoeducation, compensatory techniques and external (aids) should become a standard part of (long-term) care following neonatal critical illness.

The findings described in this thesis have underlined the necessity of broadening our focus from short-term to long-term outcome following neonatal critical illness. Given the increasing number of critically ill infants that survive today[94,95], future research directed at improving long-term outcome by protecting the vulnerable brain, particularly in the newborn period, is of utmost importance.

FUTURE RESEARCH DIRECTIONS

- Future research should aim to gain insight into the exact pathophysiological mechanisms underlying long-term neuropsychological deficits following neonatal critical illness. This may lead to the identification of clinical, iatrogenic or therapeutic factors that are most detrimental to the developing hippocampus in critically ill infants, which will improve risk stratification and better targeted treatment.
- As critically ill infants 'grow into their deficits', longitudinal studies that combine dense data collection in the perinatal period with follow-up neuroimaging and neuropsychological assessments are imperative. This will allow us to find 'windows of sensitivity' (i.e. when infants are most at risk of impairment, the optimal timing of MRI or neuropsychological assessment) as well as 'windows of opportunity' (i.e. when it is best to implement therapy or interventions).
- To improve outcome, future studies on the efficacy and safety of pharmacological therapy in critically ill infants should include the assessment of its effects on the developing brain in addition to other outcome parameters.
- Future trials on cognitive interventions following neonatal critical illness should be problem-oriented, i.e. focused on those neuropsychological domains or brain areas most at risk in survivors of neonatal critical illness.

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