DISCUSSION

General discussion and future perspectives
Sleep, the reversible state of unconsciousness we spend a third of our lives in, still holds many a mystery to modern science. Past studies have shown that sleep supports daytime cognitive performance. Conversely, experimentally induced partial and total sleep deprivation in healthy adults can lead to a host of negative consequences within the affective, cognitive, and motor domains (Pilcher & Huffcutt, 1996), suggesting that an adequate amount of sleep is essential to maintain optimal daytime functioning.

Several theories exist on the role of sleep in cognitive function. The trace reactivation or replay theory (Born & Wilhelm, 2012; Hoffman & McNaughton, 2002; Sejnowski & Destexhe, 2000; Sutherland & McNaughton, 2000; Wilson & McNaughton, 1994) proposes that sleep aids memory consolidation through reactivation of traces of neuronal activity patterns that encoded information during the prior wakeful period. The synaptic homeostasis theory (Tononi & Cirelli, 2006) suggests that synaptic downscaling during sleep counterbalances the increase in synaptic connectivity as a consequence of previous wakeful cognition. Lastly, the recently emerging overnight therapy hypothesis (Walker & Van der Helm, 2009) proposes that sleep provides a window to reset the neuronal systems involved in affect regulation and to reprocess recent emotional experiences. These hypotheses are not necessarily mutually exclusive or exhaustive. They are, furthermore, subject to ongoing discussion, elaboration, and refinement (e.g., Diekelmann & Born, 2010; Stickgold, Whidbee, Schirmer, Patel, & Hobson, 2000; Yang, et al., 2014).

Thus far, most studies of sleep and cognition are performed in adults. When starting the scientific research described in this thesis, this scientific area was still largely unexplored in children. Yet there were valid reasons, both applied and fundamental, to study the relationship between sleep and cognition earlier in development.

From an applied point of view, modern life shows a disquieting reduction in the children’s habitual sleep duration (Galland, Taylor, Elder, & Herbison, 2011; Igloiwstein, Jenni, Molinari, & Largo, 2003; Terman & Hocking, 1913). Parents, teachers, clinicians, and policy makers alike must be informed as to how this change of habits might affect cognition and behavioural problems in children. If children perform suboptimally in cognitive or behavioural domains as well as have relatively short sleep durations, it may well be beneficial to first treat the child’s sleeping problem. Early detection and treatment may be vital as detrimental effects of curtailed or disturbed sleep in children could have more, and possibly irreversible, long-term consequences than is the case in adults (Beebe, 2011; Touchette, et al., 2007), a contention that is supported by animal studies (e.g., Frank, Issa, & Stryker, 2001; Seugnet, Suzuki, Donlea, Gottschalk, & Shaw, 2011).
From a fundamental scientific point of view, studies in adults are beginning to define likely candidates for the neurobiological mechanisms by which sleep restriction affects brain function, cognition and behavioural problems. Because brain structure, physiology, and function are different in children, defining differences and similarities in the effects of sleep restriction on cognition and behavioural problems along the developmental trajectory provides a unique opportunity to further elucidate these tentative neurobiological substrates of vulnerability to sleep restriction. This, in turn, will aid our understanding of the functions of sleep for the both the developing and mature brain.

Given these important reasons, this thesis examined the relationship between sleep and cognition and behavioural problems in school-age children, using a range of different techniques. Reiterating the introduction of this thesis, the main questions we aimed to address were as follows:

1. Is sleep (duration or efficiency) related to cognition or behavioural performance in children? More specifically, which consequences can we expect following sleep restriction?
2. How does children’s sleep change in response to a shortening of its duration? Do children show a similar compensatory sleep response to sleep restriction as adults? Does this compensatory response persist in the face of a prolonged stressor?
3. Looking at children’s sleep in more depth, can we find evidence of wake-like cognitive activity, specifically during slow waves? A potential mechanism behind sleep-dependent memory consolidation is the co-occurrence of electrophysiologically measured wake-like cortical high frequency (gamma) oscillatory activity and sleep’s slow oscillations. This mechanism has been difficult to verify in adults, due to the low amplitude of their cortical gamma activity. Children may provide an optimal situation to investigate this, due to the larger amplitude of their cortical oscillations, across the frequency spectrum.
4. Can we find direct evidence for a relationship between sleep and memory performance in children? Furthermore, can we find evidence for a relationship between sleep spindles or slow waves and cognitive performance, similar to what has previously been suggested in adults?
5. How does children’s sleep change in response to a shortening of its duration—a capacity that potentially is attenuated during development into adulthood.

Below, the main findings will be discussed in a point-by-point fashion.

In chapter 2 we systematically summarised all relevant past studies relating children’s sleep (duration and/or efficiency) to cognition or behavioural problems to investigate whether we could find similar associations as previously demonstrated in adults. We performed an all-compassing meta-analysis, a scientific method to systematically and statistically evaluate all past scientific studies and their outcomes. A total of 86 studies on 35,936 children (5–12 years old) was found suitable for inclusion and statistically summed in order to answer the below five questions.

The primary question addressed by the current meta-analysis was whether sleep is associated with cognition and behavioural problems in children. We found that sleep duration shows a small but significant positive relationship with cognitive performance. Additionally, shorter sleep duration is associated with slightly more behavioural problems. The results regarding less efficient sleep were less conclusive but suggested a lack of association.

The second question, whether domains of cognition and behavioural problems are differentially sensitive to sleep, revealed specific associations of sleep duration with executive functioning, with performance on tasks that address multiple cognitive domains, and with school performance, but not with intelligence. Quite unlike typical findings in adults, sleep duration was not associated with sustained attention and memory. Furthermore, the relationship holds for both internalising and externalising behavioural problems.

The third question addressed whether the profile of differential sensitivity in children matched the profile previously described in the scientific literature of studies on adults. Although a partial overlap was seen, child studies surprisingly failed to show significant associations of sleep with sustained attention and memory, whilst these may be the two domains most robustly associated with sleep in adults. Both methodological issues and brain developmental immaturities were proposed to underlie the marked differences. Methodological aspects included whether a study was experimental or observational, the different way in which children perform tasks, floor and/or ceiling effects, participant selection criteria, and environmental and context issues. Developmentally, children possibly cannot yet fully benefit from sleep, nor fully suffer from sleep loss. Alternatively, children’s performance benefits may not be limited to sleep, but may also occur over periods of wakefulness—a capacity that potentially is attenuated during development into adulthood.

One of the key features of a meta-analysis is its ability to examine past literature and to assess which past research features were most effective in studying the topic of interest. Future studies may wish to consider the recommendations provided by our meta-analysis. First, sleep assessment methodology is of importance. Studies that measured sleep more accurately found stronger associations between sleep and cognitive performance. Likewise, studies that measured cognitive performance more accurately found stronger associations between sleep
and cognitive performance. In addition, experimental studies are more likely to reveal associations than observational studies. Finally, it is important to examine gender differences, to incorporate standard reporting methods on socioeco-
nomic status and ethnicity and to start a more in-depth investigation into develop-
mental changes (i.e., ageing from infancy to adolescence). For instance, studies using narrow age ranges can highlight effects that are concealed in studies incorporating a wide range of ages, and longitudinal studies can provide essential information on how neuronal maturation and the relation of sleep to cognition and behavioural problems interact.

In summary, this meta-analysis was the first to systematically summarise all relevant studies reporting on sleep, cognition, and behavioural problems in healthy school-age children. In practical terms, the findings suggest that insufficient sleep in children is associated with deficits in higher-order and complex cognitive functions and an increase in behavioural problems. This is particularly relevant given society's tendency towards sleep curtailment.

In chapter 3 we examined the interaction between sleep restriction and stress in children. In particular, we investigated how objectively measured sleep quantity and quality and subjectively measured stress, fatigue, and sleep quality change when children attend school (and thus are subjected to sleep restriction due to set morning get-up times), as compared to when they do not attend school. Past studies in adults have shown that a curtailment of sleep duration results in a compensatory sleep efficiency response. We wished to determine whether the imposed sleep restriction would lead to a similar compensatory sleep efficiency response. Second, we wished to determine what the additional effects of a daily stressor would be on these children's sleep by measuring stressful school weeks. Can the compensatory mechanism persist when faced with a real life challenge?

For this purpose, twenty-four adolescents aged 17.63 ± 0.10 years (M ± SEM) wore actigraphs and completed diaries on subjective stress, fatigue, sleep quality, number of examinations and consumption of caffeine and alcohol for four weeks during their final year of secondary school. This naturalistic ecologically valid quasi-experimental repeated-measures study allowed for a comparison between sleeping patterns during a week's holidays (low-stress extended-sleep) to those in a regular week of school (low-stress restricted-sleep), to two stressful examination weeks (high-stress restricted-sleep).

The resulting assessments were analysed using mixed-effect models to estimate the effects of mere school attendance and additional examination stress on sleep estimates and subjective ratings. Total sleep time decreased from 7:38 h ± 12 min during the low-stress extended-sleep period to 6:40 h ± 12 min during the low-stress restricted-sleep period. This 13% decrease in total sleep time during a regular school week elicited a partial compensation, as indicated by a 3% in-
crease in sleep efficiency and a 6% decrease in the duration of nocturnal awaken-
ings. During examination weeks total sleep time decreased to 6:23 h ± 8 min, but this was now accompanied by a decrease in sleep efficiency and subjective sleep quality, and an increase in wake bout duration.

Thus, during a normal week of school, when children are able to spend less time in bed than they may like or need, the children’s sleep showed a compensatory mechanism (i.e., the shorter length of time in bed is spent more fully asleep). However, in a stressful situation this compensation could no longer be maintained, and relatively more time in bed was spent being awake. This suggests an attenuation of the normal compensatory sleep efficiency response to sleep restriction when children are exposed to a chronic stressor.

In chapter 4 we investigated children’s sleep at a more mechanistic level, hoping to find elements within the electrophysiological patterns of sleep that could support sleep-dependent trace reactivation learning in children. Deep sleep is character-
ised by slow waves of electrical activity in the cerebral cortex. They represent alter-
ating down states and up states of, respectively, hyperpolarisation with accom-
ppanying neuronal silence and depolarisation during which neuronal firing resumes. The up states give rise to faster oscillations, notably spindles and gamma activity which appear to be of major importance to the role of sleep in brain function and cognition. Animal studies have suggested that the co-occurrences of high fre-
quency oscillations and slow waves may represent the mechanism by which new memories are transferred from the short-term store to a more long-term neo-
cortical representation (Mena-Segovia & Bolam, 2011; Mena-Segovia, Sims, Magill, & Bolam, 2008; Rosanova & Ulrich, 2005; Steriade, Amzica, & Contreras, 1996). Unfortunately, whilst spindles are easily detectable, gamma oscillations are of very small amplitude and confounded by muscle activity in surface EEG recordings.

No previous sleep study has succeeded in demonstrating modulations of gamma power along the time course of slow waves in human scalp EEG. As a consequence, progress in our understanding of the functional role of gamma band oscillations during sleep has been limited to animal studies and exceptional human studies, notably those of intracranial recordings in epileptic patients. We argued that children may provide an optimal model to investigate wake-like ac-
tivity during sleep as their developmental stage reveals more pronounced electrophysiologically detectable oscillations (Campbell, Grimm, De Bie, & Feinberg,
performed better. Those children with a higher density of slow spindles and a slower average frequency of slow waves showed lower initial and lower overall performance levels, yet the greatest overnight improvement in accuracy.

We concluded that—similar to adults—children show a strong sleep-dependent enhancement of motor skill accuracy. However, children are able to increase the speed of their motor skill performance even without sleep, a capacity that seems to disappear in adulthood. Children whose spindles and slow waves are of lower dominant frequencies performed worse initially, which allowed for a stronger increase in accuracy following subsequent sleep. Consequently, if associations between spindle and slow wave characteristics and initial performance levels are not accounted for, they may confound interpretation of sleep’s involvement in overnight consolidation. Slower spindle and slow wave frequencies may reflect immaturity of the neuronal networks involved in motor skill learning.

GENERAL CONCLUSIONS

In conclusion, the studies contained in this thesis show a number of important relationships between sleep and cognition in children.

When examining all past scientific literature on children’s sleep, the oldest article dating as far back as 1913, we found great inconsistencies in studies’ findings on the relationship between sleep and daytime functioning. By statistically summarising all past findings through meta-analysis we were able to definitively conclude that even when using the roughest estimate of sleep—namely sleep duration—mild associations could be detected with cognitive performance, in particular in higher-order and complex cognitive functions, and behavioural problems.

In practical terms, these findings imply the following: in our current society, children may on the whole sleep less than they ideally should. Given the beneficial effects of sleep for children’s complex cognitive performance, this may have detrimental consequences for both their current and future performance (Touchette, et al., 2007). Greater emphasis should be placed on the importance of sleep for cognitive development, targeting childcare professionals, teachers, healthcare professional, policy makers, and parents alike. Furthermore, it may be valuable to investigate whether interventions aimed at increasing sleep duration in school-age children could improve complex cognitive functions and school performance, whilst ameliorating internalising and externalising behavioural problems.

Our actigraphic examination, however, revealed the complex nature of the consequences of sleep restriction. We showed that, in adolescence, sleep...
restriction could be—partially—counterbalanced by an increase in sleep efficiency. However, when faced with an ongoing stressor, the compensatory response could not be maintained. The sleep efficiency response could not keep up, and thus the quality of sleep deteriorated. This may be a vital aspect. We speculate that daytime performance levels may depend on the balance between sleep duration and sleep efficiency. Daily stressors may have detrimental consequences for sleep’s beneficial effect on cognition. As different children will be differentially exposed and receptive to daily stressors, this too may help explain the relatively small size of the association between sleep and cognition previously found in the meta-analysis.

Practically therefore, an important aspect of the knowledge transfer on sleep’s importance for children’s cognitive and behavioural development, should be information on the detrimental effects of stress on sleep, and how to create healthy, stress-free sleeping conditions. Additionally, it may prove valuable to investigate whether a more dispersed schedule of examinations would interfere less with sleep and its supporting role for cognitive performance.

In summary, it appears important to consider sleep in understanding individual differences in cognition and behaviour not only in adults, but also in children. Furthermore, it may be timely to evaluate whether interventions aimed at improving sleep in children would improve complex cognitive functions—including school performance—and ameliorate behavioural problems.

Quite in contrast to what is found in adults, our meta-analysis could not find evidence for a relationship between sleep duration and memory performance in children. Our two experimental studies were therefore designed to delve deeper into the relationship between sleep and memory function.

A major hypothesis on how sleep may benefit daytime functioning is the trace reactivation theory of sleep’s effects on cognition. An important aspect of sleep appears to be its complex wave-sequences: synchronisation of spindles and slow waves may allow information to pass from short-term stores to more long-term cortical storage. Furthermore, gamma activity during sleep may be due to the reactivation of traces previously established or strengthened in the wakeful state. However, only animal studies (Steriade, 2006; Steriade & Amzica, 1998; Steriade, et al., 1996), complex intracranial studies (Csercsa, et al., 2010; Le Van Quyen, et al.; Valderrama, et al.), and a single MEG study (Aiyoub, Molle, Preissl, & Born, 2012) have been able to reveal a coupling of these sleep oscillations.

We therefore set out to examine whether or not we could provide evidence to link the two characteristic electrophysiologically detectable sleep characteristics in children: sleep spindles and slow waves. Using complex waveform analyses we were indeed able to confirm this existence in children. Furthermore, we were the first to show that gamma activity is time-linked to the positive peak of large slow waves. These results confirm the existence of complex wave-sequences in humans surface EEG. The fact we were able to find this association between different cortical oscillations during sleep, confirms our suggestion that this developmental phase is a particularly interesting time to investigate all of sleep’s typical oscillatory activity.

Furthermore, the presence of gamma-activity during slow waves suggests wake-like activity during sleep, which may be the reactivation of traces of neuronal activity patterns that encoded information during the prior wakeful period, and may thus confirm the trace reactivation theory (Born & Wilhelm, 2012; Hoffman & McNaughton, 2002; Sejnowski & Destexhe, 2000; Sutherland & McNaughton, 2000; Wilson & McNaughton, 1994). This reactivation may transfer newly acquired information from the hippocampus to the neocortex.

Finally, our last experiment examined the direct relationship between sleep and memory performance in children. We set out to test our hypothesis—formulated following our meta-analysis—that when using an implicit memory paradigm we might be able to detect sleep-dependent memory consolidation in children. Indeed, our repeated-measures motor skill learning experiment showed that children show similar sleep-dependent improvements in motor skill performance accuracy as those seen in adults.

Second, we were able to test our meta-analysis-driven hypothesis that consolidation over wakefulness might be better in children than in adults. And indeed, we found evidence for non-sleep-dependent memory consolidation in children, for children showed an improvement in motor skill speed at delayed performance compared to initial learning, regardless of whether they had slept or not in the intervening period.

Past adult studies have suggested that sleep, and especially the slow cortical oscillation, may provide a general, homeostatic downscaling of synaptic strength. This is necessary to prevent saturation and preserve cost efficiency of the neuronal networks. The synaptic homeostasis hypothesis predicts that wakefulness results in an increase in synaptic excitation of neocortical and limbic circuits, whereas sleep results in a downscaling. This downscaling could subsequently allow storage of new information, whilst enabling a return to synaptic homeostasis (Tononi & Cirelli, 2006). The current results suggest that the brains of children may not be as dependent on sleep for network maintenance through synaptic downscaling as the brains of adults. The more abundant synaptic scaling seen in children (Huttenlocher, 1979; Huttenlocher & de Courten, 1987; Paus, Keshavan, & Giedd, 2008) may act on the brain continuously—when awake and when asleep.
A closer inspection of the children’s sleep microstructure, furthermore, revealed that both spindles and slow waves were directly related to their memory performance. We were able to link both the initial performance level and the extent of performance improvement seen overnight to aspects of spindles and slow waves. The children with lower spindle and slow wave frequencies initially performed worse, yet they improved most overnight. It seemed that lower frequencies of these characteristic sleep events might reflect less mature brain networks that can still benefit maximally from sleep. Children whose frequencies are faster (and frequencies of EEG waveforms still increase in this age group), show better initial performance but are not able to improve as much, potentially due to ceiling effects. Thus the extent of maturation of neuronal connectivity patterns may underlie the degree of sleep-dependent memory consolidation. With development, the association between sleep and memory consolidation may change. The more mature brain, with stronger and more efficient connections, may benefit from sleep less than the younger immature brain that may still need more sleep to develop its vital connections. This in turn may relate to the decrease in sleep duration seen across the first decade of life.

In conclusion, we found evidence in children’s sleep recordings that could confirm both trace reactivation and synaptic downscaling methods of learning. Although these two theories were initially postulated as two opposing methods by which sleep may influence cognition, more recent studies have inferred that both methods may in fact occur simultaneously. For instance, a recent mouse study examining post-learning dendritic spine formation, reported that both methods of consolidation are not mutually exclusive, and in fact co-occur in memory consolidation (Yang, et al., 2014). The current findings confirm this co-occurrence of different types of sleep-dependent learning in children. The findings presented in this thesis show it may be of particular relevance to study the role of sleep in memory performance not only in adults but also across the different developmental stages.

**FUTURE PERSPECTIVES**

Due to the enormous size of our meta-analytic investigations, incorporating all previously reported studies on sleep and cognition in childhood, we were simultaneously able to highlight methodological aspects that are most beneficial to studying the relationship between sleep and cognition, and to emphasise the key questions remaining in the field of sleep and cognition research.

Methodologically, future studies would do well to incorporate the following recommendations. First, sleep assessment methodology is of importance, the more accurately you measure sleep, the greater the association seen with cognitive performance. Similarly, the more efficiently you measure cognition, the greater the link with sleep. Experimental studies are more effective in studying this relationship than observational studies. Finally, it is important to carefully take into account gender differences, to incorporate standard reporting methods on socioeconomic status and ethnicity and to start a more in-depth investigation into developmental changes (i.e., ageing from infancy to adolescence). For instance, studies using narrow age ranges can highlight effects that are concealed in studies incorporating a wide range of ages, and longitudinal studies can provide essential information on how neuronal maturation and the relationship between sleep, to cognition, and behavioural problems interact. Given the evidence provided in the current thesis for the role of sleep in cognition and behaviour, it appears sensible to limit the amount of sleep restriction imposed on children.

Importantly, our investigations were able to highlight the key questions that remain within the field of sleep and cognition research. In particular, the following questions remain:

- Which aspects of sleep in particular relate to cognition and behavioural problems in children—which sleep stages and which electrophysiological sleep features (e.g., sleep spindles, slow waves, or EEG time-frequency power bands)? More in-depth recordings of sleep are essential, if possible ideally obtained with ambulatory high-density EEG.
- Which specific aspects of cognition and behavioural problems relate to sleep? It would be advantageous to use validated tasks that tap into cognitive function in greater detail, for instance, by using different types of memory tasks.
- What is the direction of causation in the relationship between sleep and cognition or behavioural problems? And how exactly can we best describe the relationship? For instance, adult studies have suggested an inverted U-shape function between sleep duration and life outcomes (Cappuccio, D’Elia, Strazzullo, & Miller, 2010).
- Which neuronal structures in particular relate to the relationship between sleep and cognition or behavioural problems in childhood? Modern brain imaging techniques—such as EEG, (functional) magnetic resonance imaging (fMRI), and combinations thereof—will allow insights into the underlying neuronal structures and connectivity patterns.
- How does brain development parallel the development of sleep, cognition,
and behavioural problems? Longitudinal study designs in which participants are followed up across the crucial childhood and adolescent years could reveal if and how they co-develop.

The studies described in the current thesis were able to answer a few aspects of these questions, yet further work is essential. Ambulatory high-density EEG, functional MRI, validated cognitive tasks, and longitudinal study designs are needed to further our knowledge of the relationship between sleep, cognitive function and behavioural performance during the childhood years. Furthermore, it appears timely to consider large-scale multivariate follow-up studies to disentangle individual traits from developmental aspects in the supportive role of sleep for cognition and behaviour.

REFERENCES


