Neurobiology of Sleep in Children and Older Adults

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Neurobiology of Sleep in Children and Older Adults

De neurobiologie van slaap
in kinderen en ouderen

Thesis

to obtain the degree of Doctor from the
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by
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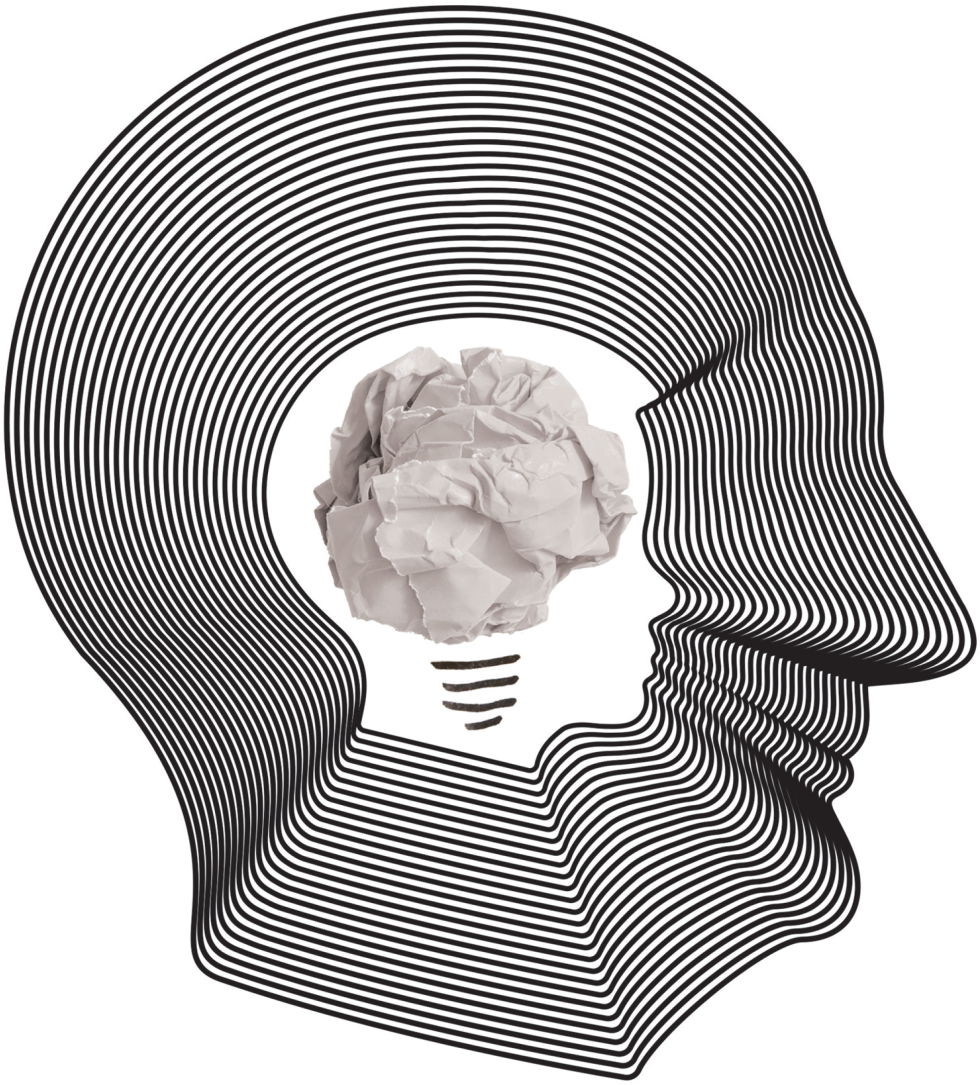
1 GENERAL INTRODUCTION



> *"People say, 'I'm going to sleep now,' as if it were nothing. But it's really a bizarre activity. 'For the next several hours, while the sun is gone, I'm going to become unconscious, temporarily losing command over everything I know and understand. When the sun returns, I will resume my life.'"*
>
> *Brain Droppings by George Carlin*

In the Greek mythology, Hypnos, the God of Sleep, is the son of Nyx ("The Night") and Erebus ("The Darkness"). His brother is Thanatos ("Death"). Hypnos lives in a big cave, where night and day meet, and where the river Lethe ("Forgetfulness") originates. Sleep is still mysterious for us in the 21st Century. A human falls asleep and wakes up at least 25,000 to 30,000 per lifetime,[1] yet few of us are concerned with the underlying mechanisms of this vital function. If one spares some time thinking about "Why do we sleep?" (E.g. a 5 years PhD project), one quickly realizes that this fundamental question is still open in 2019. This thesis does not provide an answer to this question, but this writer went back to it whenever the findings of her research were unexpected or unclear, or research questions were unanswerable.

Science has provided several, non-exclusive hypotheses about the function of sleep, however, researchers tend to only search for evidence in their own field. Neuroscientists have shown that sleep maintains synaptic homeostasis which is disturbed due to plastic changes occurring during wake.[2] Through sleep deprivation studies, experimental psychologists have shown that sleep is needed for memory consolidation.[3] Meanwhile, a group of biologists have used sophisticated imaging methods to show that during sleep, a so called glymphatic system clears the brain tissue from neurotoxic waste produced during wake.[4] All of these fields together have shown that sleep undoubtedly serves multiple vital functions to the brain.

Epidemiology, "the study of what is upon the people",[5] explores how often diseases occur in different groups of people and why.[6] Sleep epidemiology, a field not much older than your author,[7] has shown that sleep problems affect one third of the population and that poor sleep is related to numerous poor health outcomes (e.g. obesity, diabetes mellitus, hypertension, depression or cognitive deficits etc.), and even mortality.[8] This research should have brought sleep a step further on the public-health-relevance-scale, but to date no country has established definitive public health measures to improve sleep in the general population. Therefore, epidemiologists still study the etiology and consequences of poor sleep. In the past half of a century, ample cognitive and psychiatric research in the field of sleep has been conducted.[9] Since neuroimaging methods are increasingly employed in research settings, the amount of neuroimaging sleep research has also increased rapidly.[10, 11] Neuroimaging sleep studies conducted thus far, however, have been relatively small, and mostly cross-sectional. In the epidemiological studies

upon which this thesis is based, we aimed to fill this gap through a series of longitudinal studies, exploring the neurobiological determinants and outcomes of sleep patterns in childhood and later adulthood. Importantly, we did this in cohorts sampled from the general population, which is expected to more closely represent "real-life" settings and the continuum between health and disease.

Epidemiology of sleep in the Netherlands

Sleep patterns depend on demographic and cultural characteristics and are very compliant to social cues. Because circadian processes, like sleep-wake rhythms, are strongly determined by light, sleep patterns also vary geographically. Another important characteristic of sleep is that sleep patterns change with age. A newborn baby sleeps on average 16 hours per day, which is reduced by up to 25% in the 1st year, and cut in half by adult life (if you're lucky enough to be able to sleep for 8 hours as an adult!). Qualitative aspects of sleep also vary with age. Both problems with falling and staying asleep are most common in early and late life. Therefore, before studying the neurobiology of sleep we took the traditional epidemiological approach, and first estimated typical sleep patterns for the population of The Netherlands across the lifespan (**Chapter 1**). To this aim we aggregated individual participant data from 36 different population-based cohorts, including 200,358 participants aged 1 to 100 years old.

Sleep patterns change rapidly during the first several years of life, but what exactly determines these changes is not well understood. Adverse sleep patterns have been shown in children with neurodevelopmental disorders[12, 13] and preterm born children.[14, 15] Based on this, it has been hypothesized that developmental changes in sleep patterns closely correspond to the maturational state of the central nervous system.[16, 17] Others have posited that sleep is a learned behavior, and childhood sleep problems are a result of adverse external cues, such as stress or poor sleep hygiene.[18, 19] We addressed both lines of reasoning. We tested (very) early developmental biomarkers as determinants of childhood sleep patterns, namely: prenatal and neonatal head growth – a marker of early neurodevelopment and saliva cortisol levels during infancy – a marker of stress levels (**Chapter 2**). The impact of childhood sleep problems on later neurocognitive development is also not entirely clear. Therefore, in **Chapter 3** we next evaluated the associations between childhood sleep patterns and: a) magnetic resonance imaging (MRI) to define cortical morphology at age 7, and b) cognitive scores at age 6.

The pediatric studies in this thesis were performed using data from **The Generation R Study**, a population-based prospective cohort that follow children from fetal life onwards. Pregnant women living in Rotterdam, with an expected delivery date between April 2002 and January 2006 were invited to participate. From 9901 initially enrolled children, 7,893 children were followed-up in early childhood.

Changes in sleep patterns largely coincide with rapid structural brain changes that happen during early neurodevelopment and when neurodegeneration is starting to take place. Hence, we posed similar research questions at the other end of the age distribution, in older adults. In **Chapter 4** subjective and objective measures of sleep patterns in older adults were tested as determinants of microstructural integrity of cerebral white matter measured with Diffusion Tensor Imaging (DTI).

The studies in middle aged and older adults were embedded in the **Rotterdam Study**, a population-based prospective cohort. The study includes a total of 14,926 participants 45 years and older, living in the district of Ommoord, Rotterdam. Data-collection started in 1990; from 2002 onwards sleep questionnaires were implemented and from 2005 onwards MRI scanning was included in the study protocol. In a subgroup of 2063 participants sleep was measured objectively using a wrist-worn actigraph 2004 and 2007.

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2 HOW DO THE DUTCH SLEEP?

Sleep patterns across the lifespan: An individual participant meta-analysis in 200,358 persons from the general population

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In preparation

3 NEUROBIOLOGICAL DETERMINANTS OF CHILDHOOD SLEEP PATTERNS

Prenatal and early postnatal measures of brain development and childhood sleep patterns

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Abstract

Early neurdevelopment and childhood sleep

Background: Brain development underlies the maturation of sleep patterns throughout childhood. Intrauterine head growth, a marker of early neurodevelopment, has however not been related to childhood sleep characteristics. We explored associations between ultrasonographic measures of prenatal and early postnatal neurodevelopment and childhood sleep.

Methods: Six-thousand-five-hundred-twenty-eight children from a population-based birth cohort (Generation R) were included. Head circumference (HC) and lateral ventricles size were assessed with mid- and late-pregnancy fetal ultrasounds and with cranial ultrasound 3-20 weeks postnatally. Mothers reported children's sleep duration at 2 and 3 years, and sleep problems at 1.5, 3 and 6 years.

Results: Larger ventricular size, but not HC, was related to longer sleep duration at 3 years (β=0.06hrs, 95%CI:0.02;0.10 in late-pregnancy and β=0.11hrs, 95%CI:0.02;0.20 in early infancy, mid-pregnancy parameters were unrelated to sleep duration). Larger HC in mid-pregnancy was associated with a reduced risk for being a "problematic sleeper" up to age 6 (OR:0.94, 95%CI:0.89;0.99). Consistently, children with larger HC in early infancy were less likely to be "problematic sleepers" at 3 and 6 years.

Conclusions: This study shows that variations in fetal and neonatal brain size may underlie behavioral expression of sleep in childhood. Albeit small effect estimates, these associations provide evidence for neurodevelopmental origins of sleep.

INTRODUCTION

Newborns of different species undergo developmental maturation of sleep patterns.[1] The maturation of sleep patterns is considered to be an important developmental milestone for the human infant, e.g. decrease in total sleep duration and napping.[2] As brain development is intrinsically related to the sleep-wake process, it may underlie variations in sleep patterns observed throughout childhood. A full understanding of how markers of early neurodevelopment are related to childhood sleep patterns is lacking.

The second and third trimester of intrauterine life, as well as early infancy form a vulnerable period for the development of the brain.[3] Rapid maturational changes of sleep expression go alongside neurodevelopment of the fetus[4] and the infant,[5] thus impaired brain development during this period could affect later sleep patterns and problems. Several prenatal exposure studies lend support for this hypothesis. Prenatal tobacco[6] and alcohol[7] exposure is related to disturbed neonatal sleep, and maternal mood disturbances during pregnancy are related to disturbed sleep patterns in toddlerhood.[8] Birth outcomes have also been tested as determinants of childhood sleep patterns.[6] Children born preterm or small for gestational age have disturbed sleep patterns in infancy[9] and childhood.[6] However, these birth parameters are relatively crude measures of intrauterine growth and provide little or no information on fetal brain development. Intra-uterine head growth is a reliable indicator of early neurodevelopment reflecting both genetic and environmental effects, but it has not been related to childhood sleep.

Previous studies on the current sample[10, 11] and other samples[12, 13] show that measures of early brain development assessed with the fetal and neonatal cranial ultrasound are predictive of later neurodevelopmental outcomes. Prenatal head circumference is a reliable proxy for the brain volume growth of the fetus[14] associated with later cognitive functioning.[15] The size of the ventricular system is another marker of early brain development,[10, 13, 14] providing information on the growth of the cerebral hemispheres both prenatally and in neonates.

This study explores the associations of prenatal and neonatal ultrasonographic measures of brain growth with childhood sleep patterns in a large sample from the general population. We hypothesized that more advanced brain maturation, indicated by larger head circumference and larger lateral ventricles within the normal range, is associated with longer sleep duration and less sleep problems up to age 6 years. As adversities in early neurodevelopment are associated with childhood behavioral problems,[16] which in turn are often comorbid with sleep problems,[17] we also tested whether the associations are independent of behavioral problems.

METHODS

Study population

We conducted our study within the ongoing Generation R Study, which follows children born between April 2002 and January 2006 in Rotterdam, the Netherlands. The study has previously been described in detail. The Medical Ethics Committee of Erasmus Medical Centre approved the study and written informed consent was obtained from all parents. Prenatal ultrasounds were performed in 8209 women in mid-pregnancy and in 8270 women in late-pregnancy. Neonatal head circumference up to 2 months was assessed in 5558 children. Additional detailed ultrasound measurements were obtained in a random subsample of Dutch children (n=776).[11] Sleep patterns were assessed in 6808 (n=723 with postnatal cranial ultrasound) of these children (72% follow-up) after excluding twins (n=132) and lost to follow-up (n=425). We used all available information, and thus the sample size differs per analyses. The children assessed at different time points did not differ with respect to brain size; sleep indices, or other sociodemographic characteristics (data not shown).

Determinants

Fetal ultrasound

Fetal ultrasound measurements were carried out in early, mid and late pregnancy using the Aloka model SSD-1700 (Tokyo, Japan) or the ATL-Philips Model HDI 5000 (Seattle, WA), with standardized ultrasound procedures. Gestational age was established using data from the first fetal ultrasound examination.[18] As the size of the ventricles can be reliably measured only from the beginning of the second trimester, only mid pregnancy (average gestational age 21 (18 to 24) weeks) and late pregnancy (average gestational age 30 (25 to 39) weeks) measures were used. The atrial width of the lateral ventricles was measured as the widest diameter of the atrium of one of the lateral ventricles in an axial plane.[10] Based on reference growth curves from the whole study population gestational-age-adjusted standard deviation (SD) head circumference scores were constructed, which represent the equivalent of z-scores.[18] The intra- and inter-observer reliability of fetal biometry measurements in early pregnancy were excellent (intra-class correlation coefficient >0.99).

Postnatal head circumference

Infant head circumference was measured at Community Health Centers (4.5 ± 0.9 postnatal weeks) using standard procedures. Values were expressed as age and gender-adjusted SD scores using Dutch reference growth curves.[19] During the research center visit for the infant cranial ultrasound (6.9 ± 1.9 postnatal weeks) we measured the fronto-occipital head circumference (cm) at its maximum diameter through the glabella and occiput to the nearest 0.1 cm, using a flexible measuring tape.[10]

Infant cranial ultrasound

Postnatal cranial ultrasounds were performed at 6.9 ± 1.9 postnatal weeks using a commercially available multifrequency electronic transducer (3.7-9.3 MHz) with a scan angle of 146° (Voluson 730 Expert; GE Healthcare, Waukesha, WI). Infants were situated in supine position, using a probe on the anterior fontanel and a volume box at the level of foramen Monro in a symmetrical coronal section. The obtained data was analyzed with MNI Display software (Montreal Neurological Institute, McGill University, Quebec, Canada). Four raters manually traced left and right lateral ventricles using a mouse-driven cursor, after intensive training with an experienced ultrasonographer, as previously described in detail.[10] Intraobserver intraclass correlation coefficients were all above 0.99, and interobserver intraclass correlation coefficients were above 0.95.

Outcomes

Sleep duration

The usual bedtimes, wake times and the amount of daytime sleep (categories ranging between <30 minutes and >2.5 hours) were reported by the parents when children were 2 and 3 years old. Sleep duration was calculated as hours of sleep per 24 hours by adding nighttime and daytime sleep. At 3 years, separate reports for weekdays and weekends were available, and a weighted average sleep duration was computed ((5*weekday + 2*weekend)/7).

Sleep problems

At 1.5, 3, and 6 years, parents answered five items measuring dyssomnia symptoms (Doesn't want to sleep alone; Has trouble getting to sleep; Resists going to bed at night; Sleeps less than most kids during day and/or night; Wakes up often at night) from the Child Behavior Checklist (CBCL 1.5–5) on a three-point likert scale (0-not true, 1-somewhat or sometimes true, or 2-very or often true).[20] In line with a previous study,[21] we did not include the CBCL parasomnia items in the scores, as parasomnias might have a different neurodevelopmental origins. Sleep problems sum scores (range 0-10) were computed by summing the dyssomnia items. As the sleep problems scores at each time-point were strongly right skewed, we categorized children in the highest quartiles as "problematic sleepers" and studied the remainder as the reference group. Due to developmental changes in sleep patterns up to 6 years, the cut-off point of the highest quartile of sleep problems varies with age (e.g. >2.5 points at age 1.5 years, and >2 points at 3 and 6 years).

Covariates

Child Characteristics. Estimated fetal weight was calculated using the formula by Hadlock et al.[22] Information on sex, date of birth, gestational age, birth weight and Apgar score 5 minutes after birth was obtained from midwives and hospital registries. Postnatal (4.7

± 0.9 weeks) height and weight was measured at the Community Health Centers and was expressed in age- and gender-adjusted SD scores using Dutch reference growth curves.[19] Child's ethnicity was based on parent's country of birth and categorized into: Dutch (Netherlands), other Western (other European countries, United States, Canada, Australia, and Japan), Mediterranean (Turkey and Morocco), Caribbean (Dutch Antilles and Surinam), or other non-Western (Africa, Asia, non-Western America, and Cape Verde).[23] Child internalizing and externalizing problems at age 3 years were assessed with the CBCL.[20]

Maternal Characteristics. Maternal age, education and parity were assessed with questionnaires at enrollment. Educational level was classified into high, intermediate, or low.[23] History of tobacco smoking was obtained by questionnaires in early, mid- and late pregnancy and categorized into: "never smoked", "stopped smoking when pregnancy was known" and "continued smoking during pregnancy". Maternal psychiatric symptoms during pregnancy were assessed using the Global Severity Index from the Dutch version of the Brief Symptom Inventory.[24]

Statistical analyses

We tested the associations of early brain growth, i.e. fetal and infant brain ultrasound measures of head and ventricular size with sleep duration using linear regression. To study the association between early brain growth and sleep problems up to age 6 we used logistic and liner regression models for each time point separately, and general estimating equations (GEE) to study sleep problems across the follow-up. GEE models take the correlation of multiple measurements within one subject into account and yield an overall estimate of the association between early brain growth and repeatedly measured sleep problems. Moreover, they have an optimal use of available measurements by allowing for incomplete outcome data. The baseline models were adjusted for (gestational) age at ultrasound measurement, sex and head circumference (in the ventricular size models). Based on previous literature or a >5% change in effect estimate of the predictor variable, the multivariable models were additionally adjusted for child's ethnicity, gestational age and Apgar score at birth, maternal age, parity, educational level, smoking and psychiatric symptoms during pregnancy. To test whether the associations were influenced by body size we additionally adjusted the models for estimated fetal weight (prenatal models), or birth weight and infant height and weight (postnatal models). In a final step, we additionally adjusted the models for child's behavioral problems (externalizing and internalizing problems scores in separate models), to test whether the observed associations were specific for sleep problems. In sensitivity analyses, we excluded children with < 3 or >3 head circumference SD score in mid pregnancy (n=34), late pregnancy (n=25) or postnatally (n=21). We also tested if ventricular enlargement influenced our results by excluding fetuses with atrial width >10mm in mid-pregnancy (n=2) or late pregnancy (n=9), or neonates with lateral ventricular volume 3SD above the mean

(n=10). Missing values on covariates (< 10%) were imputed using multiple imputations to create ten complete datasets. Statistical analyses were run in the ten datasets and results were pooled. For non-response analysis, several socio-demographic and maternal characteristics of children, who were lost to follow-up (n=2086), were compared (chi-squared test, t-test or Mann-Whitney U test) to those with available sleep data.

RESULTS

The baseline characteristics of the children included in this study are shown in Table 1. Head circumference increased on average (SD) from 179 (14.3) mm to 286 (12.3) mm from mid to late pregnancy, and further to 376 (13.7) mm at around 5 weeks after birth. The atrial width of the lateral ventricles decreased from 5.7 (1.2) mm in mid pregnancy to 4.9 (1.7) mm in late pregnancy (Table 1). However, gestational age was not associated with the size of the ventricles within the third trimester ($\beta_{\text{gestational age, weeks}}$=0.02, p-value=0.954). The atrial width of the lateral ventricles in late pregnancy, however, was positively correlated with postnatal ventricular volume (r=0.103, p<0.001), which in turn increased further with postnatal age, i.e. older infants had a larger ventricular volume ($\beta_{\text{postnatal age, weeks}}$=0.39, p-value<0.001). Correspondingly, the correlation between different time-points of ventricular size assessments were weak (r=0.2) and decreased with time-lag (e.g. no correlation from mid-pregnancy to infancy). Average sleep duration decreased from 13.3h (1.1) at 2 years to 12.6h (1.3) at 3 years of age.

Brain growth and sleep duration

The associations of prenatal and early postnatal brain growth with sleep duration are shown in Table 2. Head circumference was not associated with sleep duration in any of the models. In contrast, larger lateral ventricles in the 3rd trimester of pregnancy and also in early infancy were related to longer sleep duration. Both associations reached statistical significance with sleep duration at 3 years of age, but not at 2 years of age. Per 1SD larger atrial width of the lateral ventricle in late pregnancy, sleep duration at age 3 years was 0.06 hours (95%CI: 0.02;0.10) longer. Consistently, per 1SD larger ventricular volume in infancy, sleep duration was 0.11, (95%CI: 0.02;0.20) hours longer. Importantly, these associations were not influenced by children with ventricular enlargement at any of the measurement rounds, and were not explained by fetal and infant body size or co-occurring behavioral problems (data not shown).

Brain growth and repeated measures of sleep problems

Table 3 shows the association between prenatal and early postnatal brain growth and sleep problems across ages 1.5 to 6 years. Fetuses with larger head circumference in mid pregnancy were less likely to be “problematic sleepers” in early childhood, independent

Table 1. Subject's characteristics (n=6808)

Main determinants: Brain growth	
Prenatal: mid pregnancy	
Gestational age, weeks	20.6 ± 1.1
Estimated fetal weight, g	380.9 ± 92.5
Head circumference, mm	179.4 ± 14.3
Atrial width of lateral ventricles, mm	5.7 ± 1.2
Prenatal: late pregnancy	
Gestational age, weeks	30.4 ± 1.1
Estimated fetal weight, g	1625.3 ± 260.9
Head circumference, mm	285.4 ± 12.3
Atrial width of lateral ventricles, mm	4.9 ± 1.7
Postnatal	
Age, weeks	4.7 ± 0.9
Weight, g	4449.6 ± 627.7
Height, cm	54.3 ± 2.4
Head circumference, mm	376.0 ± 13.6
Postnatal ultrasound (n=813)	
Age, weeks	6.9 ± 1.9
Head circumference, mm	386.7 ± 15.0
Ventricular volume, ml	0.81 (0.05-5.57)
Outcomes: Sleep patterns	
Total sleep duration, hours	
2 years	13.3 ± 1.1
3 years	12.6 ± 1.3
Dyssomnia symptoms, n(%) "problematic sleepers"	
1.5 years	1213 (25.9)
3 years	1072 (24.4)
6 years	980 (16.9)
Child characteristics	
Sex, % girls	49.7
Gestational age, weeks	39.9 (25.3-43.6)
Birthweight, grams	3441 ± 551
Apgar score 5 min after birth	10 (2-10)
Ethnicity	
Dutch, %	58.6
Other Western, %	8.6
Mediterranean, %	13.3
Caribbean, %	9.7
non-western, %	9.8
Behavioral problems, score	18 (0.0-146.9)
Maternal characteristics	
Age, years	30.5 ± 5.0
Parity, % primipara	57.5
Psychopathology score	0.15 (0.0-3.04)
Smoking during pregnancy	
% no	75.1
% until pregnancy was known	8.5
% yes	16.5
Educational level	
% low	21.9
% medium	30.6
% high	47.5

Numbers are percentages, means ± SD or median (range) pooled from 10 imputed datasets.

Table 2. Associations between prenatal and early postnatal brain ultrasounds and sleep duration up to 3 years

			Total sleep duration, hours							
			2 years				3 years			
			N	B	95% CI	P	N	B	95% CI	P
Mid pregnancy	Head circumference, SD	Model 1		-0.03	-0.04;0.09	0.464		-0.02	-0.09;0.06	0.652
		Model 2	4158	-0.02	-0.05;0.09	0.585	4123	-0.02	-0.09;0.05	0.571
	Lateral ventricle, SD	Model 1		0.03	-0.02;0.08	0.272		-0.000	-0.05;0.05	0.994
		Model 2	2260	0.02	-0.03;0.07	0.376	2151	-0.000	-0.05;0.05	0.994
Late pregnancy	Head circumference, SD	Model 1		0.02	-0.03;0.04	0.405		-0.03	-0.07;0.004	0.076
		Model 2	4239	-0.003	-0.04;0.03	0.860	4090	-0.03	-0.07;0.01	0.117
	Lateral ventricle, SD	Model 1		0.03	-0.01;0.08	0.112		0.06	0.02;0.11	0.005
		Model 2	2830	0.01	-0.04;0.05	0.753	2713	0.06	0.02;0.10	0.008*
Postnatal	Head circumference, SD	Model 1		-0.01	-0.04;0.03	0.775		-0.05	-0.09:-0.02	0.007
		Model 2	3680	0.02	-0.03;0.06	0.425	3588	-0.01	-0.05;0.04	0.716
	Head circumference, SD	Model 1		0.04	-0.04;0.12	0.357		-0.07	-0.16;0.02	0.138
		Model 2	729	0.04	-0.05;0.14	0.349	698	-0.06	-0.16;0.04	0.266
	Lateral ventricle, SD	Model 1		-0.01	-0.09;0.07	0.778		0.10	0.01;0.19	0.024
		Model 2	627	-0.01	-0.02;0.13	0.073	603	0.11	0.02;0.20	0.021*

Model 1 is adjusted for gestational age at ultrasound assessment and sex, head circumference (ventricular volume models)
Model 2 is additionally adjusted for gestational age at ultrasound measurement, child's sex, ethnicity, gestational age and Apgar score at birth, maternal age, education, parity and psychopathology score and smoking during pregnancy
*is not explained by co-occurring behavioral problems or body size (estimated fetal weight in prenatal models, and birthweight or neonatal height and weight in postnatal models)

Table 3. Associations between brain measures and repeatedly measured sleep problems

Brain measures (Z-scores)		N	"Problematic sleepers" at 1.5, 3 and 6 years			
			Model 1		Model 2	
			OR (95% CI)	P	OR (95% CI)	P
Mid pregnancy	Head circumference, SD	5825	0.91 (0.88;0.93)	<0.001	0.94 (0.89;0.99)	0.013*
	Lateral ventricle, SD	2843	0.98 (0.95; 1.02)	0.593	1.01 (0.94;1.08)	0.782
Late pregnancy	Head circumference, SD	5951	0.90 (0.88;0.92)	<0.001	0.96 (0.91;1.02)	0.169
	Lateral ventricle, SD	3631	0.90 (0.87;0.93)	0.002	0.95 (0.89;1.02)	0.161
	Head circumference, SD	4460	0.90 (0.84; 0.96)	0.002	0.95 (0.89;1.02)	0.117
Postnatal	Head circumference, SD	797	0.95 (0.80;1.11)	0.516	0.97 (0.83;1.14)	0.742
	Lateral ventricles, SD	689	0.99 (0.82;1.15)	0.872	0.97 (0.82;1.15)	0.758

Model 1: adjusted for postconceptional age at ultrasound measure, gender and head circumference (ventricles models)
Model 2: as model 1, additionally adjusted for ethnicity, gestational age and Apgar score at birth, maternal educational level, parity and maternal smoking and psychiatric symptoms during pregnancy
OR's are derived from GEE (generalized estimating equation).
*Is not explained by co-occurring behavioral problems or body size (estimated fetal weight in prenatal models, and birth weight or neonatal height and weight in postnatal models

of child and maternal characteristics (OR: 0.94, 95% CI: 0.89;0.99). Measures of brain growth during late pregnancy were also associated with later sleep problems. These associations, however, were largely explained by maternal characteristics such as educational level, smoking and psychopathology symptoms during pregnancy. Individual time-point analyses indicated that the observed longitudinal effects were similar across the different ages of sleep assessment (data not shown). However, larger head circumference in early infancy was associated with reduced risk of being a "problematic sleeper" at age 3 (OR: 0.89, 95%CI: 0.80;0.99) and 6 years (OR: 0.88, 95%CI: 0.87;0.99), but not at 1.5 years. Similar results were obtained when sleep disturbance scores were analyzed continuously (data not shown), indicating that cut-off points did not influence our results. Again, the observed associations between smaller head circumference and higher dyssomnia symptoms were not explained by behavioral problems, head circumference at the lower or upper extremes, or fetal size.

Nonresponse analysis

Mothers of children included in the study were on average 3 years older, more likely to be Dutch (32.6% vs. 57.9%, x^2 p-value<0.001), more highly educated (19.5 vs. 47.8%, x^2 p-value<0.001) and had lower psychopathology scores during pregnancy (median score 0.43 vs. 0.27, p<0.001), compared to those lost to follow-up. In addition, children included in the study had larger head circumference than those lost to follow-up in mid (Mean Difference=0.18, p=0.001) and late pregnancy (Mean Difference=0.18, p<0.001).

DISCUSSION

This study shows that prenatal and early postnatal ultrasonographic measures of brain development (i.e. head circumference and ventricular size) are related to sleep patterns across early childhood. Larger size of the ventricular system in late pregnancy and in early infancy were related to longer sleep duration at 3 years. In addition, larger head circumference in mid pregnancy and early infancy was related to a reduced risk of being a "problematic sleeper" up to 6 years of age. The association between fetal brain measures in late pregnancy and later sleep problems was explained by maternal characteristics, (e.g. mood disturbances during pregnancy). Albeit small, these effect estimates provide evidence for early neurodevelopmental origins of childhood sleep problems.

According to our knowledge this is the first study to explore prenatal brain growth as a determinant for later sleep patterns. Previous studies have reported that children born preterm have disturbed sleep patterns,[9] which is compatible with the hypothesis that early neurodevelopment plays a role in the behavioral expression of sleep.[4,25] In addition, at least one longitudinal study has shown that adverse birth outcomes (e.g. low weight and/or

length) are related to later sleep disturbances.[6] Moreover, maternal psychopathology[8] and risky substance exposure during pregnancy (e.g. nicotine,[6] alcohol,[7] benzodiazepines[26]), have been related to short or disturbed sleep later in childhood.

Although the direct biological link between delayed neurodevelopment and disturbed sleep has not been established, sleep disturbances are highly prevalent among neurodevelopmental disorders, such as ADHD and autism.[27, 28] Previous studies have also reported adverse prenatal brain development (e.g. smaller head circumference) among children with neurodevelopmental disorders.[29, 30] However, whether differences in early brain development are also present in children with disturbed sleep patterns is not known. Nevertheless, maturational delays in the sleep EEG have been observed in very preterm infants (<32 weeks of gestation)[31], in neonates with very low birth weight[31, 32] and other perinatal complications. In addition, studies using fetal magnetography have shown that sleep-like behavioral states are tightly related to the neurodevelopmental stage of the fetus,[33] and these states predict self-regulation in childhood and adolescence.[4] This indicates that although the extra uterine environment influences sleep, maturation of sleep patterns is mainly a function of brain development.

In this study, we show that early markers of brain development are related to childhood sleep patterns, which tempts us to hypothesize that impaired neurodevelopment in prenatal or early postnatal life has a long-term effect on sleep regulation. Larger head circumference prenatally and in the first two postnatal months, indicating larger brain volumes, was related to a reduced risk of being a "problematic sleeper". Similar to other studies,[34] the size of the ventricular system of the fetus showed a nonlinear relation with gestational age, resulting in a slight decrease of the average atrial width from the second to the third trimester of pregnancy followed by a an increase thereafter. Only the size of the ventricular system in late pregnancy correlated positively with ventricular volume in early infancy. Postnatal ventricular volume may indicate advanced neural maturation as suggested by the positive correlation with age. Shortly before and after birth, larger ventricles within the normal range predicted longer sleep duration at 3 years of age, which in turn is developmentally beneficial. Importantly, ventricular enlargement due medical complications has to be distinguished from larger ventricular volumes due to longer gestation and brain maturation. Previous studies by our group [10] as well as MRI studies[35] showed that in the general population a larger ventricular size in infancy indicates more advanced growth of the cerebral hemispheres. In addition, the size of the ventricular system is positively related to beneficial developmental outcomes[10] (e.g. larger ventricles before and shortly after birth were related to less temperamental difficulties[11]). As children with behavioral problems often have both adverse prenatal neurodevelopment[16] and sleep problems,[17] it is important that the results we report were not explained by co-occurring internalizing or externalizing problems. This means

that early brain development is likely to have a specific link to disturbed sleep patterns, independent of comorbid mental conditions.

The mechanisms that play a role in the relation between early brain growth and childhood sleep patterns could be in line with the developmental origins of health and disease hypothesis.[36] The brain tissue is a main substrate of the physiological and behavioral regulation of sleep (e.g. fetal rapid eye movement is considered to be an indicator and a promoter of brain development[37]), thus adversities in early neurodevelopment are likely to be reflected in disturbed postnatal sleep patterns. Along these lines, short sleep duration and dyssomnia symptoms might reflect developmental problems[21] that start prenatally and extend into childhood.[5] Indeed, longitudinal research on sleep architecture in children and adolescents has shown that slow wave sleep is a reliable marker of cortical development and maturation.[5, 32, 38] Alternately, disturbances in the neuroendocrine properties of the fetal HPA-axis that emerge towards the third trimester of pregnancy, including stress regulation, sleep, feeding, and emotion regulation,[36] could play a role in the relation between fetal brain growth and later sleep problems

Some methodological considerations need to be considered when interpreting our results. First, our results should not be generalized to clinical populations that have ventricular enlargement due to white matter damage or intraventricular hemorrhage. Second, the children included in our study had larger head circumference compared to those lost to follow-up, thus some selection bias could be present. Third, our study does not provide direct information about the growth trajectory of the nervous system. The postnatal measures were obtained only in an ethnically homogenous subsample, and the two-dimensional measures during pregnancy are not equivalent to the postnatal volumetric size of the ventricular system. Nevertheless, the atrial width of the lateral ventricle during pregnancy is predictive of the postnatal ventricular volume.[10] Finally, maternal reports on children's sleep duration might have introduced some measurement error in our sleep estimates, however, we expect any outcome misclassification to be random (e.g. independent of brain development). In addition, early brain development might also influence other aspects of sleep (e.g. sleep efficiency), which would not be captured by maternal reports of sleep problems. Future studies should include objective measures of sleep to replicate and corroborate our findings. There are also some important advantages of our study, such as the longitudinal design with repeated measures of brain development and sleep patterns in a large sample of children from the general population. Importantly, because prenatal brain growth is a rapid process, our prenatal measures were standardized based on gestational age using study specific growth curves. In addition, we were able to take key confounding factors into account, such as maternal smoking and psychopathology during pregnancy.

Conclusion

Understanding the development of sleep might be critical to understanding its functions. Previous research has mainly focused on the neurodevelopmental consequences of early childhood sleep disturbances, whereas this study shows that variations in fetal and neonatal brain development might underlie childhood sleep patterns. As sleep patterns mature along with the central nervous system, behavioral expressions of sleep might reflect neurodevelopment. Repeated measures using different imaging modalities within short time-windows should be utilized to further elucidate the early neurobiological basis of sleep in childhood.

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Infant diurnal cortisol rhythms and childhood sleep patterns

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Abstract

Infant cortisol and childhood sleep

Background: Cortisol, the end product of the hypothalamic-pituitary-adrenal (HPA) axis, plays an important role in modulating sleep. Yet, studies investigating the association between diurnal cortisol rhythm and sleep patterns in young children are scarce. We tested the hypothesis that the altered diurnal cortisol rhythm is associated with shorter sleep duration and more sleep problems across early childhood.

Methods: This study was embedded in Generation R, a population-based cohort from fetal life onwards. Parents collected saliva samples from their infant at five moments during one day. In 322 infants aged 12-20 months, we determined the diurnal cortisol rhythm by calculating the area under the curve (AUC), the cortisol awakening response (CAR), and the diurnal slope. Sleep duration and sleep behavior were repeatedly assessed across ages 14 months to 5 years. Generalized estimating equation models were used study associations between cortisol rhythms in infancy and sleep duration and sleep behavior across childhood.

Results: The diurnal cortisol slope and the CAR, but not the AUC, were associated with sleep duration across childhood. Children with flatter slopes and children with a more positive CAR were more likely to have shorter night-time sleep duration (β per nmol/L/h slope: -0.12, 95% CI: -0.19; -0.05, p=0.001; β per nmol/L CAR: -0.01, 95% CI: -0.02; -0.00, p=0.04). Cortisol measures did not predict sleep problems.

Conclusions: The present study suggests that a flatter diurnal cortisol slope and a more marked morning rise, which can indicate stress or HPA dysregulation, have long-term effects on sleep regulation.

INTRODUCTION

A good night's sleep is considered to be beneficial for the physical and mental health of children. Problems with sleeping are related to both externalizing and internalizing problems in preschoolers and older children.[1, 2] Such an association has also been found in prospective studies of older children and adolescents (see review of [3]). Longitudinal studies in young children, however, are scarce. Recently, Sivertsen et al.[4] showed that early sleep problems at 18 months predicted externalizing and internalizing problems at 5 years.

Assessment of determinants of sleep patterns in young children to understand mechanisms underlying poor sleep are particularly scarce. Epidemiological research demonstrated that sleep patterns are influenced by environmental factors such as child-rearing situation, socio-economic status and stressful events.[5] However, the biological determinants of child sleep, such as cortisol, melatonin and other hormones are mostly inferred from clinical studies.[6, 7] While cross-sectional studies have demonstrated that disturbed sleep is associated with hormonal variations, it is yet unknown whether cortisol predicts changes in sleep duration or sleep problems.

Cortisol is the hormonal end-product of the HPA-axis and is important for a wide variety of adaptive functions and is released in response to stressors. This hormone is also involved in numerous essential bodily functions which are intrinsically related to sleep.[8] In addition, cortisol shows a diurnal pattern characterized by post-waking peak (cortisol awakening response) and subsequent decline throughout the day in healthy adults.[9] Cortisol levels reach their lowest point during the first half of the sleep period.[10] During sleep, cortisol levels remain low and then rise again until morning awakening.[9, 10] Infants are born without a diurnal cortisol rhythm and this rhythm emerges during the first 18 months of life. Whereas the diurnal decrease is present in infants aged 12-18 months,[11] the CAR typically arises even later.[12]

Several studies show the close association between poor sleep and higher cortisol levels,[13] for example in infants,[14, 15] preschoolers,[16, 17] older children,[18] and children suffering from obstructive apnea syndrome.[19] However, all these studies focus on the effect of sleep patterns on cortisol changes but do not address cortisol secretion as a biological risk factor for poor sleep. Only recently, Kiel et al.[20] showed that high morning cortisol levels predict increasing sleep problems from age 2 to age 3, and to our knowledge, no studies explored the association between cortisol levels and child sleep duration. In summary, few studies assessed the association between the developing diurnal cortisol rhythm in infancy and sleeping patterns later in childhood.

In this population-based prospective study, we examined whether the diurnal cortisol rhythm in infancy, i.e. at age 14 months, is associated with night time sleep duration or sleep problems as measured repeatedly between 2 to 5 years. We tested the hypothesis that flatter slopes as part of the diurnal cortisol rhythm are associated with shorter sleep duration and more sleep problems in early childhood.

METHODS

Setting

This study was conducted in the Generation R Focus Cohort, a study investigating growth, development and health from fetal life onwards in Rotterdam, the Netherlands. The cohort has been described in detail elsewhere.[21] The Generation R Focus Study is conducted to obtain detailed measurements of the child's development in an ethnically homogeneous subgroup. Only children of Dutch national origin were included, i.e. the children, their parents and their grandparents were all born in the Netherlands. The participating children were born between February 2003 and August 2005. Written informed consent was obtained from all participants. The study has been approved by the Medical Ethical Committee of the Erasmus Medical Center, Rotterdam.

Study population

For the current study, children who visited the research center for the Focus Study around 14 months were eligible for assessment of the diurnal cortisol profile. Parents of 602 children who attended the Focus Cohort examination returned one or more saliva samples. Of these, 236 children had to be excluded, because in these children less than two morning samples or less than three samples during the day were obtained, which is insufficient to compute a cortisol composite measure. The area under the curve was calculated in 277 children, the diurnal cortisol slope in 297 children and the cortisol awakening response in 314 children. At least one of composite cortisol composite measure was available in 366 children.

Data on sleep duration and on sleep behavior at one or more time points was available in 364 (99%) of the 366 children, resulting in a study population of 277, 297, and 314, for the analyses of the AUC, the slope and the CAR, respectively.

Salivary cortisol measurements

An extensive description of the cortisol measurement and analysis was presented previously.[22] Prior to the Focus Study visit at 14 months, parents were instructed to collect five saliva samples at home using Salivette sampling devices (Sarstedt, Rommelsdorf, Germany). Parents received detailed written instructions with pictures concerning

the saliva sampling. These saliva samples were collected during one single weekday: immediately after awakening, 30 minutes later, around noon, between 1500h and 1600h, and at bedtime. For the noon saliva sample collection, parents reported a mean deviation time of 0.42h (26 minutes). Parents were asked not to let their infant eat or drink 30 min before saliva sampling to avoid disturbances of the cortisol levels. Besides these restrictions, the infants were free to follow their normal daily routines on the sampling day. Parents were asked to record information about sampling times on the Salivette tubes as well as on an enclosed schematic form. Questions assessing napping time, food intake and sleep duration were added to this form. The Salivettes were gathered at the laboratory of the Department of Epidemiology at the Erasmus MC, where the samples were centrifuged and frozen at -80°C. After completion of the data collection, all frozen samples were sent on dry ice in one batch by courier to the laboratory of the Department of Biological Psychology laboratory at the Technical University of Dresden for analysis. Salivary cortisol concentrations were measured using a commercial immunoassay with chemiluminescence detection (CLIA; IBL Hamburg, Germany). Intra- and interassay coefficients of variation were below 7% and 9%, respectively. For each time point, cortisol values that were above the 99th percentile (>200 nmol/L) were excluded (n=18, outliers from 12 children) from the analysis to reduce the impact of outliers.

We calculated three composite variables of the separate cortisol measurements within a day: the area under the curve (AUC), the diurnal cortisol slope and the cortisol awakening response (CAR). These independent variables characterize different aspects of the HPA axis activity. The AUC was used as a measure of total cortisol secretion during the day (from awakening in the morning until bedtime in the evening). It was determined by the total area under the curve given by the cortisol measurements in nmol/L on the y-axis and the time between the cortisol measurements on the x-axis, as previously described.[23] To correct for differences in length of total sampling interval time, the AUC was divided by number of hours between the first cortisol measurement at awakening and the last cortisol measurement before going to bed. The AUC was computed only for those who collected at least three saliva samples. Sleeping hours during the day were not associated with the AUC.

The diurnal cortisol slope was used as a measure of the diurnal cortisol decline. It was calculated by fitting a linear regression line for each child, which predicted the cortisol values from time since awakening. The slope was computed by using the first saliva sample and at least two other cortisol time point measures. To avoid any effect of the CAR,[22, 24] the second cortisol sample (30 minutes after awakening) was not included in this measure of the slope. Flatter slopes, as indexed by less negative betas, imply a slower cortisol decline during the day. This can be due to relatively lower morning cortisol levels or relatively higher levels in the afternoon or evening. To determine the influence of the

first and last cortisol levels on the slope, the correlation between these cortisol levels and the slope was analyzed.

The CAR was calculated as the difference between the cortisol value at awakening and the value 30 minutes after awakening.[25] The CAR was only calculated if the cortisol value 30 min after awakening was taken between 15 min and 60 min after awakening. Ninety five percent of the parents reported to have sampled the first saliva sample within 15 minutes of awakening.

Sleep duration and sleep behavior

At age 14 months information about sleep duration was derived from the schematic form enclosed with the saliva sampling. Parents were asked to report average sleep duration per night during the past week. At age 24 and 36 months parents received postal questionnaires from where information about sleep duration was assessed. At 24 months, the number of hours a child slept during the night was derived from an open question of the average hours of sleep. At 36 months, mothers were asked to report the usual bedtime and wake-up time of their child, on weekdays and on weekends. From these questions weighted average sleep duration at 36 months was calculated.

Information about sleep behavior was assessed using postal questionnaires at ages 1.5, 3 and 5 years, containing The Child Behavior Checklist[26] for toddlers (ages 18 months to 5 years). This questionnaire contains problem items on problem behavior rated on a 3-point scale: 0 (not true), 1 (somewhat or sometimes true) or 2 (very true or often true). Sleep behavior was directly derived from the Sleep Problems scale, which contains 7 items (Doesn't want to sleep alone; Has trouble getting to sleep; Nightmares; Resists going to bed at night; Sleeps less than most kids during day and/or night; Talks or cries out in sleep; Wakes up often at night). These items were summed to weighted scores according to the manual to obtain the Sleep Problems scale (30). Internal reliability of the Sleep Problems scale in the current sample, measured by Cronbach's alpha, was between 0.69 and 0.74.

Covariates

The choice of potential confounders was determined a priori and based on earlier[14] literature.[5, 14, 22, 27] Maternal age and maternal educational level were determined at enrollment using self-report. Educational level was categorized in three levels: low (no or primary education, and lower vocational training), middle (intermediate and higher vocational training) and high education (university or higher). Information about maternal smoking during pregnancy was obtained by postal questionnaires. Mothers were classified as smokers or non-smokers during pregnancy. Maternal psychiatric symptoms during pregnancy were assessed using the Brief Symptom Inventory (BSI).[28]

Date of birth, birth weight, and gender of the infant were obtained from community midwife and hospital registries at birth. Child BMI around 2 and 3 years were assessed at

child health centers and age- and sex-adjusted Z-scores were calculated using national growth curves.[29]

Maternal parenting stress was measured by the Nijmeegse Ouderlijke Stress Index – Kort (NOSIK[30]), the Dutch version of the Parenting Stress Index – Short Form. The NOSIK comprises 25 questions on two domains: parenting stress due to parental factors and parenting stress due to child factors. Only the 11 items of the parental domain were used in the present analyses, higher scores indicating greater levels of parenting stress. Harsh parenting was measured at child age 3 years and assessed through maternal self-reports based on the Parent-child Conflict Tactics Scale.[31] In a previous study, a factor analysis was conducted to identify 6 harsh parenting items.[32]

Statistical Analyses

In the non-response analysis, we compared the maternal and child characteristics of our study population with the characteristics of the eligible mothers and children with no information on the cortisol composite measures (366 vs. 236). There were only two children with information on the cortisol composite measures and no information on sleep measurements, thus we could not compare statistically compare them to our study population. For continuous variables approaching a normal distribution we used independent t-tests, for continuous non-normally distributed variables Mann-Whitney U tests and for categorical variables chi-square statistics. Analyses of missing data showed that children without information on the cortisol composite measures and without information on sleep measurements were more often girls (52.9% vs. 42.5%, chi-square=6.39, df=1, p=0.01) and had lower Apgar scores 5 minutes after birth (Apgar score below 8: 9.0% vs. 4.8%, chi-square=4.21, df=1, p=0.04). The non-responding children were more likely to have lower educated mothers as well (% low educational level: 11.6% vs. 6.3%, chi-square=5.26, df=1, p=0.02). However, these children did not differ in any other characteristics from the children in our study population.

The computed variables AUC, slope and CAR, and the CBCL scores showed a slightly skewed distribution. We did not transform these variables since regression residuals were normally distributed. We tested the associations between the composite variables of cortisol with nighttime sleep duration and sleep behavior measured at the different ages using linear regression models. First, we tested the associations adjusting for age at cortisol sampling and gender. In a next step we additionally adjusted the model for waking time at 14 months, maternal age, maternal educational level, maternal psychiatric symptoms during pregnancy, and maternal parenting stress at 18 months. We did not include maternal smoking during pregnancy, child's napping time and BMI in our models, since these covariates did not change the effect estimates meaningfully (<5%). Percentages of missing values on covariates ranged from 0% to 12% (average 7.4%). For missing values on continuous variables, the median value was imputed and for missing

values on categorical variables the median category was used for imputation. In an additional step, we also tested an interaction between the cortisol composite measures and gender on sleep duration and sleep behavior as outcome measures.

To analyze the repeated measures of sleep duration and sleep behavior during the follow-up period, we used generalized estimating equation models (GEEs). These models yield an overall estimate of the associations between the diurnal cortisol rhythm and sleep duration in the first 3 years, and with sleep behavior in the first 5 years. With GEE analyses, repeated measures over time can be analyzed, taking into account within subject correlations of the outcome. For the analysis examining associations of diurnal cortisol rhythm at 14 months and sleep duration, parent reports of nighttime sleep duration between the ages of 14 and 36 months were used as continuous outcome measures. For the analysis examining associations of diurnal cortisol rhythm at 14 months and sleep behavior, CBCL Sleep Problems weighted sum scores between 1.5 and 5 years of age were used as outcomes. This led to the following models:

$$\text{Sleep duration}_{ij} = \beta_0 + \beta_1(\text{cortisol composite measure})_i + \beta_2(\text{age})_{ij} + \beta_3(\text{sex})_i + \beta_a(\text{covariates})_i + \ldots + \text{CORR} + \text{Error}$$

$$\text{Sleep problems}_{ij} = \beta_0 + \beta_1(\text{cortisol composite measure}) + \beta_2(\text{age}) + \beta_3(\text{sex}) + \beta_a(\text{covariates}) + \ldots + \text{CORR} + \text{Error}$$

i=subject
j=timepoint (1, 2, 3)
y_{ij}= j^{th} outcome measurement on subject *i*
CORR= correction for correlation between observations (unstructured correlation matrix)

All statistical analyses were performed with the Statistical Package for the Social Sciences version 21.0 for Windows (SPSS Inc, Chicago, IL, USA).

RESULTS

Table 1 presents the characteristics of the participating mothers and children; 56.9% of this sample was male. The following median cortisol values were observed at the different time points during the day: at awakening 15.33 nmol/L (range: 0.08-51.03), 30 minutes after awakening 13.05 nmol/L (range: 0.07-55.56), at noon 5.41 nmol/L (range: 0.05-47.30), around 1600h 4.88 nmol/L (range: 0.21-40.48) and at bedtime 2.03 nmol/L (range: 0.09-58.50). These cortisol values and cortisol composite measures did not differ between girls and boys. On average, the children in our study did not show a rise of cortisol after awakening (mean CAR -1.87 nmol/L, range: -22.1; 37.6).

Table 1. Subject characteristics

	Total N=364	Mean ± SD (range) or %
Maternal characteristics		
Age (years)		31.9 ± 3.7 (16.22-43.3)
Educational level		
low	25	6.9
middle	193	53.0
high	141	38.7
Smoking during pregnancy (% yes)	39	10.7
Psychiatric symptoms (GSI-score)		0.16 ±0.18 (0.00-1.67)
Parenting stress at 18 months		0.23± 0.26 (0.00-1.82)
Child characteristics		
Gender (% boy)	207	56.9
Birth weight (grams)		3520 ±515 (1670-4795)
BMI SDS		0.19± 0.96 (-2.1;6.1)
Age of cortisol sampling (months)		14.4± 1.1 (11.7-19.3)
Cortisol values		
AUC (nmol/L)	277	8.30 ± 4.5 (0.21; 27.83)
Slope (nmol/L/h)	297	-1.04± 8.1(-3.82; 2.91)
CAR (nmol/L)	312	-1.87± 9.3 (-22.1; 37.6)
Time at awakening (hh:mm)		7:37 ± 0:45(5:35-10:00)
Napping time during the day (hh:mm)		2:54 ± 0:59(0:50-6:50)
Nighttime sleep duration (hh:mm)		
At 14 months	350	11:24 ± 0:50 (9:00-14:00)
At 24 months	349	11:13 ± 0:48 (8:30-13:30)
At 36 months	328	11:30 ± 0:37(9:00-14:36)
Sleep problems		
At 1.5 years	345	1.28± 1.8(0.00-11.0)
At 3 years	325	1.60± 2.1(0.00-12.0)
At 5 years	342	1.10± 1.73(0.00-9.00)

Values are means ± standard deviations(range) for continuous variables, and percentages for categorical variables.

GSI = Global Severity Index of the Brief Symptom Inventory, measured during pregnancy, AUC = Area under the curve, CAR = Cortisol awakening response

Sleep duration did not differ between girls and boys at 24 and 36 months, at 14 months sleep duration was longer in girls than in boys (median=12:00h in girls vs. median=11:00h in boys, Mann-Whitney Z=-2.75, df=1, p=0.006). The scores on the Sleep Problem scale did not differ between girls and boys measured at 1.5, 3 and 5 years. As none of the interactions between gender and the different cortisol composite measures were significant, the results are shown for girls and boys together.

Table 2 presents the associations between the diurnal cortisol rhythm and sleep duration reported repeatedly between 14 and 36 months. Children with flatter slopes and a more

positive CAR at 14 months were more likely to have shorter sleep duration (β per nmol/L/h slope: -0.12, 95% CI: -0.19; -0.05, p=0.001; β per nmol/L CAR: -0.01, 95% CI: -0.02; -0.00, p=0.04). The AUC at 14 months was not related to sleep duration measured between 14 and 36 months (see Table 2). Adjusting for prenatal maternal psychiatric symptoms and maternal parenting stress at child's age 18 months, both potential antecedents of the diurnal cortisol rhythm and also potential confounders, did not change any of the observed associations substantially (fully adjusted data, including maternal psychiatric symptoms and parenting stress, shown only). The appendix tables shows the separate associations between the cortisol composite measures and sleep duration at the different measurement time points.

Table 2. Associations between cortisol composite measures and repeatedly measured sleep duration

Cortisol measures	Sleep duration measured at 14, 24 and 36 months			
	Model 1		Model 2	
	Beta (95% CI)	P	Beta (95% CI)	P
AUC (nmol/L)	0.005 (-0.011; 0.020)	0.56	0.007 (-0.009; 0.023)	0.397
Slope (nmol/L/h)	-0.120 (-0.189; -0.051)	0.001	-0.105 (-0.173; -0.037)	0.002
CAR (nmol/L)	-0.008 (-0.015; -0.000)	0.041	-0.007 (-0.014; -0.000)	0.045

Model 1: adjusted for age at cortisol sampling and gender
Model 2: as model 1, additionally adjusted for waking time at 14 months, maternal age, maternal educational level, maternal psychiatric symptoms during pregnancy, and maternal parenting stress at 18 months

Betas are derived from GEE (generalized estimating equation) linear regression models.

Table 3 shows the associations between the diurnal cortisol rhythm and repeatedly measured sleep behavior between 1.5 and 5 years. The AUC, the slope and the CAR at 14 months were all not associated with Sleep Problem scores from 1.5 to 5 years (see Table 3 for details). Likewise, there were no associations between the cortisol composite measures and the Sleep Problem scores at the different measurement time points (data not shown).

Table 3. Associations between cortisol composite measures and repeatedly measured sleeping problems

Cortisol measures	CBCL sleeping problems measured at 18 months, 36 months and 5 years			
	Model 1		Model 2	
	Beta (95% CI)	P	Beta (95% CI)	P
AUC (nmol/L)	-0.002 (-0.036; 0.031)	0.89	-0.004 (-0.037; 0.029)	0.83
Slope (nmol/L/h)	0.062 (-0.111; 0.236)	0.48	0.093 (-0.066; 0.253)	0.25
CAR (nmol/L)	0.012 (-0.003; 0.028)	0.12	0.013 (-0.001; 0.027)	0.063

Model 1: adjusted for age at cortisol sampling and gender
Model 2: as model 1, additionally adjusted for waking time at 14 months, maternal age, maternal educational level, maternal psychiatric symptoms during pregnancy, and maternal parenting stress at 18 months

Betas are derived from GEE (generalized estimating equation) linear regression models.

DISCUSSION

This prospective population-based study showed that the infants with a flatter cortisol slope and those with a more marked morning cortisol rise have shorter night time sleep duration - as measured repeatedly across early childhood. However, none of these cortisol summary measures predicted pre-school sleep problems.

Although higher cortisol levels have been cross-sectionally associated with sleep problems, few studies have assessed longitudinally whether cortisol levels in infants or toddlers predict sleep problems at later age. Recently, Kiel et al.,[20] however, reported that variations in cortisol secretion patterns at ages 18-20 months predicted an increase of sleep problems from age 2 to 3 years. These authors studied a composite of diurnal, nocturnal and morning levels of cortisol. However, there was no main effect of cortisol, rather the direction of the association between cortisol and sleep depended on the parenting style; i.e. both a positive and a negative association between blunted cortisol secretion and sleep problems in this sample was observed. Thus, our results are in line with those of Kiel et al.,[20] who also observed no main effect and only detected an association when stratifying the sample on parental control.

There are different explanations possible for the negative findings on sleep problems in our study and those from Kiel et al.[20] First, it could be that we must reject our hypothesis as there is no association. Second, the association could depend to such an extent on parenting style that not accounting for an interaction in the present study obscured the association. In the present study, however, we did not observe any confounding or effect modification by parenting stress. Third, our study and that of Kiel et al.[20] may not have enough power to show a main effect association. Indeed, Kiel et al.[20] included 51 toddles in their study, which were additionally grouped, whereas our study with more than 300 children had a larger sample size, but this would still be insufficient to show very small effects. Fourth, the Child Behavior Checklist (CBCL) is not a very precise measure of sleep problems, although it is a validated instrument and the Sleep Problems scale that has repeatedly been used as a stand-alone sleep problem measure[26, 33] and had a fairly good internal reliability in our study population.

In contrast, we clearly confirmed our hypothesis that the diurnal cortisol rhythm was longitudinally associated with sleep duration. Infants with a flatter cortisol slope and those with a more marked morning cortisol rise slept shorter at night from age 14 months to age 3 years. In our study we used night time sleep duration as a separate outcome measure instead as part of a construct of poor sleep. Our findings are in line with the results of cross-sectional studies, e.g. those from Lemola et al.[34] who reported that morning cortisol secretion was negatively associated with sleep duration in children. El-Sheikh et al.,[35] as

well, found that higher afternoon cortisol levels were associated with shorter sleep duration in children. Also, Räikkönen et al.[36] found, in line with our results, that children with short sleep duration displayed higher cortisol awakening response and less decrease in cortisol across the day. These cross-sectional studies must be interpreted cautiously as consistency with the temporal direction of association cannot be evaluated. To our knowledge, only Hatzinger et al.[37] conducted a longitudinal study in preschoolers. However, they investigated whether poor sleep, objectively measured with sleep-actigraphy recordings, was prospectively associated with cortisol secretion 12 months later. Although they, like the above-mentioned studies, showed a cross-sectional association between poor sleep and high morning cortisol levels, no longitudinal association between sleep as a predictor of cortisol secretion was observed. This strongly suggests that the direction of association may be the reverse.

Several potential mechanisms could explain our findings. First, stress (acute and chronic, as well as physical and psychological) is known to alter cortisol secretion in children[38] and it has been shown that sleep, stress and cortisol secretion are highly intertwined.[39] Infants who experience more stress, such as an adverse intra-uterine environment or early life adversity due to low socio-economic status or maternal depression, are more likely to have altered cortisol secretion patterns[39-41] and sleeping patterns.[42] In this study we assessed several potential environmental stressors for the child, such as prenatal maternal psychiatric symptoms and maternal parenting stress at child's age 18 months, as potential antecedents and/or confounders. However, including these specific factors in our models did not change the observed associations and this cannot explain why cortisol is prospectively related to shorter sleep duration in early childhood. Second, and certainly not an exclusive mechanism, the diurnal cortisol secretion has a close association with the sleep-wake cycle. Mostly the impact of disturbed sleep on the diurnal cortisol rhythm has been the focus of research (review[43]). However, it is important to realize that a bidirectional relation between sleep and HPA system has been discussed repeatedly (review[44]). Yet, these studies in adults cannot easily be translated to children as sleep duration is a highly developmentally determined phenomenon.[45] Third, genetic differences could explain both different cortisol secretion patterns and later sleep problems in children (review[46]). Genetics, gene-environment interaction and other common causes can, even in a longitudinal study, not be ruled out as explanations for our results. Likewise, residual confounding is always possible, although we tried to minimize the effect of external variables by adjusting our models with several possible indicators of socio-economic background, including maternal age and maternal educational level, as well as adverse circumstances, such as maternal psychiatric symptoms during pregnancy and parenting stress at 18 months.

The results of the current study should be carefully considered in light of several methodological strengths and limitations. The strengths of our study are the large population-based sample, its prospective design, and the repeated measures over time of both sleep duration and sleep behavior. Yet, some limitations also need to be considered. First, the sampling of saliva occurred only on one single day, so day-to-day variability could not be taken into account.[47] However, to ask parents participating in a large cohort with multiple other assessments to sample on several days increases the risk of drop-out or non-response. Second, the compliance of the saliva sampling was not assessed by an objective measurement such as a timing device, for this we relied on parental report. Furthermore, we relied on parental report for sleep duration and sleep behavior which is the best available measure in large population based studies but cannot be seen as a gold standard such as polysomnography, or as objective as actigraphy. Also, at the ages of assessment we had no information on parenting style, thus stratification as performed by Kiel et al.[20] was not possible. Our analyses of missing data showed that attrition was not at random. There was a selective dropout of girls, children with lower Apgar scores and children of lower educated mothers. Due to possible selection effects, our results may be less representative of the general population.

These results have both scientific and clinical implications. Firstly, they suggest that variations in cortisol precede a shorter sleep duration and may thus help understand the biological mechanisms from stressors, such as social disadvantage and early (family) adversity, to sleep deficits. Also, it is conceivable that, if replicated, a flatter cortisol slope (implying blunted HPA axis activity) may be used as a predictor of sleep deficits in certain subgroups of stressed infants.

Conclusion

A flatter cortisol slope in infants and a more marked morning cortisol rise in infants was associated with shorter sleep at pre-school age(s). The underlying mechanism cannot easily be inferred from this study, however, if infant cortisol levels remain high across the day this may indicate stress or HPA dysregulation with long-term consequences on sleep.

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Appendix Table 1A. Associations between cortisol composite measures and sleep duration at 14 months

Cortisol measures	Sleep duration measured at 14 months			
	Model 1		Model 2	
	Beta (95% CI)	P	Beta (95% CI)	P
AUC (nmol/L)	-0.003 (-0.03; 0.02)	0.82	0.002 (-0.02; 0.02)	0.83
Slope (nmol/L/h)	-0.12 (-0.24; -0.00)	0.047	-0.11 (-0.22; 0.01)	0.07
CAR (nmol/L)	-0.01 (-0.02; 0.00)	0.04	-0.01 (-0.02; 0.001)	0.08

Model 1: adjusted for age at cortisol sampling and gender
Model 2: as model 1, additionally adjusted for waking time at 14 months, maternal age, maternal educational level, maternal psychiatric symptoms during pregnancy, and maternal parenting stress at 18 months

Appendix Table 1B. Associations between cortisol composite measures and sleep duration at 24 months

Cortisol measures	Sleep duration measured at 24 months			
	Model 1		Model 2	
	Beta (95% CI)	P	Beta (95% CI)	P
AUC (nmol/L)	0.012 (-0.011; 0.034)	0.32	0.013 (-0.011; 0.036)	0.29
Slope (nmol/L/h)	-0.161 (-0.27; -0.037)	0.006	-0.152 (-0.27; -0.037)	0.010
CAR (nmol/L)	-0.011 (-0.02; -0.001)	0.027	-0.010 (-0.02; -0.001)	0.033

Model 1: adjusted for age at cortisol sampling and gender
Model 2: as model 1, additionally adjusted for waking time at 14 months, maternal age, maternal educational level, maternal psychiatric symptoms during pregnancy, and maternal parenting stress at 18 months

Appendix Table 1C. Associations between cortisol composite measures and sleep duration at 36 months

Cortisol measures	Sleep duration measured at 36 months			
	Model 1		Model 2	
	Beta (95% CI)	P	Beta (95% CI)	P
AUC (nmol/L)	0.009 (-0.01; 0.03)	0.30	0.01 (-0.007; 0.026)	0.254
Slope (nmol/L/h)	-0.10 (-0.18; -0.01)	0.02	-0.085 (-0.165; -0.006)	0.035
CAR (nmol/L)	-0.003 (-0.01; 0.005)	0.41	-0.004 (-0.011; 0.004)	0.33

Model 1: adjusted for age at cortisol sampling and gender
Model 2: as model 1, additionally adjusted for waking time at 14 months, maternal age, maternal educational level, maternal psychiatric symptoms during pregnancy, and maternal parenting stress at 18 months

4 NEURODEVELOPMENTAL OUTCOMES OF CHILDHOOD SLEEP PROBLEMS

Early childhood sleep disturbance trajectories and brain morphology at age seven years

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Abstract

Childhood sleep and brain morhology

Background: Little is known about the impact of sleep disturbances on the structural properties of the developing brain. This study explored associations between childhood sleep disturbances and brain morphology at 7 years.

Methods: Mothers from the Generation R cohort reported sleep disturbances in 720 children at ages 2 months, 1.5, 2, 3 and 6 years. T1-weighted MRI images were used to assess brain structure at 7 years. Associations of sleep disturbances at each age and of sleep disturbance trajectories with brain volumes (total brain volume, cortical and subcortical grey matter, and white matter) were tested with linear regressions. To assess regional differences, sleep disturbance trajectories were tested as determinants for cortical thickness in whole-brain analyses.

Results: Sleep disturbances followed a declining trend from toddlerhood onwards. Infant sleep was not associated with brain morphology at age 7. Per SD sleep disturbances (1 frequent symptom or 2 less frequent symptoms) at 2 and 3 years of age, children had -6.3 (-11.7 to -0.8) cm^3 and -6.4 (-11.7 to -1.7) cm^3 smaller grey matter volumes, respectively. Sleep disturbances at age 6 years were associated with global brain morphology (grey matter: -7.3 (-12.1 to -2.6), p-value=0.01). Consistently, trajectory analyses showed that more adverse developmental course of childhood sleep problems is associated with smaller grey matter volumes and thinner dorsolateral prefrontal cortex.

Conclusion: Sleep disturbances from age 2 years onwards are associated with smaller grey matter volumes. Thinner prefrontal cortex in children with adverse sleep problems trajectories may reflect effects of sleep disturbance on brain maturation.

INTRODUCTION

The specific functions of sleep remain a scientific enigma, yet, research has provided sufficient evidence that sleep serves recovery duties to the brain.[1] Sleep patterns and problems in adults have been associated with structural and functional properties of the brain.[2-4] Compared to good sleepers, insomnia patients were shown to have smaller grey matter volumes in the orbitofrontal cortex,[5] and less activation in prefrontal cortical areas during task-based functional Magnetic Resonance Imaging (fMRI).[6] However, little is known about how sleep disturbances influence the developing brain.

Sleep problems occur frequently in children, with prevalence estimates of up to 30%.[7] The relation of sleep problems with various neurodevelopmental outcomes is well documented. Sleep disturbances have been longitudinally related to poor cognitive functioning,[8] behavioral and emotional problems,[9] autism[10] and hyperactivity,[11] in childhood and adolescence. Cross-sectional research in older children has also shown that various sleep indices correlate with brain morphology. Deep sleep brain waves underlie adolescents' cortical maturation differences[12, 13] and longer sleep duration corresponds to more grey matter in the hippocampus and dorsolateral prefrontal cortex.[14] In addition, adolescents' sleep length variability has been longitudinally related to lower white matter integrity.[15] However, it is unclear whether sleep disturbances are associated with brain morphology already in early childhood.

Most previous studies focused on distinctive sleep parameters, such as sleep duration or architecture, but sleep problems often appear in concert,[16] and show persistency from early childhood to adolescence.[17] In particular, dyssomnia symptoms such as difficulty initiating or maintaining sleep can represent misalignment in the two processes that shape sleep patterns: homeostatic processes and circadian timing.[18, 19] Animal research shows that normal sleep patterns allow the brain to reestablish the synaptic homeostasis[20] that is disturbed by plastic changes resulting from daily experiences and learning. This process is particularly important for the developing brain, as childhood is a time of concentrated learning and daily acquirement of fundamental skills.[21] Indeed, experience-dependent brain plasticity measured by the sleep electroencephalogram was shown to be higher in children than in adolescents and adults.[22] Against this background, the hypothesis that undisturbed sleep facilitates neurodevelopment and maturation has been formulated.[12, 21-23] Sleep homeostasis starts to emerge around the second month of life and rapid changes of sleep patterns continue throughout toddlerhood and childhood.[19] These changes in sleeping patterns are considered important developmental milestones, however a critical period during which sleep contributes most for neurodevelopment has not been shown. It is also unclear whether the impact of childhood sleep disturbances is more pronounced in brain areas involved in higher cognitive processing, such as the frontal cortex, which is most vulnerable to sleep deprivation.[24]

The present population-based study explored the association of repeatedly measured childhood sleep disturbances from age two months to six years with neuroanatomy as assessed by structural MRI scans at age seven. We tested if there is a critical period in early childhood when sleep disturbances are particularly important for brain development. Next, we combined repeatedly assessed sleep disturbances to define sleep problems trajectories across childhood. We hypothesized that persistent sleep disturbances during early childhood interfere with cortical development (e.g. earlier decrease in synaptogenesis) or maturation (e.g. excessive pruning) resulting in less grey matter volume, and/or thinner cortex. Specifically, we expected thinner cortex in frontal brain areas among children with persistent sleep disturbances.

METHODS

This study was conducted within the Generation R Study, a population-based prospective cohort from fetal life onwards.[25] All pregnant women living in Rotterdam, the Netherlands, with an expected delivery date between April 1, 2002 and January 1, 2006 were invited to participate. The Medical Ethics Committee of the Erasmus Medical Centre, Rotterdam, approved the study and written informed consent was obtained from all parents.

For this study, all children with maternal assessment of sleep problems on at least two time points between 2 months and 6 years, and an MRI brain scan around 7 years were eligible (n=828). After exclusion of images with insufficient quality or incidental findings, sample sizes ranged between 579-689 children in any single analysis, depending on the individual time points of sleep assessment. The trajectories of sleep disturbances across childhood were based on all children participating in the Generation R Study with sleep problems assessment on at least two time points between 2 months and 6 years of age ('The full cohort': n=5796).

Sleep assessment

Sleep problems were assessed with postal questionnaires at 2 (interquartile range [IQR] 2.2-3.6) months, 1.5 (IQR 1.5-1.6) years, 2 (IQR 1.9-2.1), 3 (IQR 3.0-3.1) years, and 6 (IQR 5.8-6.1) years. At 1.5, 3 and 6 years, mothers answered 7-questions measuring Sleep Problems from the Child Behavior Checklist (CBCL 1.5-5) on a three-point likert scale (0-not true, 1-somewhat or sometimes true or 2-very or often true).[26] Consistent with previous studies,[16, 17] exploratory factor analysis of the CBCL Sleep Problems Scale revealed two constructs measuring sleep problems of different domains with a potentially diverse neurobiological background at each time point. By computing a weighted sum score of the 5 dyssomnia items we constructed a dyssomnia scale (Doesn't want to sleep alone; Has trouble getting to sleep; Resists going to bed at night; Sleeps less than most kids during day and/or night;

Wakes up often at night), but did not study parasomnia, as symptoms like nightmares and night terrors are more transitory. To obtain information on sleep disturbances at more time points across childhood, we also used other questionnaires that evaluated children's sleep patterns to assess dyssomnia. Weighted sum scores at ages 2 months and 2 years were computed using age appropriate dyssomnia items that closely mirror those of the CBCL dyssomnia scale. To ensure that the Generation R sleep items measure the same construct as the CBCL dyssomnia scale, confirmatory factor analysis was performed in the "Full cohort" of children (n=5796) with at least two assessments of sleep disturbances. Standard model fit indices showed a very good fit (RMSEA=0.045, CFI=0.919), indicating that the same construct was measured over the follow-up time. Internal consistency per time point ranged between 0.6 and 0.7.

Next, we took a developmental approach and defined groups of children based on their patterns of sleep disturbances over time. Trajectories of sleep disturbance across childhood were defined using Latent Class Trajectory Models.[27] This is a person-centered modelling approach that estimates growth curves across unobserved subpopulations by assigning a most likely latent trajectory class to each individual. Because model accuracy depends on the sample size, this analysis was performed in all children (n=5796). There were no substantial differences in the distribution of the trajectories classes between the children that underwent MRI scanning and 'The full cohort'. Standard criteria indicated that a three-class model fit our sample best. Average posterior probability of correct class assignment was above 0.94 for each class. The largest group was the *decreasing to low sleep disturbances* class (n=363, 50.7%), which followed a normative developmental decline of sleep problems as previously reported[18] and was therefore defined as the reference. The s*table at medium sleep disturbances* class comprised of 239 (33%) children and the *increasing to high sleep disturbances* class comprised of 118 (16.3%) children.

MRI

The neuroimaging protocol in the Generation R Study has been described previously.[28] Children aged 6 to 9 years were first familiarized with the MRI environment during a mock scanning session. Structural MRI scans were obtained on a 3T scanner (General Electric Discovery MR750, Milwaukee, MI, USA). T1-weighted inversion recovery fast spoiled gradient recalled (IR-FSPGR) sequence was obtained using an 8-channel head coil, with the following parameters: TR = 10.3 ms, TE = 4.2 ms, TI = 350 ms, NEX = 1, flip angle = 16°, readout bandwidth = 20.8 kHz, matrix = 256 x 256, imaging acceleration factor of 2, and an isotropic resolution of 0.9 x 0.9 x 0.9 mm^3.

Quality assessment. Quality assessment was performed in two steps. First, all T1-weighted scans were rated on a 6-item scale for quality (unusable, poor, fairly good, good, very good, excellent). Scans rated below 'fairly good' were excluded. After processing through

FreeSurfer, all images were visually inspected for segmentation quality. Again, images that were rated as unusable or poor were excluded from analyses (n=108).

Image processing. Cortical reconstruction and volumetric segmentation were performed with the FreeSurfer image analysis suite 5.1 (http://surfer.nmr.mgh.harvard.edu/), as previously described.[29] Cortical thickness was calculated as the closest distance from the gray/white boundary to the gray/cerebrospinal fluid boundary at each vertex on the tessellated surface.[30] The surface-based map was smoothed using a 10-mm, full-width half-maximum Gaussian kernel prior to the surface-based analyses. FreeSurfer morphometric procedures have been demonstrated to show good test-retest reliability across scanner manufacturers and across field strengths.[31] Numerous studies in typical and atypical developing school aged children have used this procedure.[32-36]

Covariates

Child characteristics. Information on sex, date of birth and gestational age was obtained from midwives and hospital registries. Congenital anomalies were retrieved from perinatal and neonatal registries.[37-39] Child's ethnicity was based on parent's country of birth[40] and categorized into: 'Dutch' (Netherlands), 'Other-Western' (other European and North American countries) and 'Other non-Western'. Paroxysmal events were defined as epilepsy, neonatal seizures, febrile seizures or other seizures and reported by mothers in a screening questionnaires at ages 2,6 and 12 months.[39] Mothers reported if their child snored at 6 months of age. Child weight at 10-13 months was assessed at child health centers, and age and sex-adjusted z scores were calculated.[41] Child internalizing and externalizing problems at age 3 years were assessed with the CBCL.[26] Children's Intelligence (IQ) at age 6 years was estimated from the Mosaics and Categories subtest of the Snijders-Oomen Non-Verbal Intelligence Test-Revised.[42] Information on physician-diagnosed asthma was obtained by maternal report at 6 years.[43] Handedness was measured using a modified version of the Edinburgh Handedness Inventory.[44]

Maternal characteristic. Maternal education was assessed with questionnaires at enrollment and classified into high, intermediate, or low.[40] Tobacco smoking was obtained by postal questionnaire in early, mid- and late pregnancy and categorized into: "never smoked", "stopped smoking when pregnancy was known" and "continued smoking". Affective symptoms were assessed by averaging the depression and anxiety subscales from the Dutch version of the Brief Symptom Inventory,[45] filled in by the mother when the child was 3 years old.

Statistical analyses

First, we tested whether the dyssomnia items from 2 months to 6 years assess the same construct using confirmatory factor analysis. The sleep disturbance sum scores correlated

highly with the factor scores at each age (correlation coefficients ranging between 0.93 and 0.99), thus to simplify interpretation we used sum scores for all analyses. Because the number of items varied per time point, we standardized the scores to enable comparisons. At each time point, the associations of sleep disturbances with global brain volumetric measures at age 7 were analyzed with linear regression models. The sleep disturbances scores were log transformed to approach normality and regression residuals were normally distributed in all tested associations. The effect estimates of the associations with brain volumes (cm^3) can be interpreted as standard deviation (SD) increments or decrements of sleep disturbances. MRI outcomes (total brain volume, cortex volume, cerebral white matter volume and subcortical grey matter volume) were residualized for age at scanning (6-10 years). Based on previous literature or >5% change in effect estimate of the predictor variable, models were adjusted for child's sex, age at sleep assessment, handedness, ethnicity, snoring, BMI, presence of paroxysmal events and congenital anomalies, asthma status, and mother's history of smoking during pregnancy, education and affective symptoms score. Because of high collinearity of total brain volume with grey matter (r=0.96) and white matter (r=0.94) volumes, total brain volume was only controlled in the analyses with subcortical structures and no adjustment for multiple comparisons was performed. To explore whether cognition or behavioral problems explained the associations between sleep disturbances and brain volumes, in an additional step we adjusted the models for nonverbal IQ and externalizing or internalizing problems. Missing values on covariates (less than 15%, except for congenital malformations 20.6% and snoring 37.5%) were imputed using multiple imputations to create ten complete datasets. Statistical analyses were run in the ten datasets and results were pooled.

In addition to individual time point analyses, developmental trajectories of sleep disturbances were estimated. Differences among the trajectory groups were compared with x^2 statistic for categorical variables, ANOVA for normally distributed variables and Kruskal-Wallis for variables with skewed distribution. First, the sleep disturbance trajectory classes were entered as predictors for global brain volumes in linear regression models. The decreasing to low sleep disturbance trajectory class was used as a reference and the same covariates were controlled for. Next, regional differences in cortical morphology across the childhood sleep disturbance trajectories were explored. Vertex-wise (whole-brain) analyses were performed using the FreeSurfer QDEC module. Regions for which sleep disturbance trajectories were associated with cortical thickness, cortical surface area or cortical volume were identified using a General Linear Model. The three trajectories of sleep disturbances were entered as a continuous predictor for cortical thickness, surface area and cortical volume at each vertex (decreasing to low sleep disturbance=1, stable at medium sleep disturbance=2, increasing to high sleep disturbance=3), controlling for sex, age, handedness, prenatal smoking exposure, child's externalizing and internalizing problems, IQ, BMI, ethnicity and maternal education. Analyses were corrected for multiple

comparisons using the built-in Monte Carlo simulation at a p-value 0.05 threshold, a cluster wise correction that controls for the rate of false positive clusters. Factor and trajectory analyses were conducted in Mplus 7.1 (Muthén & Muthén, 1998-2013). Other analyses were performed in SPSS Statistics 21.0 (IBM Corporation, Somers, NY, USA).

RESULTS

Characteristics of the participating children and their mothers are shown in Table 1. Sleep disturbances followed a declining trend from toddlerhood onwards. Distributions of the sleep disturbance scores varied per time point, however, in terms of absolute symptomatology, 1 standard deviation approximately equals 1 frequent/severe symptom or two less frequent/severe symptoms at each time point.

Associations between childhood sleep disturbances and brain morphology

We tested the associations of sleep disturbances assessed at different ages with total brain volume, cortical and subcortical grey matter volume and white matter volume. We found no association for any of the brain volume measures with sleep disturbances at age 2 months and 1.5 years (Table 2). Sleep disturbances at 2 and 3 years were associated with smaller grey matter volumes at age 7, but not with white matter or subcortical grey matter volumes. Children had on average -6.3 (-11.7 to -0.8) cm^3 and -6.4 (-11.4 to -1.7) cm^3 smaller grey matter volume per standard deviation sleep disturbances at 2 and 3 years, respectively. The associations of sleep disturbances at 6 years with global brain volumes were most prominent (grey matter: -7.3cm^3, 95%CI: -12.1 to -2.6, p-value=0.01, white matter: -4.8, 95%CI: -8.0 to -1.7, p-value=0.01). Sensitivity analyses showed that adjustment for cognition or behavioral problems did not account for the observed associations between sleep disturbances and brain volumes (data not shown). The effect estimates between sleep problems at 3 years and grey matter volume slightly attenuated when correcting for co-occurring externalizing problems, however the observed association remained (-5.2 cm^3, 95%CI -10.2 to -0.1, p=0.047).

Association of sleep disturbance trajectories and brain morphology

Children in the increasing to high trajectory class were more likely to be of non-western ethnicity and had more behavioral and emotional problems at 3 years (Table 1). Mothers of these children were less educated, were more likely to smoke during pregnancy and had higher affective symptoms scores. The association of childhood sleep disturbance trajectories with global brain volumetric measures is shown in Table 3. Children in the increasing to high sleep disturbance class had smaller total brain volumes compared to children with decreasing to low sleep disturbances (-26.8cm^3, 95% CI: -50.2 to -3.4). Compared to the reference, children in the increasing to high sleep disturbance class had

Table 1. Participants' characteristics

		Sleep disturbance trajectories		
	Total Group	Decreasing to low	Stable at medium	Increasing to high
	N=720	N=363	N=239	N=118
Children characteristics				
Sex, % girls	48.5	47.1	49.0	51.7
Child age at MRI scan, years ±SD	7.9 ± 1.0	8.0 ± 1.0	7.9 ± 1.0	7.8 ± 1.0
Gestational age at birth, weeks ±SD	39.9±1.9	39.9±1.9	39.8±1.8	39.9±2.0
Congenital malformation, % yes	6.4	7.6	6.9	4.7
Sleep disturbances, score ±SD				
At 2 months	2.68 (±1.9)*	2.5±1.8	2.8±1.9	3.1±2.1
At 1.5 years	1.54 (±2.0)**	1.2±1.8	1.6±1.8	2.9±2.5
At 2 years	2.39(±2.1)**	1.9±1.8	2.4±1.9	4.0±2.4
At 3 years	1.62 (±1.9)**	0.9±1.4	1.9±1.7	3.5±2.3
At 6 years	1.17 (±1.7)**	0.0±0.0	1.4±0.5	4.4±1.6
IQ, score ±SD	103.78 ± 14.07	104.8±12.6	102.7±14.7	102.9±14.0
BMI, z-score ± SD	0.2±1.02	0.1±1.0	0.3±0.9	0.2 ±1.0
Paroxysmal events, % yes	2.3**	1.5	1.3	6.1
Asthma, % yes	6.1*	4.2	8.5	7.3
Snoring, % yes	9.8	9.7	7.7	14.2
Behavioral Problems, score ±IQR				
Externalizing	10.4 (5 - 14)**	4.0 (2.0;6.4)	10.0 (7.0;13.6)	11.2 (9.0;17,6)
Internalizing	6.1 (2 - 9)**	8.0 (4.2;12.0)	4.8 (3.0;9.0)	6.0 (4.4;10.0)
Ethnicity, %				
Dutch	74.9**	55.0	33.0	12.0
Other western	6.8	51.0	34.7	14.3
Other non-western	18.3	31.1	34.8	34.1
Maternal characteristics				
Age, years ±SD	31.2 ± 4.5	31.5±4.4	30.9±4.6	31.1±4.8
Education, %				
High	61.2**	54.7	31.9	13.4
Middle	28.5	49.8	32.7	17.5
Low	10.3	26.4	42.8	30.8
Affective symptoms, score (IQR)	0.08(0.0-0.3)**	0.08 (0.0;0.2)	0.08(0.0;0.2)	0.17(0.1;0.4)
Smoking during pregnancy, %				
Never smoked	76.3**	53.9	31.5	14.6
Smoked until pregnancy was known	7.0	49.9	34.1	16.0
Continued smoking	16.7	34.3	40.9	24.8

*P-Value<0.05; **P-value<0.01

on average -14.2 cm^3 (95%CI: -27.6 to -0.9) smaller grey matter volumes, with a consistent dose-response relationship across the sleep disturbance trajectories (p for trend=0.02). We observed no significant differences in white matter volume and subcortical grey matter volume across the sleep disturbance trajectories. Controlling for behavioral problems slightly attenuated the association between childhood sleep disturbance trajectories and grey matter volume (data not shown).

Table 2. The association between standardized childhood sleep disturbances and global brain morphology

		Total brain volume (cm^3)		Cortical gray matter volume (cm^3)		Cerebral white matter volume (cm^3)		Subcortical grey matter volume (cm^3)*	
Sleep disturbances	n	B (95% CI)	p-value	B (95% CI)	p-value	B (95% CI)	p-value	B (95% CI)	p-value
At 2 months	579	1.2 (-7.5;9.9)	0.79	1.1 (-3.9;6.0)	0.68	0.0 (-3.6;3.3)	0.92	-0.1 (-0.4;0.1)	0.35
At 1.5 years	610	-5.3 (-14.3;3.7)	0.25	-3.2 (-8.3;2.0)	0.23	-0.8 (-4.2;2.7)	0.67	-0.2 (-0.5;0.1)	0.12
At 2 years	639	-10.9 (-20.4;-1.5)	0.02	-6.3 (-11.7;-0.8)	0.02	-2.1 (-5.7;1.5)	0.26	0.1 (-0.2;0.3)	0.74
At 3 years	616	-9.3 (-17.8;-0.7)	0.03	-6.4 (-11.4;-1.7)	0.01	-1.9 (-5.1;1.4)	0.27	0.1 (-0.2;0.3)	0.56
At 6 years	689	-13.5 (-21.8;-5.2)	<0.01	-7.3 (-12.1;-2.6)	<0.01	-4.8 (-8.0;-1.7)	<0.01	-0.1 (-0.3; 0.2)	0.51

Note: Models are adjusted for child's sex, handedness, age at sleep reports, ethnicity, snoring, BMI, presence of paroxysmal events and congenital anomalies, asthma status, prenatal smoking exposure, maternal education and affective symptoms

*Adjusted for total brain volume

Table 3. Association between childhood sleep disturbances trajectories and global brain volumetric measures

	Predictors: Sleep disturbances trajectories in first 6 years			
Outcomes: Brain MRI volumetric measures (cm^3)	Decreasing to low sleep disturbances (n=363)	Stable at medium sleep disturbances (n=239)	Increasing to high sleep disturbances (n=118)	P for trend
Total brain volume, mean	1119.6	1107.5	1092.5	
B (95% CI)	Reference	-13.3 (-30.9 to 4.3)	-26.8 (-50.2 to -3.4)	0.02
Cortical grey matter, mean	535.9	526.9	521.5	
B (95% CI)	Reference	-9.6 (-19.6 to 0.4)	-14.2 (-27.6 to -0.9)	0.02
White matter, mean	375.7	371.7	366.0	
B (95% CI)	Reference	-4.4 (-11.1 to 2.3)	-9.6 (-18.5 to -0.7)	0.03
Subcortical grey matter, mean*	62.3	62.1	62.5	
B (95% CI)	Reference	-0.2 (-0.8 to 0.3)	0.2 (-0.5 to 0.9)	0.91

Note: Models are adjusted for child's sex, age, handedness, ethnicity, snoring, BMI, presence of paroxysmal events and congenital anomalies, asthma status, prenatal smoking exposure, maternal education and affective symptoms. *Adjusted for total brain volume

Next, regional differences were investigated by entering the sleep disturbance trajectories as predictors for cortical thickness, cortical surface area and cortical volume at each vertex. Adjusted for covariates, a negative association of childhood sleep disturbance trajectories with cortical thickness was identified at a cluster of the middle frontal gyrus including the right dorsolateral prefrontal cortex shown on Figure 1 (1133mm^2, $p_{corrected}$= 0.0067). Adverse developmental trajectories of childhood sleep disturbances were related to thinner cortex in the right dorsolateral prefrontal cortex, independent of child ethnicity, behavioral problems and other child and maternal characteristics. After correction for multiple comparisons, we observed no associations between sleep disturbance trajectories and cortical surface or cortical volume.

Nonresponse analysis

Based on *X*-squared test statistics the children who underwent MRI scanning (n=720) were more likely to be Dutch (75% vs. 63%, p-value<0.01) compared to 'The full cohort'. However, the samples did not differ in maternal education, sex, age or sleep disturbance score at any of the time-points.

Figure 1. Relation between sleep disturbances trajectories and cortical thickness

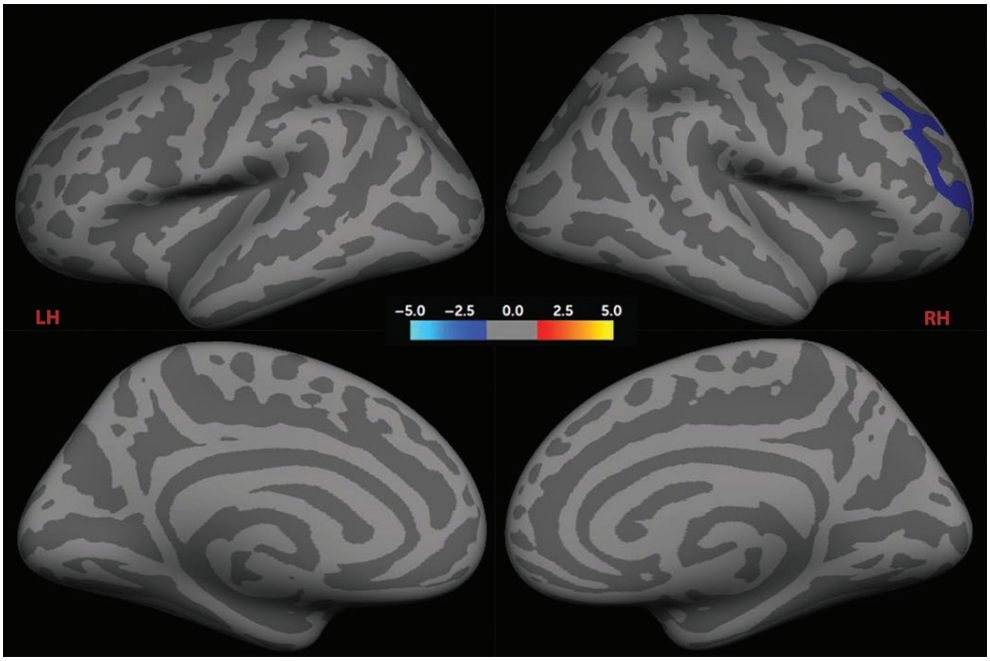

Figure 1. Vertex-wise association between sleep disturbance trajectories and cortical thickness (n=720). Adjusted for sex and age, handedness, prenatal smoking exposure, child's externalizing and internalizing problems, IQ, BMI, ethnicity and maternal education, cortical thickness was negatively associated with sleep disturbance trajectories (decreasing to low sleep disturbance=1, stable at medium sleep disturbance=2, increasing to high sleep disturbance=3) in a cluster including portions of the right dorsolateral prefrontal cortex (Monte Carlo corrected cluster-wise p<0.007). Colors represent –log10 p-value. Note: LH=left hemisphere, RH=right hemisphere

DISCUSSION

This study investigated the prospective associations between sleep disturbances throughout early childhood and brain morphology in seven-year-old children from the general population. Children with more sleep disturbances in the first six years had smaller brain volumes at seven years. From age two years onwards, sleep disturbances predicted grey matter differences, whereas sleep disturbances observed at six years were associated with global brain morphology. Children following an adverse developmental course of sleep disturbances across the first six years also had smaller brain volumes, and thinner cortex in the right dorsolateral prefrontal cortex.

To the best of our knowledge this is the first prospective study of childhood sleep problems and brain morphology. Grey matter deficits in similar regions of the prefrontal cortex have been found in adults with primary insomnia[5, 6, 46] and older children with obstructive sleep apnea.[47] In line with our results, cross-sectional studies in older children have reported

associations of sleep duration and deep sleep with grey matter in the prefrontal cortex.[12, 14] More support for our findings comes from cognitive research. Two longitudinal studies assessed sleep problems with the CBCL repeatedly across childhood and found relations with reduced working memory at age 16 years,[17] and executive control at age 17 years.[16]

During development, increase in grey matter volume indicates synaptogenesis,[48] whereas from age 6 onwards cortical pruning (elimination of redundant synapses) results in cortical thinning.[49] Therefore, different explanations for our findings are possible. First, the association between childhood sleep disturbances and smaller brain volumes could reflect an early decrease in synaptogenesis related to sleep disturbances. Genes expressed during abnormal sleep-wake cycles might affect neurodevelopment through cellular growth arrest, as a cellular stress response.[50] Indeed, animal studies have shown that the expression of genes involved in cellular stress (e.g. BiP) increase during sleep deprivation across species and brain regions.[51] Alternatively, childhood sleep problems might disturb neuroplasticity. Structural, molecular and experimental evidence shows that sleep-dependent synaptic homeostasis might be particularly important during early development.[12, 20, 22, 50, 52] Childhood sleep disturbances or the associated behavioral or cognitive difficulties might interfere with the process of experience-dependent molding of the cortical columns, resulting in less grey matter both regionally and globally.[21, 52-54]

The global trend of smaller cortical volumes among children with more sleep disturbances showed meaningful regional specificity. Childhood sleep disturbances were associated with thinner cortex in the dorsolateral prefrontal area (reduced cortical thickness). No regional differences in cortical surface area or cortical volume were found, corresponding to previously reported independent developmental paths of the different cortical measures.[36, 48, 55-59] In line with our findings, gray matter in the right dorsolateral prefrontal cortex was shown to correlate positively with sleep duration in older children.[14] The prefrontal cortex has the most protracted development during childhood,[60, 61] is involved in high order cognitive processes and is therefore most vulnerable to sleep deprivation.[62] The dorsolateral prefrontal cortex is part of the central executive network which continues developing into adolescence,[63] thus it is plausible that childhood sleep disturbances would specifically interfere with its development.[16]

The cortical maturation curve follows a U-shaped trajectory,[64] shows regional specificity[60] and developmental differences,[61] thus children with more sleep problems could be delayed in attaining peak cortical thickness or advanced on the maturation curve of the prefrontal cortex (i.e. earlier pruning).[12, 13] Longitudinal MRI data would be necessary to test this hypothesis. However, we controlled for age at sleep pattern reports and at MRI scanning, to account for any developmental differences. Given the lack of baseline MRI scans, we cannot rule out reverse causality, i.e. rather than being a consequence of sleep

disturbances, brain morphology may underlie childhood sleep disturbances. However, both our and the findings of others,[16, 17] indicate that sleep problems assessed at later ages provide more precise information compared to those at earlier ages. Sleep problems in infancy are more prevalent and likely to be part of normal development, thus less predictive of later neurobiological outcomes.

Sleep problems are more prevalent in children with psychiatric problems,[65] thus behavioral, emotional and sleep problems are likely to be associated with brain morphology in a complex and bidirectional manner. Our sensitivity analyses showed that the global differences in grey matter volume were partly explained by externalizing behavioral problems. Alternatively, using the same questionnaire to assess both sleep and behavioral problems might have introduced shared method bias resulting in over-adjustment of the studied association. Importantly, children with increasing sleep disturbance trajectories had thinner cortex in the prefrontal area regardless of the presence of behavioral problems.

This study has a distinctively suitable measurement timeline to study the prospective associations of childhood sleep disturbances and brain development up to age seven. A crucial advantage is the MRI in a big sample of children from the general population, at an important maturational stage for brain structures and functions. Although we were able to take many covariates into account, residual confounding might still be present. Some potential confounders such as sleep apnea, neural system medication use and medical comorbidities (e.g. adenotonsillar hyperplasia) were not assessed.[47] Because of the observational design of this study and the absence of baseline brain scans, causality cannot be inferred. In addition, maternal reports of sleep might have decreased the precision in our sleep disturbance estimates. Actigraphy is an objective and affordable method that can be applied to a large sample of children; however, it informs less about behavioral sleep problems, such as bedtime resistance or lack of sleep autonomy.

Conclusion

Childhood sleep disturbances are associated with smaller brain volumes, in particular of grey matter. Differences in cortical thickness among children with high or persistent sleep disturbances in the first six years were located in the dorsolateral prefrontal cortex, potentially reflecting the neurodevelopmental impact of poor sleep across childhood. Future research should explore how sleep disturbances interfere with neurodevelopment, using different neuro imaging modalities alongside with cognitive outcomes. If the link between sleep and neural maturation is confirmed, children with high and persistent sleep problems should be considered as a vulnerable group for adverse neurodevelopment.

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Early childhood sleep patterns and cognitive development at age six years

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Abstract

Childhood sleep and cognition

Background: To explore the association of sleep duration and awakening frequency with cognitive outcomes in young children.

Methods: Mothers of 2800 children from the Generation R cohort reported sleep duration and awakenings at children's age 24 months. At age six years, validated Dutch measures were used to assess children's nonverbal intelligence and language comprehension.

Results: We found a nonlinear association of total sleep time at 24 months with nonverbal intelligence (p=0.03) and language comprehension (p=0.04) at six years. Toddlers sleeping within the recommended 11-14 hours had more favorable cognitive development compared to both extremes. Frequent awakenings were negatively associated with nonverbal intelligence, but not with verbal comprehension.

Conclusion: Sleep duration in toddlerhood has an inverted-U-shaped relation with childhood cognitive measures. Frequent awakenings are associated with lower non-verbal intelligence. Given the marked decline in sleep duration and awakenings in toddlerhood, developmental changes of sleep patterns might be important for cognitive development.

INTRODUCTION

Interesting parallels between developmental patterns of sleep and cognition have been observed. A study in adolescents found relations between slow wave activity (SWA) of the sleep electroencephalogram (EEG) and variety of indices of cortical maturation derived from magnetic resonance images.[1] For example, both the quantity of SWA and synaptic density of the frontal cortex, which is most vulnerable to sleep deprivation, follow an inverted-U-shaped time course across development.[1, 2] However, the empirical evidence relating childhood sleep duration with cognition is inconclusive. Children who sleep short were found to score both higher[3] and lower[4] on IQ tests than longer sleepers. Another study found no cross-sectional association between sleep duration and cognition in seven year old children.[5] These studies hypothesized linear associations. However, several large studies in elderly population have reported nonlinear associations of sleep duration with cognitive functioning[6] and memory impairment.[7] Given that the steep developmental decline in sleep duration is coincided by maturation of complex cognitive functions of the frontal cortex,[1] a curvilinear association between sleep duration and cognition in childhood seems plausible. Correspondingly, a population-based study in 1724 adolescents aged 10-19 years, reported both short and long sleep duration to be associated with lower academic performance scores.[8] The cortical thickness of the frontal lobe starts to decline around the age of seven,[1, 2] thus the potential impact of sleep on cortical maturation may be most obvious early in development.[4] Nevertheless, previous research on sleep and cognition has primarily focused on school-aged children and adolescents.[3, 5, 8]

In addition, lack of consolidated sleep (e.g. frequent awakenings) – a proxy for maturation of sleep patterns[9] - has been related to deficits in neurobehavioral tasks, like reaction time, sustained attention and working memory. The association appeared to be stronger in young children.[10] However, most of the current knowledge about the possible adverse effects of frequent awakenings is inferred from cross-sectional studies,[11] whereas prospective findings might unravel cumulative effects.

Against this background, we examined the prospective associations of toddlerhood sleep duration and consolidation with nonverbal intelligence and language comprehension in school aged children from the general population. We hypothesized that the relation between sleep duration and cognitive outcomes would follow an inverted-U-shaped pattern; meaning that sleep duration more or less than recommendations appropriate for age would be adversely associated with cognitive development. We also investigated the number of nighttime awakenings as a determinant for cognitive outcomes at six years, expecting a negative association.

METHODS

Design and participants

The present research was conducted within the Generation R Study, a population-based cohort based in Rotterdam, the Netherlands.[12] Briefly, all pregnant women living in Rotterdam with an expected delivery date between April 2002 and January 2006 were invited to participate. The Medical Ethics Committee of the Erasmus Medical Centre, Rotterdam, approved the study and written informed consent was obtained from all participants.

Data on sleep duration and/or awakenings at 2 years was available for 5362 children. We previously published significant differences in bed sharing practices among the heterogeneous ethnic groups in the Generation R Study,[13] which influenced sleep habits and hygiene in this sample (Appendix Table 1). To reduce the chance of information bias in the maternal reports of sleep, we restricted our analyses to 3461 (64%) Dutch children. If both parents were born in the Netherlands, the child was considered 'Dutch'. Data on cognitive development at six years was available for 2800 (81%) children of which 2662 children had nonverbal intelligence assessment and 2461 children had language comprehension assessment.

Sleep assessment

Sleep variables were assessed by postal questionnaire at children's age 24 months (IQR: 23.8 - 24.7). Parents reported about their child's sleep habits in the previous week. Nighttime sleep duration was assessed with an open question of the number of hours the child slept. For daytime sleep (napping) categorical approximation of <0.5h, 0.5-1h, 1-2h and >2h, from which estimates of 0, 1, 2, and 2.5, respectively, were used to compute total sleep time (TST). First, sleep was analyzed continuously. Next, based on the American National Sleep Foundation (NSF) age appropriate recommendations,[14] we defined three categories of sleep duration, namely: optimal (11-14h/24h), short (<11h/24h), and long (>14h/24h).

Sleep consolidation was assessed with a categorical question of the average number of awakenings per night: Never, 1 to 2 times, 3 to 4 times; and more than 5 times. In the categorical analyses, the latter two categories were collapsed to "≥3 times" (>5 times = 1.5%).

Nonverbal Intelligence

When the children were 6 years old (SD=0.35), nonverbal intelligence was assessed using a validated Dutch nonverbal intelligence test: Snijders-Oomen Niet-verbale Intelligentie Test-Revisie (SON-R 2½-7).[15] Due to time constraints, two subsets were chosen: Mosaics,

assessing spatial visualization abilities, and Categories, assessing abstract reasoning abilities. A scaled total score can be calculated for any combination of subtests with the same distribution characteristics as the IQ score. A correlation of r=0.86 was found between the score derived from the Mosaics and Categories subsets, and the IQ scores derived from the complete test.[15] Raw test scores were converted into non-verbal IQ scores using norms constructed as a continuous function of age, with a sample mean value of 105 and SD of 14.

Language comprehension

Children's language development at six years was assessed using an age appropriate receptive subtest of a Dutch battery: Taaltest voor Kinderen (TvK [16]). To reduce burden to the children, 27 difficult items were selected from the full battery consisting of 40 items.[17] By choosing the correct alternative from two pictures that matches a given word, information about children's comprehension vocabulary skills was obtained. Correct answers were summed and divided by the number of items answered, yielding a correct percentage score.[17]

Covariates

Child characteristics. Information on gender was obtained from midwives and hospital registries. Gestational age at birth was established using ultrasound examination during pregnancy. To assess history of breastfeeding, mothers were asked whether they breastfed their child (yes/no), by questionnaires at 2, 6 and 12 months after birth. We dichotomized the breastfeeding information from all questionnaires in: "never breastfed" and "ever breastfed". To assess behavioral problems, we used the Child Behavior Checklist (CBCL1.5-5) questionnaire when children were 1.5 years old. This is a parent reported questionnaire, with well-established psychometric properties, containing 99 items on problem behavior rated on a 3-point scale: 0 (not true), 1 (somewhat or sometimes true) and 2 (very true or often true).[18] A total problems weighted sum score was used. Mothers reported on child's general health status at six years ("How would you describe the general state of your child's health? Excellent/Very good/Good/Fair/Bad"). Information on wheezing (no/yes) was obtained by questionnaires, which were adapted from the International Study on Asthma and Allergy in Childhood[19] at the ages of 1, 2, 3, 4 and 6 years. To assess persistence of wheezing over time, three wheezing patterns were created: early-only wheezing (wheezing at ages 1, 2 and/or 3 years), late-only wheezing (wheezing at ages 4 and/or 6 years), and persistent wheezing (reported wheezing between 1 and 6 years).[20]

Baseline cognitive function. Parent Report of Children's Ability (PARCA) was used to provide a valid estimate of children's nonverbal cognitive functioning at 2.5 years.[17] It consists of two sections: a parent-administered part (22 items assessing three functions: matching-to-sample, block building and imitation), and a parent-report part (26 questions on

quantitative abilities, symbolic play, planning and organizing, adaptive behavior and memory), which were combined in an overall sum score.[21] Early receptive language skills were assessed by maternal report when children were 1.5 years old with the Dutch version of MacArthur Short Form Vocabulary Checklist (MCDI-N), appropriate for measuring receptive vocabulary of children aged 16-30 months. The instrument contains a list of 112 words of which mothers checked the words they think their child understands and the number of positive responses was summed to a score.[17]

Maternal, socioeconomic and demographic characteristics. Parity (previous pregnancies: 0 vs. ≥1), maternal age, education and marital status (married and cohabiting vs. single) were assessed by questionnaires at enrolment. Parental education was defined by the highest completed education using the categories established by Netherlands Statistics [22] and classified as 'high' (higher vocational training or higher academic education), 'intermediate' (more than 3 years general secondary school) and 'low' (lower vocational training or 3 years general secondary school). Maternal history of tobacco smoking was obtained by postal questionnaire in early, mid- and late pregnancy. On the basis of all three questionnaires, we defined the following categories: "never smoked", "stopped smoking when pregnancy was known" and "continued smoking during pregnancy". The depression and anxiety subscales from the Dutch version of the Brief Symptom Inventory [23] were averaged to obtain an affective symptoms scale, as filled in by the mother 6 months postpartum. Monthly household income at enrollment was categorized into >2000 Euros (more than modal income), 1200-2000 Euros and <1200 Euros (below social security level). Maternal cognitive ability was assessed during the visit to the research center at child's age 5-7 years, with a computerized version of the Ravens Advanced Progressive Matrices Test, set I.[24]

Statistical Analyses

The sample for analysis consists of 2800 Dutch children with sleep assessment (duration and/or awakenings) at two years and nonverbal intelligence and/or verbal comprehension at six years. In this sample the missing values on all covariates were below 20%, except for maternal affective symptoms 6 months postpartum (26%) and childhood wheezing patterns (21%). To impute the missing values of the covariates, we used Markov Chain Monte Carlo multiple imputation technique to create ten complete datasets.[25] All statistical analyses were run in the ten imputed datasets and results were pooled. For the non-response analysis, we tested group differences in sleep and covariates between the children with and without cognitive assessments at 6 years (19%).

We used linear regression models to investigate the association of sleep duration and consolidation (awakenings) with cognitive development on a continuous scale. Two main outcomes were explored: nonverbal intelligence score and language comprehension

percentage score. The skewed language variable was transformed using natural log to approximate a normal distribution. Sleep duration and awakenings were analyzed as continuous and categorical determinants. To test nonlinear association, squared term of sleep duration (h^2) was included into the regression models. In the same manner, nighttime sleep and napping were tested as determinants of cognitive outcomes. The final model was adjusted for child's gender and age at outcome assessment, early childhood characteristics (gestational age, breastfeeding, problem behavior score at 1.5 years, baseline cognitive scores, longitudinal wheezing patterns and health status at six years), socio-demographic and maternal factors (household income, maternal age, education, IQ, parity, history of smoking during pregnancy and affective symptoms 6 months postpartum). To test the dependence of the sleep measures we mutually adjusted sleep duration and number of awakenings in the final models.

RESULTS

Characteristics of the children and their mothers are presented in Table 1. The children slept on average 13 hours per day; nearly 86 percent slept within the recommended range of 11 to 14 hours. About 50% of the two-year-old children awoke one or two times per night, and 5.7% awoke more than three times a night. Average nonverbal intelligence score was 105.2 (±SD =14.4); median verbal comprehension percentage score was 0.88 (interquartile range 0.81-0.96).

Sleep duration as a determinant of cognitive outcomes

Table 2 shows the associations of sleep duration with nonverbal intelligence and language comprehension. We found no evidence of a linear association; rather a quadratic-U-shaped model adequately described the relation between sleep duration and cognitive outcomes (Table 2). The association of the quadratic term of TST and both cognitive outcomes attenuated, but remained significant when adjusted for the child, socio-demographic and maternal characteristics (IQ points, B per h^2=-0.32; 95%CI: -0.60 to -0.04, p=0.03; language comprehension percentage score, B per h^2=-0.002; 95%CI: -0.004 to -0.0001; p=0.04). Children who slept longer than 14 hours at two years had 1.77 point (95%CI: -3.53 to -0.01, p<0.05) lower IQ scores and 2% (95%CI: -0.03 to -0.01; p<0.01) lower language comprehension scores at six years than those who slept within the recommended range of 11-14 hours. The group sleeping less than the recommended range did not reach statistically significant difference. The nonlinear association were driven by nighttime sleep, although estimates reached statistical significance only for nonverbal intelligence (IQ points, B per h^2=-0.46; 95%CI: -0.81 to -0.10, p=0.01). In contrast, napping was significantly related to language comprehension only. Children sleeping more during the day had lower language comprehension scores (B=-0.01; 95%CI: -0.02 to -0.01; p<0.01).

Table 1. Child and Parent Characteristics (n=2800)

Child Characteristics	
Gender, % girls	50.1
Age at outcome assessment, years	6.02 ± 0.36
Gestational age at birth, weeks	40 (39 – 41)
Breastfed, % never	8.7
Problem behavior at 1.5 years, score	18.7 (19 – 29)
Language comprehension at 1.5 years, score	55.47 ± 23.87
Nonverbal intelligence at 2.5 years, score	46.94 ± 4.42
Wheezing patterns up to age 6 years	
Never, %	54.5
Early-only, %	28.9
Late-only, %	4.1
Persistent, %	12.5
Sleep at 24 months	
Total Sleep Time per day, hours	13.16 ± 0.96
<11 hours, %	3.8
11-14 hours, %	85.9
>14 hours, %	10.3
Awakenings per night, %	
Never	44.1
1-2 times	50.2
≥3 times	5.7
Cognitive outcomes at 6 years	
Nonverbal Intelligence, score	105.24 ± 14.37
Language comprehension, % correct	0.88 (0.81 - 0.96)
Maternal Characteristics	
Age, years	32.0 ± 4.2
IQ, score	101.3 ± 12.5
Marital status, % single	4.8
Parity, % multiparous	38.8
Education, %	
High	63.8
Middle	26.5
Low	9.7
Pregnancy smoking, %	
Never	77.9
Until pregnancy was known	10.4
Continued smoking	11.7
Affective symptom, score	0.08 (0.00 – 0.25)
Family Income, %	
>2000 €	85.4
1200-2000 €	10.9
<1200 €	3.7

Note: Numbers are means ± SD for continuous normally distributed variables or medians (interquartile range) for variables with skewed distributions

Table 2. Association between sleep duration at 2 years and cognitive outcomes at 6 years

	Nonverbal intelligence (n=2662)*			Language comprehension (n=2461)*		
Sleep duration	B	95% CI	p-value	B	95% CI	p-value
Linear Model						
TST, h	-0.18	-0.74 ; 0.38	0.53	-0.01	-0.01 ; -0.002	0.01
Quadratic Model						
TST, h^2	-0.32	-0.60 ; -0.04	0.03	-0.002	-0.004 ; 0.00	0.04
TST, h	7.96	0.80 ; 15.12	0.03	0.05	-0.004 ; 0.11	0.07
American Sleep Foundation recommendations for TST						
<11h	-1.67	-4.50 ; 1.16	0.25	-0.01	-0.03 ; 0.02	0.60
11-14h		Reference			Reference	
>14h	-1.77	-3.53 ; -0.01	<0.05	-0.02	-0.03 ; -0.01	<0.01
Nighttime sleep						
Linear Model						
Hours	-0.27	-0.92 ; 0.39	0.42	-0.003	-0.01 ; 0.002	0.29
Quadratic Model						
$Hours^2$	-0.46	-0.81 ; -0.10	0.01	-0.003	-0.01 ; 0.000	0.06
Hours	9.63	1.81 ; 17.44	0.02	0.06	-0.01 ; 0.12	0.08
Napping, h	-0.16	-1.00 ; 0.68	0.70	-0.01	-0.02 ; -0.01	<0.01
0-1h/day	-0.81	-2.82 ; 1.19	0.43	0.02	0.01 ; 0.03	0.01
1-2h/day		Reference			Reference	
>2h/day	-0.73	-1.93 ; 0.46	0.23	-0.01	-0.02 ; -0.003	0.01

Betas are averaged from 10 imputed datasets. Note: TST denotes Total Sleep Time in 24 hours

*Models are adjusted for child's age and gender at cognitive testing, family income, maternal age, education and IQ, marital status, parity, smoking during pregnancy, affective symptoms six months postpartum, breastfeeding, child's gestational age at birth, behavioral problem score at 1.5 years, longitudinal wheezing patterns and child's general health status at six years, and baseline cognitive scores (nonverbal intelligence score at 2.5 (IQ model) or language comprehension score at 1.5 years (language model))

Nighttime awakening as a determinant of cognitive outcomes

Table 3 demonstrates the association between number of awakenings at 2 years and nonverbal intelligence and language comprehension at 6 years. Children who awake frequently (>3 times) during the night scored 1.9 IQ (95% CI: -4.23 to 0.42) points lower than those waking up 1-2 times a night and 2.4 IQ (95% CI: -4.71 to -0.03) points lower than those reported to sleep through the night. Mutual adjustment of sleep duration and number of awakenings in a single model did not attenuate the associations with cognitive outcomes (Appendix Table 2).

Table 3. Association between nighttime awakenings at 2 years and cognitive outcomes at 6 years

	Nonverbal intelligence (n=2662)*			Language comprehension (n=2461)*		
	B	95% CI	p-value	B	95% CI	p-value
Number of awakenings	-0.86	-1.7 ; -0.02	<0.05	-0.000	-0.01 ; 0.01	0.99
Never		Reference			Reference	
1-2 times	-0.53	-1.63 ; 0.56	0.34	0.001	-0.01 ; 0.01	0.83
≥3 times	-2.44	-4.79 ; -0.09	0.04	0.000	-0.02 ; 0.02	0.99

Betas are averaged from 10 imputed datasets.

Nonresponse analysis

The Dutch children with missing data on nonverbal intelligence and language comprehension at 6 years (n=661) differed slightly from the children included in the analyses (n=2800). Children included in the analyses were less likely to awake during the night (44% vs. 38%, p=0.03) and slept on average 5 minutes longer than those excluded. The children included also had less behavioral problems (p=0.04) and were more likely to be breastfed (92% vs. 89%, p=0.01). Non-respondent mothers were younger (mean difference of 7 months, p=0.006), lower educated (13% vs. 9% low education, p=0.003) and were more likely to have continued smoking during pregnancy (15% vs. 11.6%, p=0.003) compared to mothers of children included in the analysis. Our study population did not differ from those excluded from the analysis in gender distribution, baseline cognition and parental characteristics including family income, marital status, affective symptoms and intelligence score.

DISCUSSION

This large population-based study explored the prospective associations of sleep duration and nighttime awakenings in toddlers with cognitive outcomes at six years. We found evidence for a nonlinear relation of sleep duration with nonverbal intelligence and verbal comprehension. The nonlinear association of sleep duration was driven by nighttime sleep for both cognitive outcomes, whereas daytime napping was negatively related only to language comprehension. Frequent awakenings (>3 times per night) were independently associated with lower nonverbal intelligence, but not with language comprehension.

Several previous studies found an association between sleep duration and cognition in early childhood. However, most of them reported linear associations.[3, 4] For example, Touchette, Petit [4 26] reported that children with persistently short sleep duration from the age of two scored lower on vocabulary test at five years. Additionally, short sleepers who "improved" sleep duration in the third year still scored lower on the nonverbal intelligence test at six years. They interpreted this as residual effect of insufficient sleep early in cognitive development. In the study of Touchette, Petit [4], the long sleepers were the reference, hypothesizing a linear association between sleep duration and cognition, the more-the better. In contrast we tested a nonlinear association and took the children sleeping within the recommended range (11-14 hours per day [14]) as the reference group. Interestingly, Geiger, Achermann [3] found a negative association between sleep duration and intelligence in a cross-sectional study of 7-11 years old adolescents. The authors posited that results were consistent with an extension of the cognitive efficiency theory; "intelligence is not a function of how hard, rather how efficient the brain works".[27] However, our analyses

were controlled for pre-existing cognitive differences, which reduce the possibility that pre-existing cognitive development influenced sleep (e.g. children with higher cognitive ability being more efficient in recovering neuronal synapses during sleep). In line with several large studies in elderly population[6, 7] and one study in older children[8], our data suggest that both short and long sleep duration in toddlers are risk factors for unfavorable cognitive outcomes at age six years. However, the short sleeping group in our sample may have been too small to detect statistically significant differences. Consistent with previous evidence,[28] we found that the proportion of sleep consolidated during the night resembles cognitive development; the nonlinear relation between sleep duration and cognitive outcomes was driven by nighttime sleep. In agreement with findings of two studies in a twin sample of similar age, daytime napping was inversely related to language development. These studies reported higher ratio of daytime vs. nighttime sleep to be associated with language delays,[29] and most the variance in consolidated nighttime sleep was shown to be genetically determined.[30]

In line with previous research reviewed by O'Brien [11] our results showed that frequent awakenings are associated with lower nonverbal intelligence scores. However, in the present study frequent awakenings appeared to be unrelated to verbal comprehension, which might be because we only measured receptive vocabulary. Lack of sleep consolidation might have more pronounced adverse effects on expressive verbal skills.

A common underlying factor affecting both the developmental changes of sleep patterns and cognitive development, could explain why toddlers who sleep longer score lower on cognitive tests at six years. A recent meta-analysis showed a developmental declining trend in sleep duration and number of awakenings, which is steeper at younger ages.[31] Thus, children who sleep longer than expected and lack sleep consolidation at two years of age, might lag behind the typical developmental changes of sleep patterns.[28] Correspondingly, toddlers who slept more during the day had lower language comprehension scores at six years. However, if a factor contributed to both, sleeping patterns and cognitive delay, then adjustment for previous cognitive development could attenuate the observed association. Given that measures of cognition in early childhood are only moderately predictive of later cognitive outcomes,[17] our baseline cognitive measures might have been unsuccessful in fully capturing all underlying effects. Alternately, sleep efficiency might be underpinning the relation of sleep duration with cognition. Yet, mutual adjustment of sleep duration and consolidation did not attenuate the relation.

Lower cognitive performance following short or fragmented sleep in children has been previously reported.[32, 33] Support for this association also comes from non-respiratory sleep disorders research, although the pathology behind remains a puzzle [reviewed in 34] In our study, we found no differences in cognitive performance between the short sleepers and the reference group, possibly due to lack of power. However, in a semi-experimental

study Sadeh, Gruber [35] showed that sleep restriction of only half an hour, already affects daytime performance in children. Children of 1-3 years who awake during the night, lose on average 1.5 hours of sleep.[30]

The inverted-U-shaped association between sleep and cognitive outcomes might represent a measure of general well-being of the child, affecting both sleep and cognitive development.[5] It was previously shown in children[36, 37] and adults[38, 39] that sleep duration at both extremes is associated with negative health-related outcomes. This means sleeping less or more than the average is negatively related to children's cognitive development, possibly accounted for by impact on health or wellbeing. However, adjusting for mother reported health status did not explain the observed associations. Likewise, respiratory complaints might be a plausible explanation; however, childhood wheezing patterns also did not attenuate the association.

In terms of absolute difference in cognitive scores, effect estimates are modest. Children that awoke frequently or slept longer than current recommendations scored 2.4 points (15% of a SD) and 1.8 points lower (10% of a SD) on the IQ scale, respectively. Although a difference of this size might not be clinically relevant for a single child, on a population level small effects often reflect large differences. Small changes on the natural log scale of language comprehension can be interpreted as percentage changes,[40] thus compared to the reference group, children that slept more than 14 hours per day scored on average 2% lower on the language test.

The main strength of our study is the prospective design, which captures the long-term consequences of early childhood sleep disturbance on cognitive outcomes. Moreover, the study is population-based, and we were able to account for various confounding variables, including cognitive development at baseline. Furthermore, we expect that by including the cognitive ability of the mother into our models, we corrected for a part of the heritability of cognitive traits. Another major strength of this study is the large sample of toddlers, an age group for which paucity of sleep data has been pointed out.[31] Finally, age-appropriate and validated batteries were used to obtain information on child cognition.

Our study also has several limitations. Because cognitive development and sleep practices are greatly influenced by the cultural context in which they occur, we restricted our sample to children of Dutch national origin, thus our findings cannot be generalized to other ethnic groups. The non-response analysis indicated that children with behavioral problems at 18 months are less likely to participate at follow-up. Non-respondent mothers were younger, less educated and were more likely to have continued smoking during pregnancy compared to those included in the analyses. A methodological limitation is

that the measures used and the longitudinal timeframe were not designed specifically for this study. Repeated measures of sleep are needed to thoroughly understand the longitudinal relation between sleep and cognition. Although maternal reports give a fairly clear picture of toddlers' habitual sleep,[33] this may have reduced the precision of our sleep estimates. Future studies should measure sleep objectively (e.g. actigraphy, a relatively cheap method suitable for large samples).

Conclusion

Sleep duration and nighttime awakenings in toddlerhood were associated with cognitive outcomes at age six after controlling for prior cognitive development, child, maternal and demographic characteristics. Given the marked decline in sleep duration and awakenings in toddlerhood, developmental changes of sleep patterns might influence cognitive development. Rather than assuming that only short sleep has adverse effects on cognition, sleep duration at both extremes should be considered in future studies of cognitive development.

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Appendix Table 1. Ethnic differences in sleep hygiene

	Falling asleep (n=4206)	Bed sharing (n=4200)	Bedtime rituals (n=4186)
Ethnicity	In own bed, %	Own bed, %	Yes, %
Dutch (n=2803)	93	94	95
Other Western (n=408)	82	88	94
Other European countries (n=340)	83	87	95
USA & Canada (n=30)	89	93	85
Australia (n=11)	100	100	100
Japan (n=6)	100	50	83
Indonesia (n=21)	75	100	80
Mediterranean (n=387)	58	85	73
Turkey (n=240)	58	83	77
Morocco (n=147)	58	87	66
Caribbean (n=307)	56	66	80
Dutch Antilles (n=71)	51	64	79
Surinam (n=236)	57	67	81
Other non-western (327)	68	79	79
Africa (n=78)	71	78	77
Asia (n=106)	69	79	77
Non-western America (n=64)	78	82	95
Cape Verde (n=79)	58	76	70
P-value	<0.01	<0.01	<0.01

Note: Numbers are percentages. P-value was derived from a X^2 test

Appendix Table 2. Mutual adjustment of sleep indices and cognitive outcomes at 6 years

	Nonverbal intelligence*			Language comprehension*		
Exposure	B	95 CI	p-value	B	95 CI	p-value
Sleep duration						
TST, h^2	-0.32	-0.60 ; -0.04	0.03	-0.002	-0.004 ; 0.000	<0.05
TST, h	7.77	0.60 ; 14.93	0.03	0.05	-0.01 ; 0.11	0.07
Awakenings						
Never		Reference			Reference	
1-2 times	-0.58	-1.72 ; 0.55	0.31	-0.001	-0.01 ; 0.01	0.70
>3 times	-2.53	-5.00 ; -0.07	0.04	-0.002	-0.02 ; 0.01	0.83

Betas are averaged from 10 imputed datasets. Note: TST denotes Total Sleep Time in 24 hours

* Models are adjusted for child's age and gender at cognitive testing, family income, maternal age, education and IQ, marital status, parity, smoking during pregnancy, affective symptoms six months postpartum, breastfeeding, child's gestational age at birth, behavioral problem score at 1.5 years, longitudinal wheezing patterns and child's general health status at six years, and baseline cognitive scores (nonverbal intelligence score at 2.5 (IQ model) or language comprehension score at 1.5 years (language model))

5 SLEEP AND CEREBRAL WHITE MATTER IN MIDDLE-AGED AND OLDER ADULTS

Sleep complaints and cerebral white matter: a prospective bidirectional study

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Objectively measured sleep patterns and microstructural integrity of cerebral white matter

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6 GENERAL DISCUSSION

Most people and all health care practitioners realize that sleep in toddlers is very different than sleep in older adults, but I doubt even Hypnos, the Greek God of Sleep, knows exactly why. Sleep is a physiological process from the brain to the brain; hence changes in sleep patterns closely resemble aging processes happening in the brain. To date, very few sleep studies of any discipline have aimed to explore whether brain properties are determining sleep patterns, or conversely, whether changes in sleep patterns lead to structural brain changes. This thesis brings together several studies aiming to disentangle the neurobiological correlates of sleep patterns and their variations across the lifespan, with a particular focus on directionality. In this chapter, the main findings of this thesis will be discussed. I will address some methodological considerations that are of interest for this field of research, and will discuss the implications for future research and clinical practice.

MAIN FINDINGS

How do the Dutch sleep?

Before digging into the neurobiological determinants and outcomes of sleep patterns, we took a traditional epidemiological approach and estimated the "extent of the problem". To this aim, we conducted a systematic literature review to identify all population-based studies conducted in the Netherlands that have collected data on sleep patterns. By pooling individual participant data (IPD) from 36 studies including 200,358 participants we described typical sleep patterns for the Dutch population across the lifespan. We showed that the average Dutch person generally sleeps long enough according to the current recommendations of the American Sleep Foundation.[1] However, sleep problems are common in similar to other industrialized countries.[2-4] Teenagers are the most sleep deprived age group, whereas insomnia symptoms are most common in adult women. As shown in other populations[2] and in smaller Dutch samples,[5] smoking and overweight are risk factors for short sleep duration and low sleep efficiency. In this study we also aimed to describe age-specific sleep duration and compare these values to the recommendations of American Sleep Foundation. Therefore, we estimated percentiles of sleep duration across the lifespan, which can be used to estimate age-specific reference intervals for quantitative traits. Except for pediatric populations[6-8] such percentile curves across age are rarely calculated for sleep duration. The current recommendations of appropriate sleep duration are based on a systematic literature review exploring various mental and physical health outcomes as a function of sleep duration.[1] In this study, a large expert panel rated the appropriate sleep duration for every age group, based on the findings from the identified studies. We took an alternative approach. As optimal sleep might be of different duration for different individuals, and what optimal sleep is probably differs per outcome, we provided estimates that enable a comparison of a person's sleep duration

with the population distribution of persons of the same age and sex group. Using these reference curves, both patients and practitioners can determine whether a person sleeps at the lower or higher end of the sleep duration distribution. This information together with other daytime or nighttime complaints can help determine if a person is sleeping "enough". Importantly, we also showed that sleep complaints are least common in persons spending 7-8 hours in bed. The guidelines of the American Sleep Foundation provide recommendations for sleep duration, a heritable phenotype that is hard to adapt.[9-11] We propose that optimizing time in bed could be a potential target for indirectly improving sleep duration and efficiency in the general population, as it is recommended in clinical samples.[12] Taken together this largest descriptive sleep study to date charted sleeping patterns for the Dutch population of all ages and can be used for future reference both in research and clinical medicine.

Neurobiological determinants of childhood sleep patterns

In lack of early brain imaging, previous longitudinal studies in children have mostly focused on the effects of early sleep duration and sleep problems on neurodevelopmental outcomes.[13-18] However, the causality may be the reverse, early neurobiological factors could underlie childhood sleep patterns.[19] To fill this gap, we tested the hypothesis that very early neurobiological factors determine childhood sleep patterns in Chapter 3. We first showed that fetuses and neonates with larger head circumference, and larger lateral ventricles within the normal range, had longer sleep duration in toddlerhood and less sleep problems across childhood. Next, we showed that infants with a more marked morning cortisol rise and a flatter diurnal slope sleep shorter at night during toddlerhood. The findings of the two studies indicate two different, mutually nonexclusive mechanisms of the etiology of childhood sleep patterns. The heritability estimates of head size in infancy have been estimated to 90%,[20] whereas very low heritability of 0% to 9%[21] have been estimated for both saliva and plasma cortisol levels. This indicates that faster head growth in utero can point towards a genetically determined beneficial neurodevelopmental trajectory that is later reflected in better sleep patterns in childhood, i.e. longer sleep duration and less sleep problems. On the other hand, shorter sleep duration in infants with altered cortisol secretion might indicate environmental influences on sleep duration. Adverse intra-uterine environment or early life adversity due to low socio-economic status or maternal depression, could lead to both higher cortisol morning peaks and insufficient decrease before bedtime,[22, 23] resulting in shorter sleep duration.[24] However, faster developing infants might develop the cortisol awakenings response earlier.[25] Due to advanced maturation, sleep duration of these infants could decrease sooner, resulting in shorter sleep in toddlerhood. This will have implications on how decreases in sleep duration in toddlerhood are interpreted in pediatric clinics. Regardless, our results show that behavioral expressions of sleep in early childhood can be considered important developmental milestones that correspond to the neurodevelopmental status of the child and the appropriateness of the rearing environment.

Neurodevelopmental outcomes of childhood sleep patterns

The relation of childhood sleep problems with later neurocognitive development is not entirely clear; therefore, we next explored neurocognitive outcomes of early sleep patterns in Chapter 4. We showed that children with persistent sleep problems across the first six years have less grey matter volume and a thinner cortex in areas involved in high-order cognitive functions. We also demonstrated that sleep problems during infancy, i.e. before the age of 2, are not predictive of brain morphology, and thus might be considered part of normal development. In addition, toddlers with less consolidated sleep, i.e. more nighttime awakenings had lower IQ scores at age six than those sleeping through the night. These findings indicate that sleep disturbances in childhood might have an impact on children's brain structure and function. Sleep disturbances could have a direct relation with brain development, but these associations could also go through inefficient daytime functioning of tired children, which limits their learning opportunities and consequent neurocognitive development.[26] It is yet unknown how much cognitive benefits of last night's sleep help shape children's brain on micro-longitudinal scale. In addition, we showed that toddlers sleeping longer than their peers have lower verbal and nonverbal cognitive scores. This, like the relation between higher cortisol awakening response and shorter sleep duration, might support a maturational view on the decrease in sleep duration. Long sleep duration would not necessarily be considered developmentally beneficial, rather toddlers sleeping longer than their peers would be behind in overall neurocognitive development, including in the developmental decrease in sleep duration. Taken together with the findings from Chapter 3, these results indicate a complex bidirectional relation between sleep and neurodevelopment and maturation in childhood. In other words, early cerebral structures determine sleep patterns, but sleep patterns also shape the brain. There are very few longitudinal studies exploring changes in sleep duration and patterns across childhood, and virtually none relating it to neuroimaging or cognitive outcomes. Future longitudinal research with repeated measures would be necessary to answer these important developmental questions.

Sleep and cerebral white matter in older adults

The most substantial structural brain changes happen in the first and last years of life, during neurodevelopment and neurodegeneration. Therefore, we next explored the relation between sleep patterns and brain structure at the other end of the age distribution. As sleep maintains the function of oligodendrocytes, which support white matter in the adult brain, we focused specifically on changes of cerebral white matter in the aging brain. Using repeated measures of both MRI and self-reported sleep patterns, we formally tested the direction of the association between sleep complaints and white matter properties after the age of 45. We found that sleep complains in middle-aged and older adults were not a cause or a consequence of white matter changes in the aging brain. This could indicate that 1) subjective sleep reports do not reflect objective sleep

in older persons and are therefore not related to brain changes; 2) substantial changes in sleep patterns or brain properties only take place at a very old age not captured in our age cohort or 3) the average follow-up of 5 years is not long enough for changes to be statistically significant. Fortunately, we were able to test this hypothesis using objective sleep parameters, though only in the direction that sleep would cause changes in the brain. We showed that objective sleep parameters are related to white matter integrity. Poor sleep, as indicated by more wake after sleep onset, lower sleep efficiency, and consequent shorter sleep duration, was related to lower white matter microstructural integrity, but not with changes in white matter microstructure across time. This could indicate that although poor sleep is associated with worse white matter integrity consistently over a follow-up of up to 11 years, it does not influence neurodegenerative changes in the brain. Lacking temporal associations, the association might actually be the reverse; white matter microstructure could underlie poor sleep in older adults. From a more neurological perspective, sleep disturbances could be indicative of underlying white matter changes. Regardless, this study points towards an important role of objective sleep parameters for white matter microstructural integrity, on a global level, but also more specifically in white matter tracts that could have a role in initiating and maintaining sleep.

METHODOLOGICAL CONSIDERATIONS

Individual Participant Data Meta-analysis

Most meta-analytical methods quantify results using pooled group-level summary statistics (aggregate data) from previously published literature. This approach summarizes the aggregate summary statistics into a weighted average. However, when synthesizing published aggregate data even rigorously conducted meta-analyses can sometimes be of limited value, because it cannot properly take subject-level characteristics into account. Additional problems arise when aggregate data are not available, poorly reported, derived or presented differently across studies (for example, continuous vs. categorical sleep duration), and likely to be published or reported in greater detail when statistically or clinically significant. In contrast, IPD meta-analysis is particularly useful when the effects are expected to vary across subgroups,[27] e.g., sleep duration differs substantially among sexes and ethnic groups. Through IPD meta-analysis it becomes possible to disentangle subject-level and study-level sources of heterogeneity and effect modification and to consistently adjust for confounding variables.[28] This has led investigators of different fields to embark into pooling IPD in order to estimate effects of associations in large samples.[29,30] In the sleep field, however, pooling IPD has not been applied often. Some associations have been studied in many individual studies, e.g. the relation of sleep duration with obesity and BMI,[31-34] or the relation of sleep disturbances with cognitive decline and dementia;[35-37] however, meta-analytical studies have only summarized aggregate published data to

pool the effects across studies. This limits the opportunity to explore effect modification, or confounding by baseline characteristics, e.g. smoking or educational status. To give an example, it has often been discussed that the relation between sleep duration and BMI differs with age,[34, 38, 39] but the association has been studied in individual studies including only narrow age-bins. An alternative approach is to pool data on sleep duration, BMI and background variables across studies of different age cohorts, and formally explore effect modification by age. In this example, pooling IPD would allow to further explore subgroups (e.g., three-way interactions sleep duration*sex*age). Despite more efforts, the conclusions from an IPD meta-analysis might often lead to similar conclusions as meta-analysis of aggregate data. This evidence, however, provides better basis for identifying specific vulnerable groups and potentially aid development of targeted interventions.

Pooling aggregate data for descriptive purposes is particularly difficult, as the published estimates are unlikely to be reported in a way that can be systematically summarized, e.g. different cutoffs for categorizing short sleep duration. Another challenge for describing group-specific sleep patterns from published data, is that the stratified estimates, if published, are often presented in groups that do not correspond to the posed research questions, e.g., age bins that do not correspond to those of the American Sleep Foundation. Access to IPD may help improve data quality, through standardization of definitions and analyses. With the IPD meta-analysis conducted in Chapter 2, we showed that this meta-analytical approach could be used to generate descriptive data in large samples. Nevertheless, standard definitions and protocols cannot fully account for heterogeneity between studies due to sampling strategies, assessment batteries or regions. In addition, this approach despite is exceptionally work intensive and fairly novel in the field. Yet, as a result of distinctive collaborative spirit among research groups working in the field of sleep, our effort resulted in a study level response rate of over 80%.

The perks and perils of longitudinal data

One of the most important advantages of prospective cohort study is the availability of repeated measurements of the outcome and/or the exposure. This allows researchers to study changes and trajectories across time, sensitive periods and a myriad of associations. In almost all chapters of this thesis we used prospective data addressing questions on temporal relations between sleep and various indices of neural health. Using repeated measures has led to important conclusions, but also highlighted many analytical challenges. In this part, I will discuss several of these challenges and propose potential remedies.

Repeated measurements of the exposure

With the emergence of prospective studies with long follow-up, epidemiological training is increasingly focusing on repeated measurements. However, most courses address

repeated measures of the outcome, whereas in the research setting the opposite is often the case, i.e. the exposure is repeatedly measured and the outcome only once. In traditional regression models, repeated measures of the exposure can be addressed in two ways: 1) by taking the average value of the repeated measures across the follow-up; or 2) by analyzing each time-point separately.[40] Both these approaches have important pitfalls, the first does not take into account important information derived from the within person variance, and the second faces problems with multiple testing and interdependence of the assessments. Traditional longitudinal models, such as Linear Mixed Model and General Estimating Equations were also not primarily designed to address repeated measurements of the exposure. Estimates are actually incorrect when the covariance matrix of the outcome equals 1, i.e. when the outcome has only been measured once. In this case, it has been suggested that the outcome and the exposure are flipped such that the effect estimates represent the association between changes (or trajectories) in the exposure as a function of increments on the scale of the outcome.[40] This approach, however, ignores the directionality of the association, and is not suitable for testing interactions, i.e. differential effects of the exposure across groups. Different statistical methods can be used to deal with this. First, in Chapter 3.1 I have used a latent variable method to define latent class trajectory groups based on repeated measures of the exposure (e.g. sleep disturbance scores). This person-centered modeling approach estimates growth curves across unobserved subpopulations and assigns a most likely latent trajectory class to each individual.[41] The number of latent groups is determined by well-defined model fit indices, and these groups can then be used as an independent variable in a simple regression model. This fairly simple data-driven approach has the advantage of using all available information, even if the number of observations per individual is unequal, while accounting for baseline differences. This approach, however, could potentially ignore information on subtle individual changes on a continuous scale, as it classifies individuals in a categorical fashion. Alternatively, a number of methods that fall under the Structural Equation Modeling approach offer the opportunity to estimate individual intercept and slope of the exposure, that are then used as predictors for the outcome within the same model.[42] A similar result can also be achieved by estimating individual intercepts and slopes of the exposure within the Linear Mixed Model, following a two-step approach, i.e. estimation of individual trajectories of the exposure is performed using age of the assessments as independent variable.[40] Finally, a somewhat more complicated method to model repeated measures of the exposure falls under the Causal Inference Methods, namely the G-formula.[43] This method is designed to provide estimates of associations of repeated measures of the exposure with a single outcome measure, while also allows controlling for time-varying confounders, a major elephant in the epidemiology room. Unfortunately, this method, despite being very intuitive and applicable for many clinical questions, has not been used much outside of the field of Epidemiological and Statistical methods development. All of these methods should be summarized and taught in basic Repeated Measurement Courses to make them more approachable for young researchers.

Studying changes of the outcome

In longitudinal research we often aim to answer questions about changes of the outcome over time. Intuitively, we do this by computing a delta measure (difference between the value of the outcome at follow-up minus the value of the outcome at baseline). Alternatively, adjusting for baseline values of the outcome in a regression model where the outcome at follow-up is the dependent variable should lead comparable results.[44] However, in large epidemiological dataset, we sometimes note differences between the estimates from these two methods at times, which leave us with the question what method is correct. The latter method includes the baseline values of the outcome in the minimization of sum of the squared residuals, which would intuitively, would bring us to the correct estimate. In political science and econometrics, however, scientist have argued that correcting for baseline values of the outcome results in a high predictive value of the overall model that is a clear over-adjustment leading to alteration of the effect estimates of other predictors of interest, sometimes even reversing the sign.[45] Others have argued that adjustment for baseline values of the outcome is a good way to adjust for unmeasured confounding; however the temporal conclusions that can be made based on these models are limited.[44] When delta is used as outcome measure other problems arise: 1) outliers can greatly influence the distribution of the changes, and 2) changes are always interpreted as equidistant, ignoring potential non-linear increments on the outcome scale, for example the difference between increasing the sleep duration from 5 to 6 hours may not be the same as increasing from 10 to 11 hours.[46] In contrast, psychologists studying cortisol levels,[47] cognitive decline,[48] as well as some sleep researchers studying cortical atrophy[49] have taken an approach that combined the two methods. Changes in grey matter volume were studied in a model where the delta volume is the outcome, while adjusting for the grey matter volume at baseline. Using this method is recommended when the amount of change is expected to depend on the baseline values (as is typically is for cortisol levels for example). However, if the exposure predicts the baseline level of the outcome, conditioning on this baseline measure induces a spurious correlation between the exposure and change score.[48]

Our approach in Chapter 4.1., where I studied changes in sleep complaints and white matter properties over time, was to perform both models. I used delta and follow-up values adjusted for baseline, and if any differences were found I further explored the underlying reasons. In this case, the null findings were consistent across the models, leading us to the conclusion that sleep complaints do not underlie white matter changes in the aging brain, nor are a result of white matter changes. However, in chapter 4.2., the two regression models differed and we took an alternative approach and studied changes in a Linear Mixed Model. These results indicated that the interaction of the exposure variable (objective sleep parameters) with follow-up time was not statistically significant, meaning that poor sleep does not influence the trajectories of white matter integrity

over time. Objectively disturbed sleep is related to white matter integrity, but despite the longitudinal design the temporality could not be inferred.

In conclusion, there are multiple ways to study changes in an epidemiological setting, and the right method depends on the research question and the variables of interest. One should first understand the phenotype of interest and study its changes over time independent of other variables. Next, one should explore if changes in the variable of interest depend on the baseline values, i.e. are only the high values decreasing or are only the low values increasing. Finally, one should pose the question are the baseline values of exposure expected to influence changes of the outcome or are the exposure changes influencing outcome changes. One paper or a PhD thesis of course does not hold the space for discussing all available methods and results, but as a rule of thumb one should always explore alternatives and flag inconsistencies between comparable methods.

Risk factor, prodrome, symptom or consequence: the complicated relation between sleep and brain health

As sleep patterns are intimately related to virtually all aspects of health and wellbeing, disentangling what came first, the sleep problem or the disease is particularly difficult. Traditionally, sleep disturbances have been considered a consequence or a symptom, rather than a risk factor or a prodrome. Clinicians would therefore oversee sleep problems, as these would typically be considered to be a consequence of the primary disease, combined with psychological changes that happen when a person is ill. Disorders where sleep is disturbed, but not considered crucial vary from depression,[50] bipolar disorder,[51] schizophrenia, to cardiovascular disease,[52] dementia,[35] Parkinson's disease,[53, 54] neurodevelopmental disorders[55, 56] and many more. Whereas for some conditions the directionality is clearer, for other conditions the story would be somewhat more complex. For example, pain disturbs sleep,[57] whereas, insomnia increases the risk for suicide,[58, 59] accidents and fatal injuries.[60, 61] Recent research has shed light on several conditions presenting with changes in sleep patterns at a very early stage, such as long sleep in cognitive decline[62, 63] and reduced sleep need and insomnia in bipolar disorder.[51, 64, 65] In depression, sleep problems are part of the definition,[50] whereas in neurodevelopmental disorders like ADHD insomnia disorder is present in up to 80%.[66] This makes it particularly difficult to disentangle if sleep is a prodrome or a symptom. Nevertheless, sleep is a potentially modifiable factor that in the case of being a prodrome, could prevent the incidence of the disorder (like it has been shown for manic attacks[67, 68]), or in the case of being a symptom could improve the course of the disease (treating sleep problems in ADHD improves symptoms of hyperactivity[69]).

Addressing causality can be challenging in an observational setting, but longitudinal designs do help. One of Hill's Criteria for Causation that can only be addressed in a

longitudinal study is temporality, i.e. does the effect occur after the cause. In particular, when repeated measures of both the exposure and the outcome are measured, the direction of the association can be explored. Baseline values of one variable can be used to predict changes in another variable, and vice versa. Additionally, it can be explored whether changes in one variable determine changes in another variable, i.e. does an increase in sleep duration predict cognitive decline. This way the temporality of the association is examined, but reverse causality, a major problem in cross-sectional studies, is also addressed. For example, if sleep disturbances at baseline predict changes in white matter integrity, we would intuitively infer that this is the direction of the association. However, if the reverse association is not tested, we cannot be sure about the temporal sequel of the relation. It may be that brain changes that happened prior to the baseline measurement determined sleep patterns which are associated with the future trajectories of white matter integrity. Only if there is no association between white matter integrity at baseline and changes in sleep patterns across time, then we can more confidently conclude about the direction of the association. As sleep is likely to be bidirectionally related to many indices of brain health, future studies should focus on disentangling directionality of associations and address reveres causality.

CLINICAL IMPLICATIONS

The studies conducted within this thesis could have implications for public health policies and daily clinical work. First, we propose that time spent in bed is an important determinant of poor sleep that has often been ignored both in research and in the clinical practice. Second, in Chapter 3 we showed that behavioral sleep patterns in childhood are a good indicator of the overall neurodevelopmental trajectory of the child, and therefore should receive attention in pediatric clinics. This is further supported by the findings of Chapter 4, where we demonstrate that childhood sleep disturbances have long-term associations with later neurocognitive development. The importance of developing early interventions for promoting healthy sleeping habits at a young age is clear. Third, our studies show that long sleep in childhood is not necessarily developmentally beneficial and should also be considered as important anamnestic information in the pediatric clinic. If slower decrease in sleep duration in toddlerhood is shown to be related to unfavorable developmental outcomes in future studies, pediatricians and developmental psychologists will have to address the current "the more the better" conviction about sleep duration in childhood. Finally, good news from our thesis comes for older persons and physicians working with them. Although subjective sleep complaints are quite common in the older population, these do not seem to negatively influence cerebral white matter health. In addition, the increase in sleep complaints with aging is not indicative of underlying white matter damage. However, when sleep disturbances exist objectively, these could be indicative of,

or lead to, white matter changes. Actigraphy is an affordable and user-friendly method to measure sleep and can easily be implemented.

FUTURE DIRECTIONS

Answering research questions tends to open many more questions, and with every new method used another one is discovered. The work presented in this thesis points towards bidirectionality in the relation between sleep and brain structure from childhood to older age. This interpretation of the findings, however, warrants explicit confirmation of the posed hypotheses.

First, as a proof of principle that sleep supports development, indices of fetal sleep and neurodevelopment should be systematically recorded in large samples and charted across intrauterine development. Understanding the ontogeny of sleep and its relation to the differentiation of the central nervous system as early as fetal life might answer important questions about the function of sleep during development and beyond.[70] If one can show that fetal cyclic changes in activity, for instance, stimulate neurons to set up complex neural networks,[71, 72] then it would be easier to understand why sleep is disturbed in many neurodevelopmental disorders. Measures of fetal sleep, however, can only be inferred from fetal movement, heartbeat and eye movement, it is yet to be determined how precisely these indices measure sleep.[73] Vice versa, more precise early markers of brain development, such as neonatal brain MRI, could also be used to predict later sleep patterns. Here, repeated measures of both would help disentangle the parallel developmental trajectories of these two phenomena (e.g. joint trajectory modeling of grey matter volume and sleep duration). Ideally, EEG measurements would inform about the changes in sleep architecture across early life,[15, 16, 74-76] but cruder measures such as changes in sleep duration, timing and consolidation, would also be highly applicable in the general practice.

Complementary approaches could be used in adults. First of all, bidirectional associations of white matter properties with objective sleep parameters should be explored. Although there was no association with subjective sleep complaints, white matter could underlie changes in sleep patterns that happen with aging. In addition, more subtle changes in sleep, such as the decrease in slow wave activity with aging[77, 78] should be explored as a determinant and outcome of structural brain changes in the aging brain.

Research questions in the field of sleep problems and disorders should particularly focus on sleep as a trait. Several large genome-wide studies have shown that many aspects of sleep (sleep duration,[11, 79] insomnia,[80, 81] chronotype[82]) are genetically determined, which highlights the importance of studying why some people for instance cannot get to sleep

and other do this with ease. Additionally, multimodal-imaging approaches that create links between brain structure and function and subsequently relate it to sleep characteristics would help disentangle neural basis of good and bad sleep (e.g. combining sleep EEG with resting state MRI, spectroscopy or magnetoencephalography).

Conclusion

I finish this thesis as I started it, wondering why we sleep and what happens inside the skull in the meanwhile. If you have read the book, you must have realized that my research did not provide an answer to this question, but in lack of a better summary I will list three general messages that can be taken forward by future researchers who would like to pursue this research. First, sleep comes from the brain and serves to the brain; bidirectionality in these processes is very likely, but the pathways are unknown. Second, the structural and functional brain changes that happen in children and older adults are related to their sleep patterns, potentially providing the opportunity to improve brain health. Finally, in clinics sleep should be considered important anamnestic information in the context of other indices of mental and physical health.

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7 SUMMARY/SAMENVATTING

SUMMARY

Sleep problems affect one third of the population and are particularly common during early life and later adulthood. At this time, during early neurodevelopment and when neural aging processes are starting to take place, the most substantial structural brain changes occur. Although it is known that sleep serves vital duties to the brain, the parallels between changes in sleep patterns and brain changes have not been thoroughly studied (**Chapter 1**). The aim of this thesis was to explore the neurobiological determinants and outcomes of sleep patterns in childhood and older adulthood. To explore temporality and directionality we conducted several longitudinal studies in early childhood, and in older adulthood.

In **Chapter 2** we first describe typical sleep pattern for the Dutch population across the lifespan. To this aim, we assembled individual participant data from 36 population-based cohorts creating a sample of 200,358 persons aged 1 to 100. Using this sample, we first provided age-specific percentile curves for self-reported sleep duration and sleep efficiency. Our results also showed that Dutch people on average seem to be getting the recommended hours of sleep, but teenagers, adult women, overweight persons and smokers are particularly prone to experiencing sleep problems. Finally, we showed that spending 7 to 8 hours in bed is related to lowest prevalence of sleep problems in the general population. These findings may guide public health interventions to improve sleep on larger scales.

Next, in **Chapter 3**, we explored early neurobiological determinants of childhood sleep patterns and problems. It has been hypothesized that developmental changes in sleep patterns closely correspond to the maturational state of the central nervous system. Others have posited that sleep is a learned behavior, and childhood sleep problems are a result of adverse external cues, such as stress or poor sleep hygiene. We addressed both hypotheses, tested (very) early developmental biomarkers as determinants of childhood sleep patterns, namely: prenatal and neonatal head growth – a marker of early neurodevelopment, and saliva cortisol levels during infancy – a marker of stress levels. We showed that fetuses and neonates with larger head circumference, and larger lateral ventricles within the normal range, had longer sleep duration in toddlerhood and less sleep problems across childhood. This could indicate that faster head growth in utero reflects genetically determined beneficial neurodevelopmental trajectories that are later reflected in better sleep patterns in childhood. Next, we showed that infants with a more marked morning cortisol rise and a flatter diurnal slope sleep shorter at night during toddlerhood. This could indicate that adverse intra-uterine environment or early life adversity due to low socio-economic status or maternal depression, could lead to both higher cortisol morning peaks and insufficient decrease before bedtime, resulting

in shorter sleep duration. Taken together these results show that behavioral expressions of sleep in early childhood can be considered important developmental milestones that correspond to the neurodevelopmental status of the child and the appropriateness of the rearing environment.

In **Chapter 4,** we explored the opposite direction, how early childhood sleep problems relate to later brain structure and function. First, we showed that sleep problems from age two onwards are related to lower grey matter volumes at age seven. In addition, children with increasing sleep disturbance trajectories in the first six years had thinner lateral prefrontal cortex, a region involved in higher-order cognitive functions. We also showed that children who awake frequently during the night at the age two years have lower IQ scores that those sleeping through the night. Importantly, this study also showed that toddlers sleeping longer than recommendations have lower verbal and nonverbal cognitive scores. This, might point against "the more the better" idea for sleep duration in childhood. Toddlers sleeping longer than their peers might lag behind in the developmental decrease in sleep duration, and potentially in overall neurodevelopmental trajectories later in childhood.

Next, in **Chapter 5** we explored similar hypotheses at the other end of the age distribution, in middle-aged and older adults. As sleep supports the function of oligodendrocytes and sleep loss may destabilize axonal integrity and disturb white matter, we focused on cerebral white mater. First, we studied the bidirectional relation between subjective sleep complaints and micro- and macro-structural integrity of white matter. We found that sleep complaints do not underlie or are a sequel to white matter changes in the aging brain. However, when we measured sleep objectively the story was different. We showed that actigraphic estimates of poor sleep, indicated by more wake after sleep onset, a lower sleep efficiency, and a shorter sleep duration, are associated with worse microstructural integrity of white matter. Microstructural alterations seem most pronounced in tracts involved in brain's judgment of comfort, gating of sensory information, motor control and the synchronization of activity between the two hemispheres.

Finally, in **Chapter 7** we summarize the findings, point out some methodological considerations and propose some research and clinical applications of our research.

SAMMENVATTING

Een derde van de bevolking heeft last van slaapproblemen en deze komen vooral veel voor tijdens de vroege kindertijd en in de latere volwassenheid. In deze periodes, zowel tijdens de vroege neurologische ontwikkeling alsmede wanneer neurale verouderingsprocessen beginnen, treden de meest substantiële structurele hersenveranderingen op. Hoewel bekend is dat slaap essentiële taken voor de hersenen heeft, zijn de parallellen tussen veranderingen in slaappatroon en hersenveranderingen nog niet grondig bestudeerd (hoofdstuk 1). Het doel van dit proefschrift was om de neurobiologische determinanten en uitkomsten van slaappatronen in de kindertijd en op de oudere volwassen leeftijd te exploreren. Om de timing-specifieke effecten en de richting hiervan te onderzoeken, hebben we verschillende longitudinale onderzoeken uitgevoerd in de vroege kindertijd en op de oudere volwassen leeftijd.

In hoofdstuk 2 beschrijven we eerst het normale slaappatroon voor de Nederlandse bevolking gedurende het gehele leven. Om dit te doen hebben we de individuele deelnemersgegevens van 36 bevolkingsonderzoeken verzameld, waarmee er een groep van 200.358 personen van 1 tot 100 jaar oud ontstond. In deze groep hebben we eerst leeftijdsspecifieke percentielcurven voor zelf-gerapporteerde slaapduur en slaapefficiëntie bekeken. Onze resultaten toonden aan dat Nederlanders gemiddeld de aanbevolen slaapuren lijken te halen, maar dat tieners, volwassen vrouwen, mensen met overgewicht en rokers vooral gevoelig zijn voor slaapproblemen. Ten slotte toonden we aan dat 7 tot 8 uur in bed doorbrengen gerelateerd is aan de laagste prevalentie van slaapproblemen in de algemene bevolking. Deze bevindingen kunnen de volksgezondheidszorg helpen bij de ontwikkeling en verbetering van slaap interventies.

Vervolgens hebben we in hoofdstuk 3 de vroege neurobiologische determinanten van slaappatronen en -problemen in de kindertijd onderzocht. Er wordt verondersteld dat ontwikkelingsveranderingen in slaappatronen sterk corresponderen met de ontwikkeling van het centrale zenuwstelsel. Aan de andere kant wordt er beweerd dat slaap aangeleerd gedrag is en dat op jonge leeftijd slaapproblemen het gevolg zijn van negatieve externe gebeurtenissen, zoals stress of slechte slaaphygiëne. We hebben beide hypotheses onderzocht, eerst bestudeerden we de biomarkers in (zeer) vroege ontwikkeling, zoals: prenatale en neonatale hoofdgroei – een marker van vroege neurologische ontwikkeling – en cortisolspiegels verkregen uit speeksel tijdens de kindertijd – een marker van stress. We toonden aan dat foetussen en pasgeborenen met een grotere hoofdomtrek en grotere laterale ventrikels (binnen de normale afkappunten), een langere slaapduur hadden in de peuterjaren en minder slaapproblemen gedurende de kindertijd. Dit zou erop kunnen wijzen dat een snellere hoofdgroei in utero de weerspiegeling is van een genetisch bepaalde gunstige neurologische ontwikkeling, wat later in de kinderleeftijd tot uiting komt als betere slaappatronen. Vervolgens lieten we zien dat baby's met een opvallende stijging in ochtendcortisol en een vlakkere cortisolspiegel gedurende de dag,

als peuters 's nachts korter slapen. Dit kan erop wijzen dat een ongunstige intra-uteriene omgevingsfactoren of negatieve levensgebeurtenissen gedurende de eerste kinderjaren, zoals een lage sociaaleconomische status of depressie van de moeder, kan leiden tot zowel hogere cortisol ochtendpieken als onvoldoende afname van de cortisolspiegel voor het slapen gaan, wat resulteert in een kortere slaapduur. Samengenomen tonen deze resultaten aan dat slaapgedrag in de vroege kindertijd kan worden beschouwd als belangrijke ontwikkelingsmijlpalen die samengaan met de neurologische ontwikkeling van het kind, en dat een passende opvoedingssituatie belangrijk is.

In hoofdstuk 4 verkenden we de andere kan van het verhaal: hoe slaapproblemen in de vroege kinderjaren zich verhouden tot de latere hersenstructuur en -functie. Ten eerste hebben we laten zien dat slaapproblemen vanaf de leeftijd van twee jaar verband houden met lagere grijze stof volume op zevenjarige leeftijd. Bovendien hadden kinderen met toenemende slaapproblemen in de eerste zes levensjaren een dunnere hersenschors van de laterale prefrontale cortex, een regio die belangrijk is voor hogere cognitieve functies. We hebben ook aangetoond dat 2-jarige kinderen die 's nachts vaker wakker worden, lagere IQ-scores hebben dan de kinderen die de nacht doorslapen. Belangrijk is dat uit deze studie ook bleek dat peuters die langer slapen dan de aanbevolen hoeveelheid, een lager verbaal en non-verbaal IQ hebben. Dit is in tegenspraak met de hypothese dat hoe langer kinderen slapen, hoe beter hun ontwikkeling. Peuters die langer slapen dan hun leeftijdsgenoten lopen mogelijk achter in de slaapduurvermindering die normaliter in de kinderontwikkeling wordt gezien, en mogelijk lopen ze ook achter in de algemene neurocognitieve ontwikkeling.

Vervolgens hebben we in hoofdstuk 5 vergelijkbare hypotheses onderzocht aan het andere einde van het leven, bij volwassenen van middelbare en oudere leeftijd. We hebben ons gericht op de witte stof van de hersenen, omdat slaap de functie van oligodendrocyten positief beïnvloedt en omdat slaapverlies de axonale integriteit kan destabiliseren en zodoende witte stof kan verstoren. Eerst hebben we de bi-directionele relatie bestudeerd tussen subjectieve slaapklachten en de micro- en macro-structurele integriteit van witte stof. We ontdekten dat slaapklachten niet ten grondslag liggen of een gevolg zijn van de veranderingen in de witte stof in het ouder wordende brein. Echter, dit was anders toen we de slaap objectief hadden gemeten. We toonden aan dat actigrafische metingen van slechte slaap, aangegeven door vaker wakker worden gedurende de nacht na het in slaap vallen, een lagere slaapefficiëntie en een kortere slaapduur, geassocieerd zijn met een slechtere micro-structurele integriteit van de witte stof. Microstructurele veranderingen leken het meest uitgesproken in hersenbanen die betrokken zijn de beoordeling van comfort, interpreteren van sensorische informatie, motorische controle en de synchronisatie van activiteit tussen de twee hemisferen.

Ten slotte vatten we in hoofdstuk 7 de bevindingen samen, wijzen op enkele methodologische overwegingen, en stellen we enkele toekomstige wetenschaps- en klinische toepassingen van ons onderzoek voor.

8 ADDENDUM

Publications and Manuscripts

PhD Portfolio

Words of thanks

About the author

PUBLICATIONS AND MANUSCRIPTS

The bidirectional association between sleep problems and autism spectrum disorder: a population-based cohort study.
Verhoeff ME, Blanken LME, **Kocevska D**, Mileva-Seitz VR, Jaddoe VWV, White T, Verhulst F, Luijk MPCM, Tiemeier H.
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Pediatr Res. 2018 Jan 17

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Kocevska D, Muetzel R, Luik AI, Luijk MP, Jaddoe VW, Verhulst FC, White T, Tiemeier H.
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Macronutrient Intakes in Infancy Are Associated with Sleep Duration in Toddlerhood
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Zuurbier LA, Vernooij MW, Luik AI, **Kocevska D**, Hofman A, Whitmore H, Ikram MA, Tiemeier H
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A

Three authors reply
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Bidirectional associations between objectively measured sleep and body mass index: a prospective study in middle-aged and older adults.
Koolhaas CM*, **Kocevska D***, te Lindert BHW, Erler NS, Franco OH, Luik AI, Tiemeier H.
Under revision

Sleep complaints and cerebral white matter: a prospective bidirectional study
Kocevska D, Cremers LGM, Lysen TS, Luik AI, Ikram MA, Vernooij MW, Tiemeier H.
Under revision

The prospective association of objectively measured sleep with microstructural integrity of cerebral white matter in middle-aged and older persons
Kocevska D, Tiemeier H, Lysen TS, Muetzel RL, Van Someren EJW, Ikram MA, Vernooij MW**, Luik AI**
Submitted

Sleep patterns across the lifespan: An individual participant meta-analysis in 200,358 persons from the general population
Kocevska D, Lysen TS, Lifelines, Luijk MPCM, Antypa N, Biermasz N, Blokstra A, Brug J, Comijs HC, Corpeleijn E, de Bruin EJ, de Graaf R, Derks I, Dewald-Kaufmann J, Elders PM, Gemke RJBJ, Grievink L, Hartman CA, Heijnen CJ, Huisman MA, Huss A, Ikram MA, Jaddoe VWV, Klein Velderman M, Koning M, Noordam R, Oldehinkel TAJ, Oude Groeniger J, Penninx BWJH, Picavet SJ, Reijneveld SA, Reitz E, Renders CM, Rodenburg G, Rutters F, Singh A, Snijder MB, Stronks K, ten Have M, Twisk JWR, Van de Mheen D, van der Ende J, van der Heijden KB, van der Velden PG, van Lenthe F., van Litsenburg RRL, van Oostrom SH, van Schalkwijk FJ, Verheij R, Verhoeff ME, Verhulst FC, Vermeulen MCM, Vermeulen R, Verschuren MWM, Vrijkotte TGM, Wijga AH, Willemen AM, Wissink IB, ter Wolbeek M, Xerxa Y, Franco OH, Bramer WM, Luik AI, Van Someren EJW**, Tiemeier H**
In preparation

Childhood Sleep Disturbances and White Matter Microstructure in pre-adolescence.
Mulder T, **Kocevska D**, Muetzel RL, Verhoeff ME, Jaddoe VWV, Hillegers MHJ, White T, Tiemeier H
Submitted

Gestational age at birth and sleep duration in early childhood in three population-based cohorts
Luijk MPCM, Broekman BFP, **Kocevska D**, Tham EKH, Gaudreau H, Reiss IKM, Jaddoe VWV, Tiemeier H, Meaney MJ, El Marroun H.
Submitted

Neuroanatomy of child callous traits: a population-based study
Bolhuis K, Viding E, Muetzel RL, El Marroun H, **Kocevska D**, White T, Tiemeier H, Cecil CAM.
Accepted in Biol Psych

Objectively measured sedentary time and mental and cognitive health: cross-sectional and longitudinal associations in The Rotterdam Study
Koolhaas CM, van Rooij FJA, **Kocevska D**, Ikram MA, Franco OH, Tiemeier H
Under revision

During Day and Night: Psychotic-Like Experiences and Nightmares in Childhood.
Verhoeff ME, Bolhuis K, Cecil CAM, **Kocevska D**, Hudziak JJ, Hillegers MHJ, Mileva-Seitz VR, Jaddoe VWV, Verhulst FC, Tiemeier H, Luijk MPCM.
Accepted in Schizophrenia Research

Family Irregularity Disturbs the Development of Sleep in Children
Verhoeff M, Serdarevic F, **Kocevska D**, Bodrij FF, Mileva-Seitz VR, Jaddoe VWV, Hillegers MHJ, Tiemeier H, Cecil CAM, Verhulst FC, Luijk MPCM.
Submitted

**equal contribution*
***shared senior authorship*

PHD PORTFOLIO

Name PhD student: Desana Kocevska
Erasmus MC Department: Child & Adolescent Psychiatry
Research School: NIHES
PhD period: Aug 2013 – Jun 2018
Promotors: Henning Tiemeier
Eus Van Someren
Copromotor: Annemarie Luik

PhD training	Year	ECTS
MSc-program Clinical Epidemiology		
Principles of Research in Medicine	2013	0.7
Clinical Decision Analysis	2013	0.7
Methods of Public Health Research	2013	0.7
Health Economics	2013	0.7
Social Epidemiology	2013	0.7
Markers of Prognostic Research	2013	0.7
The Practice of Epidemiologic Analysis	2013	0.7
Study Design	2013	4.3
Clinical Epidemiology	2013	1.4
Methodologic Topics in Epidemiologic Research	2013	1.4
Biostatistical Methods I: Basic Principles	2013	5.7
Biostatistical Methods II: Classical Regression Models	2013	4.3
Elective courses, MSc		
Women's health	2014	0.9
Nutrition & Physical Activity	2014	1.4
Health Services: Research and Practice	2014	0.9
Preventing Failed Interventions in Behavioral Research	2014	1.4
Public Health in Low and Middle-Income Countries	2014	0.7
DSc-program		
Advances in Epidemiologic Analysis	2014	0.4
Bayesian Statistics	2015	1.4
Missing Values in Clinical Research	2015	0.7
Causal Mediation Analysis	2015	0.7
Principles of Epidemiologic Data-analysis	2015	0.7
Conceptual Foundation of Epidemiologic Study Design	2016	0.7
History of Epidemiologic Ideas	2018	0.7
Elective courses, DSc		
Repeated Measurements in Clinical Studies	2015	1.4
Psychiatric Epidemiology	2015	1.1
Maternal and Child Health	2015	0.9

PhD training	Year	ECTS
Skills Courses		
English Language	2013	1.4
Introduction to Medical Writing	2013	1.1
Courses for the Quantitative Researcher	2013	1.4
Endnote, Medical Library, Erasmus MC	2014	0.3
Systematic literature search, Erasmus MC Library	2014	0.6
Research Integrity	2014	2.0
Other courses		
The two-process model revisited	2016	0.2
Sleep and Circadian Rhythm Neuroscience	2017	1.4
International conferences		
23rd congress of ESRS, Bologna, Italy (*oral presentation*)	2016	1.4
European Insomnia Network meeting (*oral presentation*)	2016	0.7
Epilepsy, Sleep and Neurocognition Symposium, Heeze	2017	0.2
SLEEP, *Boston, MA, USA (oral and poster presentation)*	2017	1.4
SLAAP, *Ermelo, the Netherlands (oral presentation)*	2017	1.0
SLAAP, *Ermelo, the Netherlands (poster presentation)*	2016	1.0
Symposia, Meetings & Workshops		
Psychiatry Research Meetings, Rotterdam (*oral presentation*)	2016	1.0
Generation R Research Meeting (*oral presentation*)	2016	1.0
KJP Colloquium, Erasmus MC (*oral presentation*)	2016	0.2
NSWO Symposium , *Ermelo (oral presentation)*	2017	0.2
Teaching and educational activities		
Research project supervisor – 4th year medical students	2014	2
Master thesis supervision		
Tessa Mulder – *sleep problems and white matter integrity*	2016-2017	2
Tim Vulkers – *objective sleep and white matter integrity*	2017	1
Selma Meinderts – *prenatal brain development and sleep*	2016	1
Research Grants		
ERAWEB MSc scholarship, 1 year	2013	
ERAWEB PhD scholarship, 3 years	2014	
TrustFonds Erasmus University, *travel grant*, €250	2016	
Hersenstichting Nederland, *co-applicant*, €100,000	2016	
European Sleep Research Society, *personal grant*, €2,800	2017	
KNAW Ter Meulen Beurs, *personal grant*, €2,900	2017	
KNAW, Startimpuls Grant, *co-applicant*, €260,000	2018	
Other activities		
Generation R general task	2014-2015	
Internship at Netherlands Institute for Neuroscience, Sleep and Cognition Group	2016	
Incidental Findings on brain MRI, rater	2016-2017	
Research fellowship at Child Development Center and Pediatric Sleep Disorders Center, University Children's Hospital Zurich, Switzerland	2017	
Peer review (BMC Public Health, J. Pediatr.)	2018	

WORDS OF THANKS

Disclaimer: I wrote this part well after the deadline for printing, just to be in line with the rest of my PhD work. I am sorry if I forgot to thank someone and am very grateful to anyone that listened my sleep/brain/development/research/blah-blah stories over the past 5 years.

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Цимери, педерчиња, нечу да ве именувам, само вие ја разбирате и цените маката што е потрошена на ова. Бевте таму на почетокот на шизофренијата, и уште сте си мои, најмои! Алавју!

Сите милион фини, и не толку фини, но добри луѓе што преспаа ноќ-две, до десет, фала ви за прљавите постелини. Најбитно, фала ви што ме слушавте да мудрувам за тоа каков е животот во Холандија. А да, по таа линија, исто би сакала да се заблагодарам на сите барови и најт шопови во Ротердам, и тие си одиграа битна улога.

На баба Деса, баба Новка и дедо Санде, можам да им се заблагодарам што се живи и здрави и што секое доаѓање ми се радуваат како на првото кокиче.

Мајка ми и татко ми, прво и основно, фала што рано ми покажавте што е PhD и дека е кул ствар да се има. Второ, што сте одличен пример за како треба и како не треба, во перфектен баланс. Трето, фала што од проблематично дете создадовте жена што брани докторат. (Океј, и јас имам заслуга за последново, да не бидам толку скромна.)

Брат ми, најбитниот човек во мојот живот. Океј, вториот најбитен, пошо јебига, пораснавме, живот. Цел живот си ми репер, дека нели попаметен, машко. Еве ќе почнам да читам, ветувам. Веќе почнав и ок музика да слушам. Фала ти бате што си ми прав брат, и што без збор дозволуваше да викам 20 души да ти пијат и пушат у дневна. А и на Стефанче фала, што те прави среќен, а и нас богами.

Јован, љубов моја, какво чудо што во право време одлучивме да се сакаме. И какво олеснување за да можам на раат да го завршам ова чудо. Едвај чекам да видам што не чека во заедничкиот живот. Те сакам.

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ABOUT THE AUTHOR

Desana Kocevska was born on 27 August 1989 in Skopje, Macedonia. She started her secondary education in 2004 in Skopje and graduated in 2007 in Mesa, Arizona, USA. The same year she enrolled at the Ss. Cyril and Methodius University in Skopje where she obtained her Medical Doctor's degree in 2013. Right after obtaining her university diploma Desi was granted an ERAWEB scholarship financed by the European Commission to follow a Master's program in Clinical Epidemiology at the Netherlands Institute of Health Sciences (NIHES) in Rotterdam. During her Master's program Desi's interests in research developed and in 2014 she enrolled in the PhD program at the Department of Epidemiology and the Department of Child and Adolescent Psychiatry of Erasmus MC in Rotterdam. During her PhD trajectory she also followed a Doctor of Science program and did research internships at the Netherlands Institute of Neuroscience (NIN) in Amsterdam and at the Child Development Center at University Children's Hospital Zurich. Together with her mentors, Desi has applied for several grants that funded her PhD project, her internships abroad and her future project. Desi will pursue her research career as a postdoctoral researcher in the Sleep and Cognition group at the NIN and hopes to eventually start a residency in Psychiatry at one of the University Hospitals in the Netherlands. This way she hopes to build bridges between clinical medicine and research.