

# Sleep, 24-hour activity rhythms, and brain structure

A population-based study



Lisette A. Zuurbier

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and brain structure:**

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# **Sleep, 24-hour activity rhythms, and brain structure:**

A population-based study

Slaap, het 24-uurs bewegingsritme en hersenstructuur:  
Een populatiestudie

## **Proefschrift**

ter verkrijging van de graad van doctor aan de  
Erasmus Universiteit Rotterdam  
op gezag van de  
rector magnificus

Prof. dr. H.A.P. Pols

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op  
vrijdag 29 april 2016 om 11.30 uur  
door

**Lisette Anna Zuurbier**  
geboren te Langedijk

The logo of Erasmus University Rotterdam, featuring the word 'Erasmus' in a stylized, cursive script.

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## **Manuscripts based on the studies described in this thesis**

### **Chapter 2.1**

Luik AI, Zuurbier LA, Hofman A, Van Someren EJ, Tiemeier H. Stability and fragmentation of the activity rhythm across the sleep-wake cycle: the importance of age, lifestyle, and mental health. *Chronobiol Int* 2013;30:1223-30.

### **Chapter 2.2**

Zuurbier LA, Luik AI, Hofman A, Franco OH, Van Someren EJ, Tiemeier H. Fragmentation and stability of circadian activity rhythms predict mortality: the Rotterdam study. *Am J Epidemiol* 2015;181:54-63.

### **Chapter 2.2a**

Zuurbier LA, Kocavska D, Tiemeier H. Three Authors Reply. *Am J Epidemiol* 2015;182:470-1.

### **Chapter 2.3**

Zuurbier LA, Luik AI, Leening MJ, Hofman A, Freak-Poli R, Franco OH, Stricker BH, Tiemeier H. Associations of heart failure with sleep quality: The Rotterdam Study. *J Clin Sleep Med* 2015;11:117-21.

### **Chapter 3.1**

Zuurbier LA, Ikram MA, Luik AI, Hofman A, Van Someren EJ, Vernooij MW, Tiemeier H. Cerebral small vessel disease is related to disturbed 24-h activity rhythms: a population-based study. *Eur J Neurol* 2015;22:1482-7.

### **Chapter 3.2**

Zuurbier LA, Vernooij MW, Luik AI, Kocavska D, Hofman A, Whitmore H, Ikram MA, Tiemeier H. Apnea-hypopnea index, nocturnal arousals, oxygen desaturation and structural brain changes: A population-based study. Submitted.

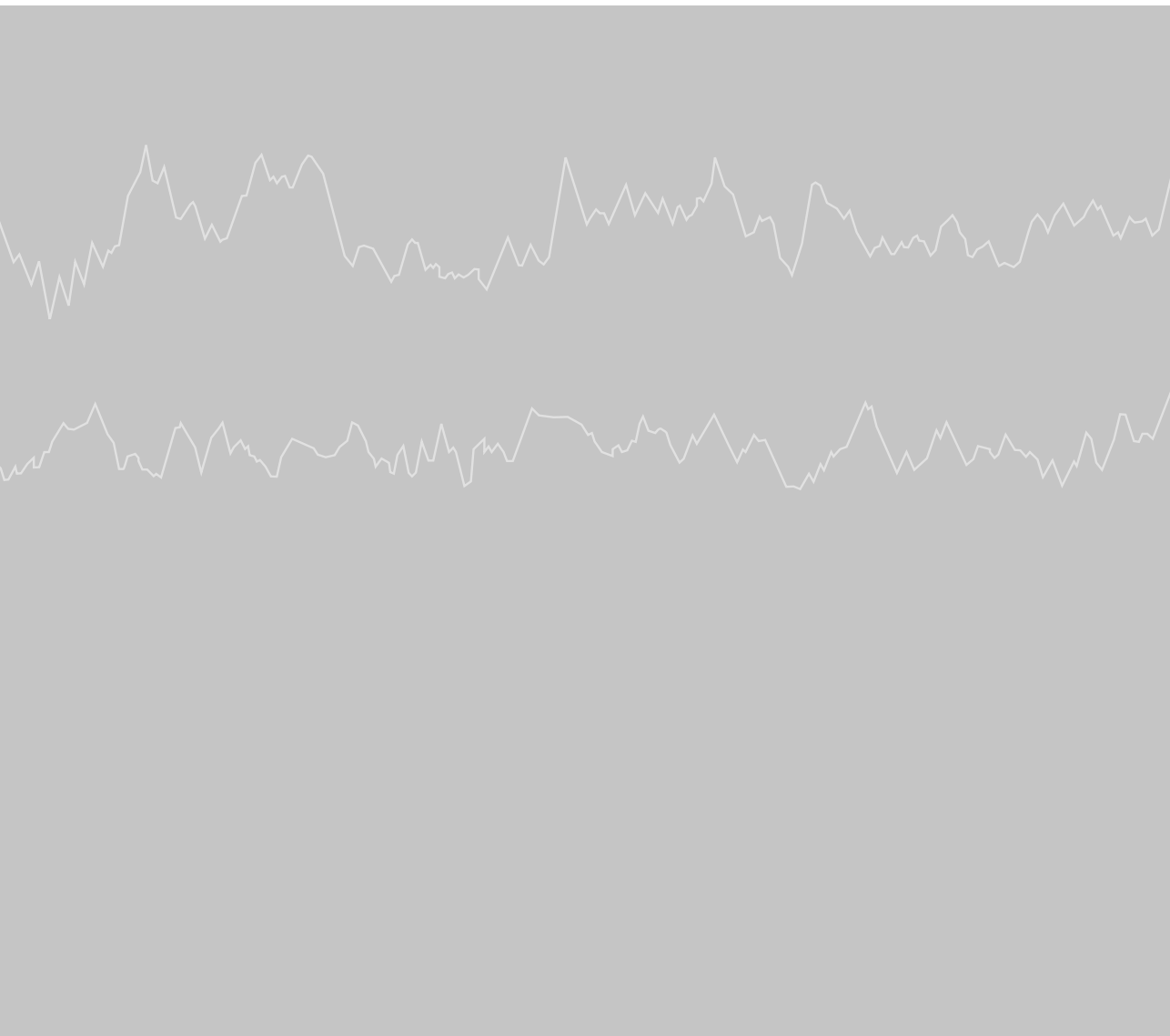
### **Chapter 3.3**

Zuurbier LA, Luik AI, Tiemeier H, Niessen WJ, Whitmore H, Ikram MA, Vernooij MW. Brain structure, EEG activity during sleep and sleep quality: A population-based study of middle-aged and elderly persons. Submitted.

### **Chapter 3.4**

Zuurbier LA, Vernooij MW, Spiegelhalter K, Hofman A, Niessen WJ, Van Someren EJ, Ikram MA, Tiemeier H. Gray matter, white matter and hippocampal volume in middle-aged and older adults with insomnia: A population-based study. To be submitted.





# Chapter 1

## General Introduction





A good night of sleep is very important for a healthy life. Many people probably only realize this after a night of poor sleep, feeling physically, cognitively and emotionally busted. However, sleep problems are very common, especially in the older population.

In elderly persons, about 80% have sleep complaints.<sup>1</sup> For example, elderly persons report trouble falling asleep, waking up too early, a low sleep quality, napping during the day, and obtaining not enough sleep during the night.<sup>2,3</sup> These sleep problems can partly arise due to reduced physical activity during the day, less exposure to light, and more physical and mental problems, such as cardiovascular disease, diabetes and depression. Many, if not most, chronic physical and mental health problems are established risk factors for sleep problems.<sup>3,4</sup> Furthermore, two specific sleep disorders are common in older age; insomnia and sleep apnea. Over 10% of the adult population suffers from clinically relevant insomnia. Insomnia is a sleep disorder characterized by problems of falling or staying asleep. Daytime impairment, typically due to fatigue during the day, is another core characteristic.<sup>5</sup> Sleep apnea is characterized by repetitive respiratory events (apneas and hypopneas) during sleep, leading to sleep fragmentation, nocturnal intermittent oxygen desaturation, arousals and also to daytime fatigue. Over 10% of the adult population (30 – 70 years old) have an apnea-hypopnea index (AHI)  $\geq 15$ , indicating sleep apnea, but the prevalence increases to about 20% in adults aged 60-70 years old.<sup>6</sup> Insomnia and sleep apnea have both been prospectively related to physical and mental health such as cardiovascular disease, cognitive decline and mortality.<sup>7-11</sup> The resulting fatigue can impact social and occupational life.

The sleep changes with ageing are paralleled by structural brain changes that are also more prevalent in elderly populations. Typical changes observed in elderly populations are loss of brain tissue (i.e. cerebral atrophy), cerebral vascular lesions, and reduced connectivity between brain areas.<sup>12</sup> A good night of sleep is important for a healthy and functioning brain. There are several mechanisms through which poor sleep may negatively affect the brain. First, during sleep potentially neurotoxic waste products are removed from the brain interstitial space.<sup>13</sup> Chronic dysregulation of this process could lead to brain structural changes. Second, sleep deprivation has been related to impairment of the neuroendocrine system, including emotional and cognitive problems. Functional impairment in the neuroendocrine system may precede long-term structural and functional changes in the brain. For example, sleep deprivation is related to an impaired ability to form new memories, to an inappropriate emotional response to negative aversive stimuli, and to decreased reaction speed and concentration.<sup>14-16</sup> This is evident, for example, from the many car accidents occurring because of sleepiness. Third, sleep apnea causes disturbances in the sleep-wake pattern, such as fragmentation during sleep, and has been related to incident neurological diseases such as stroke.<sup>17</sup>

Thus sleep is important for the brain, but the reverse relation is equally important; a healthy mind is important for a good sleep. In diseases that affect the brain, like Alzheimer's disease,

sleep problems are highly prevalent.<sup>18</sup> Furthermore, the brain works as an internal clock, regulating the 24-hour rhythm of the sleep-wake cycle. An important regulator of this 24-hour or circadian rhythm is the suprachiasmatic nucleus, a structure in the hypothalamus of the brain. This structure is also called the biological clock of the brain. It integrates internal and external signals (e.g. light), to create stable circadian rhythms.<sup>19</sup>

Many brain changes in the elderly occur unnoticed. However, even small brain changes can affect health and behavior. To date, there is only scarce knowledge whether brain changes typically observed in an ageing population relate to altered sleep patterns. This is important to understand, in view of the high prevalence of sleep problems at old age and the potential important clinical consequences.

The aim of this thesis was to investigate the associations between sleep, 24-hour activity rhythms and health parameters in a middle-aged and elderly population, particularly focusing on the relation between sleep and age-related structural brain changes. The analyses performed in this thesis were based on data from the Rotterdam Study, a large population-based cohort among adults aged 45 and over living in Rotterdam, the Netherlands, in which both sleep parameters and neuro-imaging data are acquired.<sup>20</sup>

In the Rotterdam Study, sleep is measured with three different methods. First, sleep is measured subjectively with questionnaires. This is important for the assessment of how a person experiences sleep. However, personality or emotional states impact self-report, and even in healthy persons answers on sleep habits and times do not reflect their true sleep patterns well. Therefore, we also measured sleep objectively, by polysomnography (PSG) and actigraphy. PSG is the gold standard in sleep research. During PSG brain activity (electroencephalography), eye movements (electrooculography), muscle tone (electromyography), heart function (electrocardiography), breathing and oxygen desaturation are measured. The Rotterdam Study is one of the few cohort studies acquiring PSG. However, PSG cannot be used to study day-night rhythms. Therefore, actigraphy was used to measure sleep and the 24-hour activity rhythm on a seven-day basis. Activity rhythms were quantified by calculating the interday stability and the intraday variability. Interday stability is a measure of how stable the rhythm is over multiple days, that is, how similar the individual day-night patterns are over time. Intraday variability reflects the fragmentation of the rhythm, that is, the rate of shifting between rest and activity.

Furthermore, brain imaging is included in the core protocol of the Rotterdam Study, enabling us to study a spectrum of age-related structural brain changes in relation to sleep.

In this thesis, Chapter 2 focuses on sleep, 24-hour activity rhythms and health. Chapter 2.1 describes the influence of demographics, lifestyle and sleep on 24-hour activity rhythms. In Chapter 2.2 sleep and 24-hour activity rhythms are used to predict mortality. This chapter is extended with Chapter 2.2a in which the addition of information on circadian misalignment (the difference between the timing of sleep on workdays and weekend days) was discussed.

In Chapter 2.3 I present a study on the differential effects of clinical heart failure and echocardiographic indicators of cardiac dysfunction on sleep quality.

Chapter 3 focusses on sleep, 24-hour activity rhythms and structural brain parameters. In Chapter 3.1 I present our research on the relation of cerebral small vessel disease with disturbed 24-hour activity rhythms. In Chapter 3.2 I investigated different aspects of sleep apnea in relation with brain structure. Chapter 3.3 describes the associations between brain structure and sleep parameters, measured both subjectively and objectively with PSG. In Chapter 3.4 differences in brain morphology between insomnia cases and good sleepers were explored.

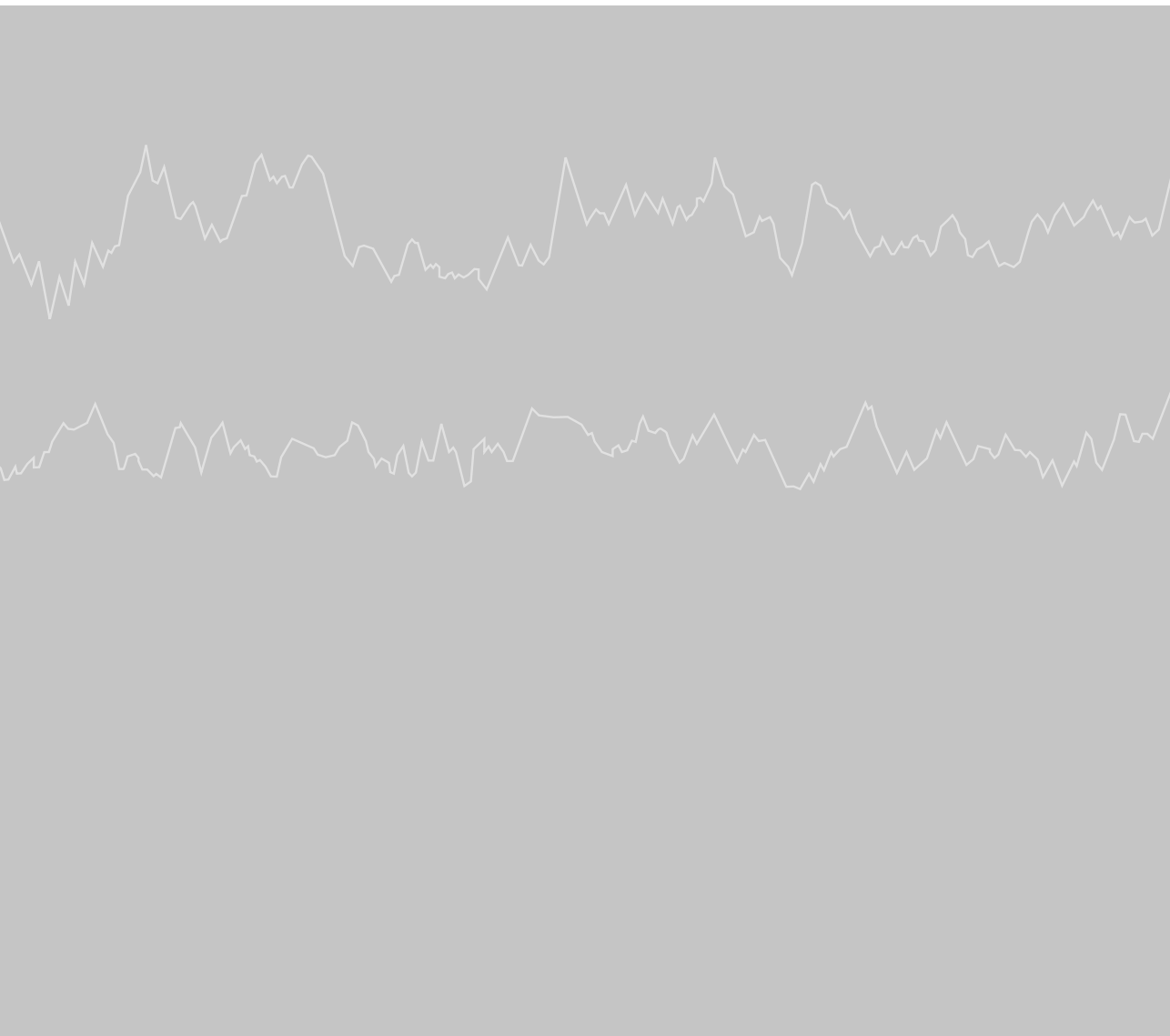
Finally, in Chapter 4 I discuss the main findings of this thesis, together with methodological considerations, clinical implications and suggestions for future research.

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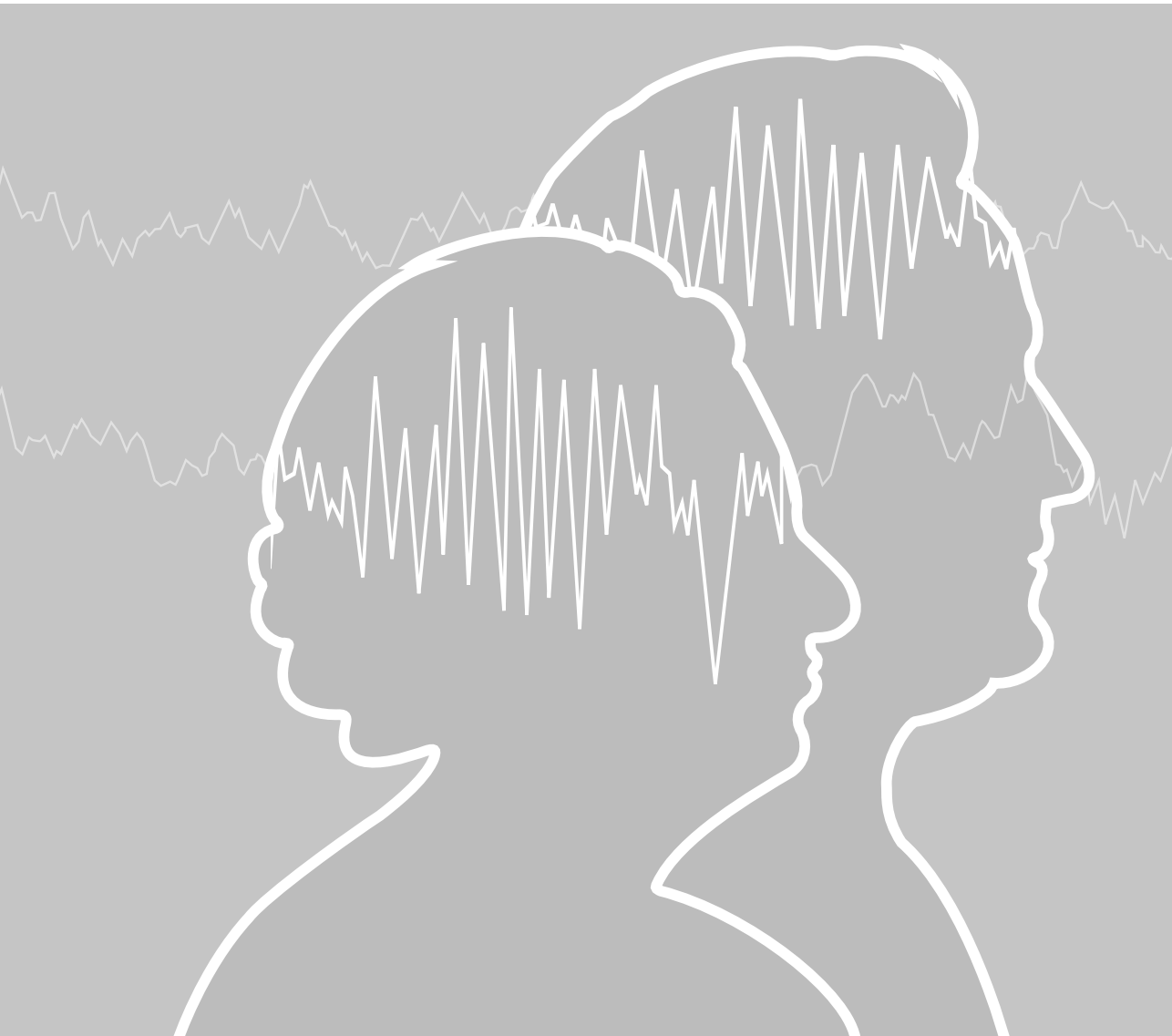






# Chapter 2

## **Sleep, 24-hour activity rhythms and health**







# Chapter 2.1

## **Stability and fragmentation of the activity rhythm across the sleep-wake cycle: the importance of age, lifestyle and mental health**

Annemarie I. Luik, Lisette A. Zuurbier, Albert Hofman, Eus J.W. van Someren,  
and Henning Tiemeier

## Abstract

The rhythms of activity across the 24-hour sleep-wake cycle, determined in part by the circadian clock, change with aging. Few large-scale studies measured the activity rhythm objectively in the general population. The present population-based study in middle-aged and elderly persons evaluated how activity rhythms change with age, and additionally investigated socio-demographics, mental health, lifestyle and sleep characteristics as determinants of rhythms of activity. Activity rhythms were measured objectively with actigraphy. Recordings of at least 96 hours ( $138 \pm 14$  hours, mean  $\pm$  SD) were collected from 1734 people (age  $62 \pm 9.4$  years) participating in the Rotterdam Study. Activity rhythms were quantified by calculating interdaily stability, i.e. the stability of the rhythm over days, and intradaily variability, i.e. the fragmentation of the rhythm relative to its 24-hour amplitude. We assessed age, gender, presence of a partner, employment, cognitive functioning, depressive symptoms, Body Mass Index, coffee use, alcohol use and smoking as determinants. The results indicate that older age is associated with a more stable 24-activity profile ( $\beta=.07$ ,  $p=.02$ ), but also with more fragmentation of activity and inactivity ( $\beta=.20$ ,  $p<.001$ ). Having more depressive symptoms was related to less stable ( $\beta=-.07$ ,  $p=.003$ ) and more fragmented rhythms ( $\beta=.10$ ,  $p<.001$ ). A high Body Mass Index and smoking were also associated with less stable rhythms (BMI:  $\beta=-.11$ ,  $p<.001$ , smoking:  $\beta=-.12$ ,  $p<.001$ ) and more fragmented rhythms (BMI:  $\beta=.09$ ,  $p<.001$ , smoking:  $\beta=.11$ ,  $p<.001$ ). We conclude that with older age the 24-hour activity rhythm becomes more rigid, while the ability to maintain either an active or inactive state for a longer period of time is compromised. Both characteristics appear important for major health issues in old age.

## Introduction

Circadian rhythm changes are commonly observed in middle-aged and elderly persons and have been attributed to functional changes in the suprachiasmatic nucleus, the biological clock of the brain.<sup>1</sup> Observed age-related changes typically include alterations in the 24-hour cycle of sleep and wakefulness. Previous studies have demonstrated several changes with increasing age: more frequent and longer napping,<sup>2</sup> a higher fragmentation of the rest and activity pattern,<sup>3</sup> a tendency to fall asleep earlier,<sup>4</sup> and a tendency to wake up earlier.<sup>5</sup> While large population-based studies have objectively assessed nocturnal sleep, objective assessment of the circadian organization of the sleep-wake cycle in the elderly is scarce. The studies available have mostly focused on rhythm alterations in relation to disease,<sup>6,7</sup> determinants of circadian alterations and variations in the sleep-wake cycle in the general population have remained largely unclear.

Changes in sleep and their determinants have been studied extensively. For example, lifestyle and dietary habits are known to affect sleep. In particular, alcohol consumption initially improves sleep, but more awakenings and lighter sleep are seen during the latter part of the night.<sup>8</sup> Smokers report greater difficulty initiating and maintaining sleep and a lower sleep quality. Research on objective sleep data confirms these results in a clinical study.<sup>9</sup> In a cross-sectional, population-based study, regular daily caffeine intake was also, albeit less clearly associated with disturbed sleep and daytime sleepiness.<sup>10</sup> However, it is not known how these habits are related to the circadian organization of the sleep-wake cycle in middle-aged and elderly persons in the general population.

Mental health is intimately related to sleep and circadian rhythms. Depressive symptoms have been related repeatedly to sleep, the available literature suggests depressive symptoms to be associated with disturbed circadian rhythms as well.<sup>11</sup> Disturbances in the sleep-wake rhythm are also more common in persons with poor cognitive functioning. The sleep-wake rhythm has been found to relate to cognitive functioning, even independent of age<sup>12</sup> and as well in demented elderly people.<sup>13</sup>

In this study, we examined the circadian organization of the sleep-wake cycle in a large population-based study of middle-aged and elderly persons with actigraphy. Activity rhythms can be considered as an indicator of the circadian organization of the sleep-wake cycle<sup>14,15</sup> and allows assessment of the rhythm over longer periods of time. Participants in the present study were asked to wear the actigraph for one week. We assessed whether age, lifestyle factors and mental health indicators were related to objectively assessed activity rhythms, independent of self-reported sleep. In addition, we studied whether self-reported sleep characteristics and sleep quality were associated with the activity rhythm across the sleep-wake cycle.

## Materials and methods

### *Study population*

The current study was embedded in the Rotterdam Study, a population-based cohort of older persons which started in 1990 in the district of Ommoord, Rotterdam, The Netherlands. The Rotterdam Study targets cardiovascular, endocrine, hepatic, neurological, ophthalmic, psychiatric, dermatological, oncological, and respiratory diseases. In 2000, the study population was extended with a second cohort by inviting inhabitants of the same district aged 55 and over. In 2006, a new cohort with inhabitants aged 45 and over was added. No health-related exclusion criteria were used. A more detailed description of the study can be found elsewhere.<sup>16</sup> The Medical Ethics Committee of the Erasmus University Rotterdam approved the Rotterdam Study and written informed consent was obtained from all participants. All procedures were conform international standards.<sup>17</sup>

From December 2004 until April 2007, in total 2634 consecutive participants were invited to enter the actigraphy study; of these 2084 (79%) agreed. The actigraphy study comprised participants of the second cohort (second examination, December 2004 - December 2005), and the new cohort (baseline, January 2006 - April 2007). No exclusion criteria were used, although participants had to be able to understand the instructions for participation. Due to technical problems and the requirement that the recording must be a minimum of 4 consecutive days and nights, the recordings of 1734 participants (83%) were available for further analyses.

### *Assessment of the activity rhythm*

All participants were asked to wear an actigraph around the wrist (Actiwatch model AW4, Cambridge Technology Ltd, Cambridge, United Kingdom) continuously for 7 consecutive days and nights. The actigraph had to be removed from the wrist while bathing only. Actigraphs were measured in 30-second epochs. 24-hour periods with more than 3 continuous hours missing were excluded to prevent a time-of-day effect. Recordings of less than 4 complete days and nights were also excluded from the analyses.

Activity rhythms were quantified using non-parametric indicators.<sup>18</sup> This allows us to describe the rhythm without making untenable assumptions about the shape of the rhythm. We calculated three variables, the interdaily stability, the intradaily variability and amplitude of the rhythm. The interdaily stability (IS) indicates the stability of the rhythm, i.e. the extent to which the profiles of individual days resemble each other. Intradaily variability (IV) quantifies how fragmented the rhythm is relative to its 24-hour amplitude; more frequent alterations between an active and an inactive state lead to a higher intradaily variability. The amplitude is calculated as the normalized difference between the most active 10 hours and the least active 5 hours. We do not report data using the amplitude as it correlated highly with

interdaily stability ( $r=.68$   $p<.01$ ) and intradaily variability ( $r=-.69$   $p<.01$ ) and it is less specific than interdaily stability and intradaily variability. Data are available upon request. Interdaily stability and intradaily variability were moderately and inversely correlated ( $r=-.49$ ,  $p<.01$ ).

#### *Assessment of sleep parameters*

Sleep parameters were assessed subjectively with a sleep diary and objectively by actigraphy on the same days. The sleep diary included questions about sleep characteristics, sleep medication, sleep quality and dietary habits for each day. To evaluate use of sleep medication, participants filled out whether they had used sleep medication and which medication they used. Sleep onset latency was assessed by asking participants how long it took them to fall asleep. For total sleep time participants estimated how long they slept during the night. Participants also filled out if they had been awake during the night, and if so, how often. Daily values were generally averaged over the week; only napping indicates how many days per week participants had taken a nap during the day. We also used actigraphy to estimate total sleep time, sleep onset latency and wake after sleep onset objectively. The procedure to calculate total sleep time and sleep onset latency has been described in more detail elsewhere.<sup>19</sup> Wake after sleep onset is calculated as the total time of the epochs scored as wake between sleep start and sleep end, all defined according to the Actiwatch manual.<sup>20</sup> A threshold of 20 was used to distinguish sleep from waking.<sup>21</sup> Possible sleep apnea was assessed with two questions from the Pittsburgh Sleep Quality Index.<sup>22</sup> We considered apnea possible when participants reported (1) loud snoring at least two nights per week and at least occasional respiratory pauses or (2) respiratory pauses during sleep with a frequency of at least 1–2 nights weekly.<sup>23</sup>

#### *Assessment of sleep quality*

Sleep quality was assessed with the sleep diary. Participants answered the dichotomous questions, “Did you sleep well this night?”, “Do you feel well rested after getting out of bed?” and “Do you have the feeling that the amount of sleep was too little?”. Perceived sleep quality indicates the average of these three questions (range 0-7). Perceived impairment indicates how many days of the week participants felt so tired that it impaired activities during the day.

#### *Assessment of demographics, mental health and lifestyle*

Partnership, employment status, cognitive functioning, depressive symptoms and Body Mass Index (BMI) were routinely collected in the Rotterdam Study. During a home interview all participants were asked about partnership and employment status. Cognitive functioning was measured using the Mini Mental State Exam (MMSE)<sup>24</sup> during one of the visits to our center. Depressive mood was assessed using the Center for Epidemiologic Studies



Depression (CES-D) scale<sup>25</sup> as part of a home interview. Height and weight were measured without shoes and heavy clothing during a center visit to calculate the Body Mass Index (kg/m<sup>2</sup>). Coffee use was defined as the number of days coffee was consumed after 18:00 h per week. Alcohol use indicated how many units of alcohol were used after 18:00 h summed up over the week. Current smoking assessed whether the participant smoked cigarettes, cigars or pipe at the time of the interview.

#### *Assessment of confounders*

Education and general health were studied to control for confounding. Education was assessed routinely in the home interview and subdivided in low, intermediate and high education. As an indicator of general health we assessed the ability to perform activities of daily living (ADL) with the Stanford Health Assessment Questionnaire<sup>26</sup>, questions were answered in a 0 to 3 range.

#### *Analyses*

All parameters were assessed quantitatively, except the use of sleep medication, possible apnea, employment and education. Sleep medication was dichotomized as no use of sleep medication versus any use of sleep medication during the week of actigraphy. As the number of missing values per parameter never exceeded 5%, missing values in quantitative predictors were replaced by the median. For qualitative predictors a separate missing category was used. Interdaily stability, intradaily variability and the actigraphically assessed sleep parameters were winsorized at 4 standard deviations from the mean. To obtain normally distributed values, interdaily stability, intradaily variability, actigraphic sleep onset latency and actigraphic wake after sleep onset were transformed using a Box-Cox transformation<sup>27,28</sup>. Continuous dependent variables were standardized to facilitate comparison.

Correlations between the activity rhythm, sleep parameters and sleep quality parameters were computed using a Pearson Correlation coefficient. A point-biserial correlation was computed to assess the correlation between quantitative and binomial data.

We assessed the relation of the demographic, mental health and lifestyle with interdaily stability and intradaily variability using multivariate linear regression analyses. Analyses included all demographic, mental health and lifestyle parameters and were thus mutually adjusted. The relation between sleep characteristics and interdaily stability and intradaily variability were assessed in a separate model adjusted for demographic, mental health and lifestyle parameters. In addition, we controlled for education and ADL in all models. Analyses were performed using SPSS Statistics (version 20).

## Results

The population characteristics are described in Table 1. Of the total sample of 1734 participants, 53% was female and the mean age was 62.3 years  $\pm$  9.4 years. Participants were generally in good health as indicated by the low average ADL-score ( $0.29 \pm 0.42$ ) and medical records; 2.6% of participants had a cardiovascular accident, 2.0% had a myocardial infarction and 12.9% was diabetic. 11.8% reported having cancer at least once in their lives. The mean interdaily variability was  $.80 \pm .10$ , the mean intradaily variability was  $.42 \pm .14$ . Interdaily stability and intradaily variability were moderately negatively correlated ( $r = -.49$ ,  $p < .01$ ). This negative correlation is also reflected in the mostly inverse association patterns of several demographic, mental health, lifestyle and sleep parameters with intradaily variability and interdaily stability.

Table 2 shows the association of demographics, mental health and lifestyle with the stability and fragmentation of the activity rhythm. All analyses were fully adjusted, thus the different possible determinants were mutually corrected for the other risk factors. Older age was associated with a high interdaily stability ( $\beta = .07$ ,  $p = .020$ ), i.e. more stable rhythms. Male gender ( $\beta = -.11$ ,  $p < .001$ ) and being employed ( $\beta = -.11$ ,  $p = .001$ ) were related to a low interdaily stability. Persons with better cognitive functioning ( $\beta = .08$ ,  $p = .001$ ) and less depressive symptoms ( $\beta = -.07$ ,  $p = .003$ ) were more likely to have a high interdaily stability. A high Body Mass Index ( $\beta = -.11$ ,  $p < .001$ ) and smoking ( $\beta = -.12$ ,  $p < .001$ ) were associated with less interdaily stability. Individuals who had a high coffee intake ( $\beta = .09$ ,  $p < .001$ ) had a more stable rhythm, reflected in a high interdaily stability. Additional adjustment for possible apnea did not change the results for Body Mass Index and the stability ( $\beta = -.10$ ,  $p < .001$ ) of the activity rhythm.

Next, we studied the associations of demographics, lifestyle and mental health indicators with the intradaily variability, i.e. the fragmentation of the rhythm (see also table 2). Older age ( $\beta = .20$ ,  $p < .001$ ) was associated with more intradaily variability, thus a high interdaily stability was accompanied by high intradaily variability for older age. In contrast, female gender ( $\beta = -.13$ ,  $p < .001$ ) and being employed ( $\beta = -.07$ ,  $p = .010$ ) were associated with a low intradaily variability. Persons with depressive symptoms ( $\beta = .10$ ,  $p < .001$ ) were more likely to have a high intradaily variability. A high Body Mass Index ( $\beta = .09$ ,  $p < .001$ ) and smoking ( $\beta = .11$ ,  $p = .001$ ) were also related to a high intradaily variability. Additional adjustment for possible apnea did not change the effects of Body Mass Index on intradaily variability ( $\beta = .08$ ,  $p < .001$ ). In summary, findings for intradaily variability corresponded to those observed for the interdaily stability (i.e. reversed direction of association), except for age and employment.

**Table 1** Population characteristics\*, N=1734

<b>Demographic</b>		
	Female, %	53.4
	Age (years)	62.25 ± 9.35
	Partner, %	77.3
	Employment, %	33.1
	Education, %	
	Low	15.2
	Intermediate	63.3
	High	19.7
	ADL (score)	0.29 ± 0.42
<b>Health indicators</b>		
	Cognitive functioning (score)	27.98 ± 1.75
	Depressive symptoms (score)	5.49 ± 7.13
	Stroke, %	2.6
	Myocardial infarction, %	2.0
	Cancer, %	11.8
	Diabetes Mellitus, %	12.9
<b>Lifestyle</b>		
	BMI (score)	27.86 ± 4.16
	Coffee (days per week)	4.36 ± 2.91
	Alcohol (units per week)	9.47 ± 9.34
	Current smoking, %	20.6
<b>Subjectively assessed sleep<sup>†</sup></b>		
	Sleep medication, %	14.5
	Possible apnea, %	29.7
	Sleep onset latency (minutes)	17.72 ± 11.74
	Total sleep time (hours)	6.85 ± .95
	Awakenings after sleep onset <sup>†</sup> (number)	1.51 ± 1.10
	Napping (days per week)	1.66 ± 2.05
<b>Objectively assessed sleep<sup>‡</sup></b>		
	Sleep onset latency (minutes)	14.56 ± 12.39
	Total sleep time (hours)	6.38 ± 0.87
	Wake after sleep onset (minutes)	69.46 ± 25.88
<b>Sleep quality<sup>†</sup></b>		
	Perceived sleep quality (average per week)	5.54 ± 1.61
	Perceived impairment (days per week)	.75 ± 1.44
<b>Activity rhythm<sup>‡</sup></b>		
	Interdaily stability (score)	.80 ± .10
	Intradaily variability (score)	.42 ± .13

\*Mean ± SD, unless stated otherwise

<sup>†</sup>Assessed on daily basis within one week of actigraphy through self-report<sup>‡</sup>Assessed by one week of actigraphy

**Table 2** Associations of demographics, mental health and lifestyle with interdaily stability and intradaily variability\*

	Interdaily Stability				Intradaily Variability			
	B	SE of B	$\beta$	p-value	B	SE of B	$\beta$	p-value
<b>Demographic</b>								
Age	.01	.00	.07	.019	.02	.00	.20	<.001
Sex (ref=men)	.21	.05	.11	<.001	-.27	.05	-.14	<.001
Partner (ref=no)	.19	.06	.08	.002	-.13	.06	-.06	.024
Employment (ref=no)	-.23	.06	-.11	<.001	-.15	.06	-.07	.011
<b>Mental Health</b>								
Cognitive functioning	.05	.01	.08	.001	-.02	.01	-.04	.08
Depressive symptoms	-.01	.00	-.07	.005	.01	.00	.10	<.001
<b>Lifestyle</b>								
BMI	-.03	.01	-.11	<.001	.02	.01	.09	<.001
Coffee (days/week)	.03	.01	.09	<.001	-.02	.01	-.04	.07
Alcohol (units/week)	-.01	.00	-.05	.06	-.00	.00	-.03	.25
Current smoking (ref=no)	-.27	.06	-.11	<.001	.26	.06	.11	<.001

\*Multivariate linear regression analyses mutually adjusted for sex, age, partner, employment, education, ADL, cognitive functioning, depressive symptoms, BMI, coffee use, alcohol use and current smoking.

Several indicators of poor sleep were consistently associated with less interdaily stability and more intradaily variability (see table 3). More daytime napping ( $\beta=-.25$   $p<.001$ ), use of sleep medication ( $\beta=-.09$ ,  $p<.001$ ), and less subjective total sleep time ( $\beta=.07$ ,  $p=.003$ ) were all associated with less interdaily stability in the analyses fully adjusted for demographic, mental health and lifestyle parameters. Sleep parameters assessed by actigraphy had stronger associations with the stability of the rhythm; less objective total sleep time ( $\beta=-.15$   $p<.001$ ), longer objective sleep onset latency ( $\beta=.25$   $p<.001$ ) and more objective wake after sleep onset ( $\beta=-.15$   $p<.001$ ) were all related to less stable rhythms. Persons who perceived their sleep as good ( $\beta=.08$ ,  $p<.001$ ) and who experienced less impairment due to tiredness ( $\beta=-.11$ ,  $p<.001$ ) had a high interdaily stability. In line with these results, associations of the determinants with intradaily variability mostly showed a reverse pattern (see table 3). Possible apnea was neither related to interdaily stability ( $\beta=-.02$   $p=.37$ ) or intradaily variability ( $\beta=.03$ ,  $p=.21$ ) after full adjustment including BMI.

**Table 3** Associations of sleep characteristics and sleep quality with interdaily stability and intradaily variability\*

	Interdaily Stability				Intradaily Variability			
	B	SE of B	$\beta$	p-value	B	SE of B	$\beta$	p-value
<b>Sleep disorders</b>								
Sleep medication (ref=no)	-.26	.07	-.09	<.001	.16	.07	.06	.014
Possible apnea (ref=no)	-.04	.06	-.02	.51	.07	.05	.03	.21
<b>Subjectively assessed sleep</b>								
Sleep onset latency	.00	.00	.01	.34	.00	.00	.00	.96
Total sleep time	.07	.03	.07	.003	-.11	.02	-.10	<.001
Awakenings after sleep onset	.02	.02	.02	.34	.05	.02	.05	.024
Napping	-.12	.01	-.25	<.001	.19	.01	.39	<.001
<b>Objectively assessed sleep</b>								
Sleep onset latency	-.22	.04	-.15	<.001	.12	.04	.08	.003
Total sleep time	.29	.03	.25	<.001	-.33	.03	-.28	<.001
Wake after sleep onset	-.06	.01	-.15	<.001	.08	.01	.18	<.001
<b>Sleep quality</b>								
Perceived sleep quality	.05	.02	.08	.001	-.04	.02	-.07	.006
Perceived impairment	-.08	.02	-.11	<.001	.07	.02	.10	<.001

\*Multivariate linear regression analyses adjusted for sex, age, partner, employment, education, ADL, cognitive functioning, depressive symptoms, BMI, coffee use, alcohol use and current smoking.

## Discussion

In a large population-based sample of middle aged and elderly persons, we assessed activity rhythms across the sleep-wake cycle. Older age was related to a more stable, but also to a more fragmented activity rhythm. Several demographic and lifestyle factors were associated with less stability and more fragmentation of the rhythm. The relation of a high Body Mass Index and smoking with less stable and more fragmented rhythms was most pronounced. In addition, sleep estimates derived from actigraphy had a consistent association with the stability and fragmentation of the rhythm.

Older age was associated with more stable rhythms, yet with more fragmented rhythms in middle aged and elderly persons, independent of several demographic, mental health and lifestyle factors. More fragmented rhythms in older participants are possibly explained by morbidity. For example, disrupted circadian organization of activity rhythms have been related to cerebral changes,<sup>29</sup> cardiovascular disease<sup>6</sup> and mortality.<sup>30</sup> Possibly the fragmentation of the rhythm is a nonspecific health indicator and reflects the presence of different clinical and subclinical diseases. In contrast, the positive association between older age and more stable rhythms can better be explained by health-related behavior – as non-optimal health itself should not stabilize the circadian rhythm. Non-optimal health might underlie a stable rhythm in old age as disease necessitates certain routines. Moreover, adjustment for indicators of disease did not attenuate the association of age with the

stability of the activity rhythm. Secondly, the observed changes in circadian rhythms with age could be due to changes in behavior. For example, older age is most likely accompanied by lower activity levels. Lower levels of activity can lead to more fragmentation due to more awakenings during the night and naps during the day. In addition, the elderly are more stringent in their daily structure, as they adhere to more stable routines than younger persons, who tend to be more flexible with bedtimes. Lastly, more fragmented rhythms with older age could be due to biological aging. The aging process is known to be accompanied by biological changes which can disturb the circadian rhythm. The suprachiasmatic nucleus, which represents the biological clock of the brain, shows functional changes with age<sup>1</sup> which have been related to more fragmented rhythms in a postmortem study on demented elderly people.<sup>31</sup> Importantly, the relation between aging and the circadian organization of the sleep-wake cycle is assumed to be bidirectional. Factors associated with the sleep-wake cycle can lead to more disturbed circadian rhythms, but a disturbed circadian rhythm can also increase changes in mental health and lifestyle.

Our study also suggests that lifestyle is important for rhythm disturbances. A high Body Mass Index was associated with a less stable and a more fragmented activity rhythm, which indicates a disturbed circadian organization of the sleep-wake cycle. As a cross-sectional design does not allow assessment of the direction of the effect, we can only infer carefully that Body Mass Index influences the activity rhythm. This association might be due to breath-related diseases such as apnea, which is known to be more prevalent in persons with a high Body Mass Index and can disturb sleep severely. In our study, possible apnea did not explain the association between Body Mass Index and the activity rhythm. Smokers were less likely to have more stable and less fragmented rhythms. Since smoking in elderly persons is mostly a longstanding addictive behavior, it is more likely to be a cause of poor sleep than to be induced by poor sleep in middle and old age. Pressure to smoke accumulates during sleep as the hours of not smoking lead to withdrawal effects which can disturb the sleep-wake cycle. Smoking might be an amenable determinant of rhythm disturbances if this behavior can be lastingly changed. Coffee use after 18:00h was related to less fragmentation of the rhythm. Earlier population-based studies found the opposite; high daily caffeine intake was related to poor sleep.<sup>10</sup> This could be due to the different assessed times of intake, but can also reflect behavioral adaptations. Participants with a poor circadian rhythm probably have reduced their caffeine intake, since caffeine is widely known as a wake promoting agent and not as addictive as smoking. Only persons resilient to the effects of coffee on sleep may uphold the habit to drink coffee in the evening.

Cognitive status was associated with the stability of the activity rhythm, but not with the fragmentation. Stable activity rhythms and good global cognitive functioning may be indicators of a healthy brain. A previous study found that cognitive functioning was not related to the stability of the rhythm, while it was related to the fragmentation of the sleep-

wake cycle.<sup>12</sup> However, this study focused specifically on executive functioning, while in our study we assessed a global indicator of cognition. Depressive symptoms were related with both the stability and the fragmentation of the rhythm, which is as expected since disturbed sleep is one of the DSM-IV criteria for depression. In addition, in several studies depression has been linked to disturbed circadian rhythms<sup>11,13</sup> and even more extensively to sleep characteristics.<sup>32</sup>

Sleep characteristics were consistently related to circadian rhythm in our study. Of the subjectively assessed sleep-related behaviors, napping had a particularly strong association with stability and fragmentation. Napping is inherently related to intradaily variability, but not necessarily to the stability of the rhythm. Shorter sleep onset latency, more total sleep time and less wake after sleep onset were, when assessed with actigraphy, associated with more stable and less fragmented rhythms. This confirms the strong relation between sleep and the circadian organization of the activity rhythm.

The current study was embedded in an existing population-based study; this allowed us to assess a large number of variables and makes our results generalizable. Lastly, we used non-parametric measures of the activity rhythm, instead of more commonly used parametric indicators. The main advantage of a non-parametric indicator is that it does not make assumptions about the nature of the rhythm, which is problematic in elderly populations with less pronounced circadian rhythms. However there are some limitations that should be considered. Collection of sleep-related data was limited on sleep disorders, such as apnea or restless legs, which could have been possible confounders in our study. In addition, our study was cross-sectional, therefore we can only carefully infer temporal effects. Lastly, effect sizes were, although significant, sometimes small in this large population-based study. However, effect sizes in population-based samples tend to be smaller than in case-control or clinical studies which compare more extreme groups.

We conclude that with older age the circadian organization of the sleep-wake cycle is more fragmented, but also more stable. The fragmentation of the rhythm is more related to health and biological processes, whereas the stability of the rhythm seems to be driven by behavior. In addition, mental health and lifestyle factors, in particular smoking and a high Body Mass Index, are important for the circadian rhythm. Known risk factors for common disease in middle aged and elderly persons were associated with disturbances in the circadian organization of the sleep-wake cycle. This strengthens the hypothesis that disturbances in circadian rhythms, more specifically disturbances in the activity rhythm, can be seen as sensitive markers of the effects of general aging.<sup>33</sup> Future studies must show if changing our lifestyle is a way to reduce circadian disturbances across the sleep-wake cycle; this needs to be assessed longitudinally.

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# Chapter 2.2

## **Fragmentation and stability of circadian activity rhythms predict mortality: The Rotterdam study**

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## Abstract

Circadian rhythms and sleep patterns change as people age. Little is known about the associations between circadian rhythms and mortality rates. We investigated whether 24-hour activity rhythms and sleep characteristics independently predicted mortality. Actigraphy was used to determine the stability and fragmentation of the 24-hour activity rhythm in 1,734 persons (aged 45–98 years) from the Rotterdam Study (2004–2013). Sleep was assessed objectively using actigraphy and subjectively using sleep diaries to estimate sleep duration, sleep onset latency, and waking after sleep onset. The mean follow-up time was 7.3 years; 154 participants (8.9%) died. Sleep measures were not related to mortality after adjustment for health parameters. In contrast, a more stable 24-hour activity rhythm was associated with a lower mortality risk (per 1 standard deviation, hazard ratio = 0.83, 95% confidence interval: 0.71, 0.96), and a more fragmented rhythm was associated with a higher mortality risk (per 1 standard deviation, hazard ratio = 1.22, 95% confidence interval: 1.04, 1.44). Low stability and high fragmentation of the 24-hour activity rhythm predicted all-cause mortality, whereas estimates from actigraphy and sleep diaries did not. Disturbed circadian activity rhythms reflect age-related alterations in the biological clock and could be an indicator of disease.

## Introduction

Most physiological processes, including body temperature, hormone secretion, and sleep-wake timing, are regulated in cycles that last approximately 24 hours, called circadian rhythms. Circadian rhythms and sleep patterns change as people age.<sup>1</sup> Elderly people sleep less during the night, have more fragmented sleep, have more difficulty in falling asleep, tend to fall asleep and wake up earlier, take more naps, and report a lower sleep quality.<sup>2-6</sup> The longitudinal associations of these changes with adverse health consequences and mortality are not well understood. In previous studies, investigators found that people who slept for short durations each night ( $\leq 6$  hours) and had poor sleep quality had higher risks of diabetes and cardiovascular diseases.<sup>7,8</sup> Studies in which the association between sleep and mortality have been investigated were mainly focused on sleep duration. The results suggested that the association between sleep duration and mortality is U-shaped; both subjective short and long sleep durations are predictors of all-cause mortality.<sup>9,10</sup>

Few studies have investigated the associations of circadian rhythms with mortality. In the elderly, the amplitude of several physiological circadian rhythms is reduced compared with that of younger people, as is the stability of the day-night rhythm.<sup>1,4,11</sup> This could be explained by an age-related decline in circadian organization. The aging process affects central and peripheral oscillators differently, possibly leading to suboptimal peripheral physiological functioning.<sup>12</sup> The circadian rhythm of physical activity in elderly is better characterized by 2 nonparametric variables that do not assume the 24-hour cosine-like shape that is present in, for example, core body temperature and hormones. Two nonparametric variables quantify stability and fragmentation. A stable activity rhythm is characterized by a 24-hour profile that remains very similar from day to day. This gives an indication of the strength of synchronization between the activity rhythm and zeitgebers, which are environmental cues with a 24-hour pattern. Fragmentation gives an indication of the frequency of alterations between rest and activity relative to its 24-hour amplitude.

In 2 previous studies in which 24-hour activity rhythms were analyzed parametrically, investigators found that older men and women with the least robust 24-hour activity rhythms had a 1.5–2-times higher risk of all-cause mortality.<sup>13,14</sup> In addition, abnormal sleep-wake cycles were associated with a 3-times higher mortality rate in elderly persons older than 85 years of age.<sup>15</sup> These studies assessed 24-hour activity rhythms in very old persons and had relatively short follow-up periods (average follow-up of 4.1, 3.5, and 2 years, respectively). In these activity rhythm and mortality studies, a few subjective sleep parameters were taken into account. One study considered sleep medication and disturbed sleep due to pain as potential confounders.<sup>14</sup> Anderson et al.<sup>15</sup> assessed sleep quality using the Pittsburgh Sleep

Quality Index (PSQI) and daytime sleepiness using the Epworth Sleepiness Scale in relation to mortality but did not find an association. However, none of these studies accounted for objectively assessed sleep characteristics, such as sleep duration, sleep onset latency, and waking after sleep onset. In a large population-based study with a longer follow-up period, we aimed to evaluate the association between nonparametric measures of the 24-hour activity rhythm, stability, and fragmentation, and mortality in middle-aged and elderly people. We also ran analyses in which we excluded deaths that occurred in the first 2 years to minimize the risk of reversed causality. Furthermore, actigraphic and subjective sleep diary estimates of sleep duration, sleep onset latency, waking after sleep onset, and sleep quality were studied in relation to mortality to investigate whether circadian rhythms and sleep characteristics predicted mortality independently.

## Methods

### *Participants*

The present study was conducted within the Rotterdam Study, a prospective study of persons 45 years of age or older living in Rotterdam, The Netherlands, that started in 1990. The aim of the study was to examine the incidence of and risk factors for neurological, cardiovascular, psychiatric, and other chronic diseases. Details of the study have been published previously.<sup>16</sup> The Rotterdam Study was approved by the medical ethics committee according to the Wet Bevolkingsonderzoek ERGO (Population Study Act Rotterdam Study), executed by the Ministry of Health, Welfare and Sports of the Netherlands, and conforms to the Declaration of Helsinki. All participants provided written informed consent.

From December 2004 to April 2007, we invited 2,632 successive participants to participate in the actigraphy study; of these, 2,063 (78%) agreed. There were no exclusion criteria besides being able to understand the instructions for this study. After exclusion of recordings that failed because of technical problems or that contained fewer than 4 consecutive days and nights, 1,734 (84%) recordings (mean recording duration, 138 (standard deviation (SD), 14) hours) were available for analyses.<sup>17</sup> No participants were lost to follow-up.

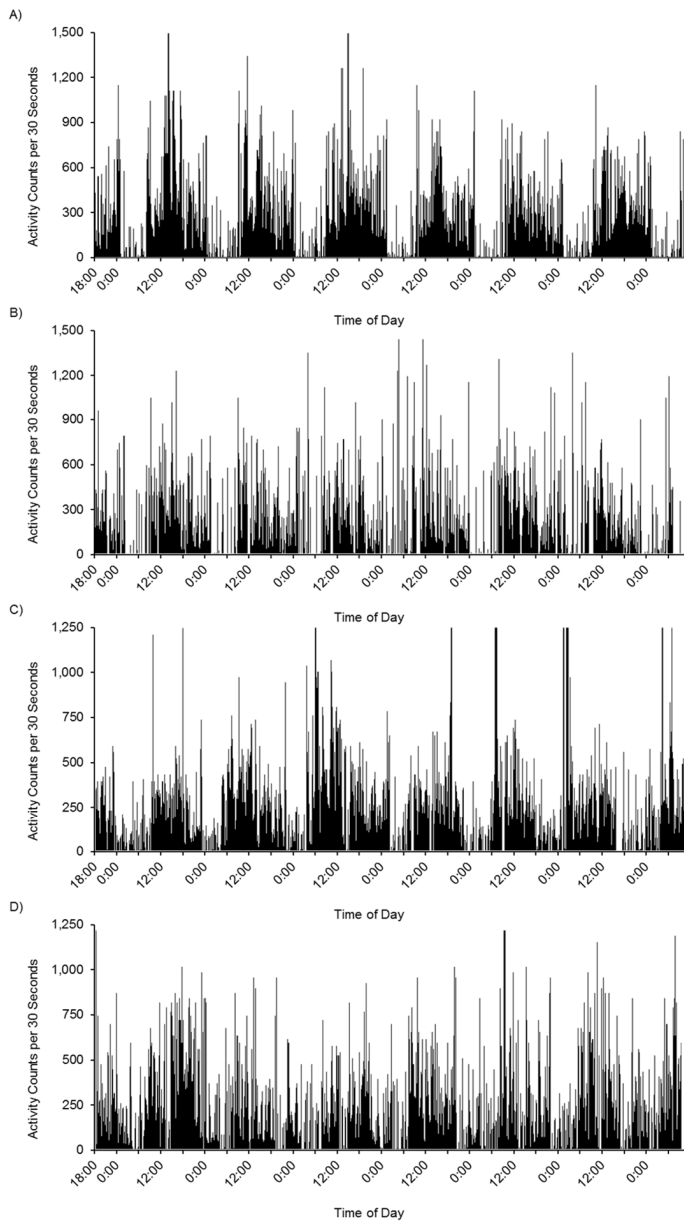
### *Actigraphy*

We measured the 24-hour activity rhythm using an actigraph unit (Actiwatch model AW4, Cambridge Technology Ltd., Cambridge, United Kingdom) worn on each subject's nondominant wrist, as described previously.<sup>18</sup> In brief, participants were asked to wear the actigraph unit for 7 consecutive days and nights and to remove it only before bathing. Recordings were obtained in 30-second intervals. Because the elderly can have less distinct

circadian rhythms, subjects' 24-hour activity rhythms were analyzed nonparametrically; thus, no assumptions were made about the underlying shape of the circadian rhythm. The actigraph unit was used to calculate two 24-hour activity rhythm variables (interday stability and intraday variability<sup>19</sup>) and 3 sleep variables (sleep duration, sleep onset latency, and waking after sleep onset<sup>20</sup>). Interday stability is a measure of how stable the rhythm is over multiple days, that is, how similar the individual day-night patterns are over time. It is calculated as the ratio of the variance of the average activity patterns around the mean and the overall variance.<sup>21</sup> Intraday variability reflects the fragmentation of the rhythm, that is, the rate of shifting between rest and activity. It is calculated as the ratio between the mean squares of the difference between all successive hours (first derivative) and the mean squares around the grand mean.<sup>21</sup> The variables have been shown to be sensitive in observational and experimental studies on aging.<sup>22,23</sup> Examples of activity rhythms characterized by a high or low stability and fragmentation are given in Figure 1.

### *Sleep diaries*

During the week of actigraphy assessment, each participant kept a sleep diary comprising data on sleep characteristics, sleep quality, use of sleep medications, napping, and alcohol consumption. To assess subjective sleep duration and sleep onset latency, participants were asked to answer the questions, "In total, how many hours did you sleep last night?" and "How long did it take you to fall asleep?" We averaged a weeks' worth of daily values. Sleep quality was measured using 3 dichotomous questions: "Do you think you slept well last night?", "Do you think the amount of sleep was not enough?", and "Did you feel rested after getting out of bed?" We reverse-coded the answers to the second question so that a score of 1 indicated good sleep quality. The score for sleep quality was created by summing the 3 dichotomous questions assessed each day (range, 0–3), taking the daily average of this score (range, 0–1), summing these scores over the days of participation, and taking into account the total number of days a person participated (range, 0–7). Higher scores represent a better sleep quality. Each day, participants specified which sleep medications were used, if any. In all analyses, use of sleep medication was dichotomized into no use of sleep medication or any use of sleep medication during the week of actigraphy. Napping was evaluated by asking whether the participant had taken 1 or more naps. The total number of days during which the participant took a nap, adjusted for the total number of days for which the participant contributed data, was used in analyses. Alcohol consumption was evaluated as the sum of cups of alcohol after 18:00 hours on day 1 in the week of actigraphy.



**Figure 1** Four examples of activity plots, the Rotterdam Study, the Netherlands, 2004–2007. Plots show activity rhythms of participants from the Rotterdam Study. The x-axis represents time (0:00, midnight; 12:00, noon) and the y-axis represents activity counts per 30 seconds. Participants started wearing the actigraph unit at 18:00 hours on day 1. These activity counts are scaled relative to the individual means and cannot be compared easily across persons. A) Activity rhythm with high stability and low fragmentation in a 73-year-old man; B) activity rhythm with high fragmentation in an 84-year-old woman; C) activity rhythm with low stability in a 47-year old man; D) activity rhythm with low stability and high fragmentation in a 62-year-old man.

*Pittsburgh Sleep Quality Index*

The PSQI was used to measure subjective sleep quality (global PSQI score) and possible sleep apnea.<sup>24</sup> Higher scores represent poorer sleep. We considered participants to have sleep apnea if they reported that they snored loudly at least 2 nights per week and if they reported occasional respiratory pauses or respiratory pauses during sleep at least 1–2 nights per week.<sup>25</sup>

*Assessment of outcome*

Records of general practitioners and hospitals were used to continuously assess death from any cause. In addition, information on vital status was acquired bimonthly from death certificates from the municipality. The number of person-years was calculated from the date of actigraphy start to the date of death or end of follow-up on September 27, 2013. The mean follow-up time was 7.3 years.

*Covariates*

We assessed the following variables as possible confounders based on previous literature<sup>14,17</sup>: sex, age, use of sleep medication, possible sleep apnea, napping, activities of daily living (ADL), educational level, cognitive functioning, depressive symptoms, body mass index, employment status, current smoking, alcohol consumption, myocardial infarction, diabetes, and stroke. Use of sleep medication, napping, and alcohol consumption were estimated using the sleep diaries. Information on possible sleep apnea, ADL, educational level, depressive symptoms, employment status, and current smoking (cigarettes, cigars, or pipe) was obtained in a home interview. ADL were measured with the Stanford Health Assessment Questionnaire and were used to indicate general health.<sup>26</sup> Depressive symptoms were measured using the Center for Epidemiologic Studies-Depression scale.<sup>27</sup> During a visit to our research center, cognitive functioning was measured using the Mini Mental State Examination<sup>28</sup>; height and weight were measured with the participants wearing light clothing and no shoes to calculate body mass index (weight (kg)/height (m<sup>2</sup>)). Myocardial infarction, diabetes, and stroke were determined using medical records.

*Statistical analyses*

The number of missing values for a variable never exceeded 5% (the maximum amount of missing data was 4.2% for ADL). Missing values for continuous variables were replaced with the median, and missing values for categorical variables were put into a separate category. Interday stability and intraday variability were standardized and Winsorized at 4 standard deviations from the mean. We analyzed the curvilinear association between sleep duration and mortality by adding a squared term of sleep duration to the model. In addition, we tested a nonlinear association by defining 3 categories of sleep duration (<6, 6–7.5 (reference group), and >7.5 hours).



**Table 1** Baseline Characteristics by Status at End of Follow-up in 1,734 Men and Women, Rotterdam Study, the Netherlands, 2004-2007

	Status at end of follow-up			Test Statistic <sup>a</sup>
	Total (n=1734)	Alive (n = 1580)	Dead (n = 154)	
	Mean (SD) N(%)	Mean (SD) N(%)	Mean (SD) N(%)	
Age (years)	62.2 (9.3)	61.2 (8.6)	72.6 (10.4)	-13.1 <sup>d</sup>
Gender (male)	808 (46.6%)	723 (45.8%)	85 (55.2%)	5.0 <sup>b</sup>
Employment (yes)	574 (33.1%)	558 (35.3%)	16 (10.4%)	39.9 <sup>d</sup>
Education: low	264 (15.2%)	227 (14.4%)	37 (24.0%)	
intermediate	1097 (63.3%)	1000 (63.3%)	97 (63.0%)	14.5 <sup>c</sup>
high	341 (19.7%)	322 (20.4%)	19 (12.3%)	
Activities of daily living (score)	0.29 (0.4)	0.25 (0.4)	0.66 (0.6)	-8.3 <sup>d</sup>
Cognitive functioning (score)	28.0 (1.8)	28.0 (1.7)	27.3 (1.9)	5.0 <sup>d</sup>
Depressive symptoms (score)	5.5 (7.1)	5.4 (7.0)	6.6 (7.9)	-2.1 <sup>b</sup>
Myocardial infarction (yes)	67 (3.9%)	52 (3.3%)	15 (9.7%)	15.7 <sup>d</sup>
Diabetes (yes)	205 (11.8%)	167 (10.6%)	38 (24.7%)	26.8 <sup>d</sup>
Stroke (yes)	45 (2.6%)	30 (1.9%)	15 (9.7%)	34.1 <sup>d</sup>
Body mass index <sup>e</sup>	27.9 (4.2)	27.9 (4.1)	27.6 (4.1)	0.82
Current smoking (yes)	358 (20.6%)	328 (20.8%)		0.29
Alcohol (cups per week)	5.7 (1.3)	5.8 (7.3)	5.3 (6.9)	0.80
Interday stability (score)	0.80 (0.1)	0.80 (0.1)	0.77 (0.1)	3.2 <sup>c</sup>
Intraday variability (score)	0.43 (0.1)	0.42 (0.1)	0.52 (0.2)	-7.7 <sup>d</sup>
Sleep duration (hours)	6.4 (0.9)	6.4 (0.9)	6.4 (1.0)	-0.58
Sleep duration: <6 hours	523 (30.2%)	480 (30.4%)	43 (27.9%)	
6-7.5 hours	1069 (61.6%)	977 (61.8%)	92 (59.7%)	3.9
>7.5 hours	142 (8.2%)	123 (7.8%)	19 (12.3%)	
Sleep onset latency (minutes)	14.6 (12.4)	13.8 (11.8)	22.6 (14.7)	-8.6 <sup>d</sup>
Wake after sleep onset (minutes)	69.5 (25.9)	68.9 (25.2)	74.5 (30.4)	-2.6 <sup>b</sup>
Sleep medication (yes)	252 (14.5%)	218 (13.8%)	34 (22.1%)	8.6 <sup>b</sup>
Apnea (yes)	507 (29.2%)	448 (28.4%)	59 (38.3%)	6.8 <sup>b</sup>
Napping (days per week)	1.7 (2.0)	1.6 (2.0)	2.7 (2.4)	-5.6 <sup>d</sup>
Sleep duration: <6 hours	277 (16.0%)	244 (15.4%)	33 (21.4%)	4.1
6-7.5 hours	1048 (60.4%)	964 (61.0%)	84 (54.5%)	
>7.5 hours	409 (23.6%)	372 (23.5%)	37 (24.0%)	
Sleep onset latency (minutes)	17.7 (11.7)	17.7 (11.7)	17.8 (11.7)	-0.09
Sleep quality sleep diary (score)	5.5 (1.6)	5.6 (1.6)	5.5 (1.6)	0.74
Global PSQI score <sup>§</sup>	3.7 (3.5)	3.6 (3.5)	3.9 (3.7)	-0.78
Poor sleep (PSQI score >5)	398 (23.0%)	353 (22.3%)	45 (29.2%)	-1.80

Abbreviations: PSQI, Pittsburgh Sleep Quality Index; SD, standard deviation.

<sup>a</sup> Statistical test to compare the means of the alive or dead status at end of follow-up; t-test for continuous,  $\chi^2$  for categorical variables.

<sup>b</sup>  $P < 0.05$ .

<sup>c</sup>  $P < 0.01$ .

<sup>d</sup>  $P < 0.001$ .

<sup>e</sup> Weight (kg)/length (m)<sup>2</sup>.

Cox proportional hazards models were used to determine the hazard ratios and 95% confidence intervals for the associations of circadian rhythm and sleep parameters with mortality. We included a covariate in the model if it changed the estimate of the main determinants by more than 10%, if the covariate predicted mortality ( $P < 0.05$ ), or if it was an important a priori confounder. On the basis of these criteria, educational level, employment status, and alcohol consumption were not included in the full model. We tested 2 different models. Model 1 was adjusted for age and sex. Model 2 was adjusted for age, sex, ADL, current smoking, diabetes, myocardial infarction, stroke, cognitive functioning, depressive symptoms, body mass index, use of sleep medication, possible sleep apnea, and napping. All statistical tests were 2-sided, and a  $P$  value  $< 0.05$  was considered statistically significant. We tested the proportional hazards assumption using Schoenfeld residuals.<sup>29</sup> The residuals did not significantly deviate from zero slope. Analyses were performed in SPSS, version 20 (SPSS Inc., Chicago, Illinois).

## Results

### *Baseline characteristics*

The average follow-up time for the 1,734 participants was 7.3 (SD, 1.3) years. The mean age was 62.2 (SD, 9.3) years, and 47% of subjects were male. In total, there were 154 deaths (8.9%) during the follow-up period. The participants' baseline characteristics stratified whether they were alive at the end of follow-up are summarized in Table 1.

Interday stability and intraday variability were moderately correlated ( $r = -0.49$ ,  $P < 0.001$ ). The global PSQI score was moderately correlated with sleep quality as assessed using a sleep diary ( $r = -0.45$ ,  $P < 0.001$ ). Circadian rhythm and sleep variables were only weakly to mildly correlated, with the highest correlation between interday stability and objective sleep duration ( $r = 0.31$ ,  $P < 0.001$ ). All correlations between 24-hour activity rhythm and sleep parameters can be found in Supplemental Table 1.

### *Cox proportional hazards model*

Both circadian rhythm variables were significantly related to mortality (Table 2). After full adjustment, interday stability was associated with a lower mortality risk (per SD, hazard ratio (HR) = 0.83, 95% confidence interval (CI): 0.71, 0.96), and intraday variability (i.e., fragmentation) was associated with a higher mortality risk (per SD, HR = 1.22, 95% CI: 1.04, 1.44). To show the cumulative survival graphically, interday stability and intraday variability were divided into quartiles (Figure 2). Because interday stability and intraday variability were moderately correlated, we also analyzed a model adjusted for age and sex that included

both 24-hour activity rhythm variables (per SD of intraday variability, HR = 1.25, 95% CI: 1.07, 1.47; per SD of interday stability, HR = 0.84, 95% CI: 0.71, 1.00).

Actigraphically measured sleep onset latency and waking after sleep onset were marginally related to mortality in an age- and sex-adjusted model, but these associations were nonsignificant in the fully adjusted analysis (per minute of sleep onset latency, HR = 1.01, 95% CI: 1.00, 1.02; per minute of waking after sleep onset, HR = 1.01, 95% CI: 1.00, 1.01). The actigraphic estimates of sleep duration, both continuous and categorical, were not related to mortality. Subjective sleep duration was quadratically associated with mortality in the age- and sex-adjusted model. However, this association was not significant after further adjustment (per hour, HR = 1.07, 95% CI: 0.97, 1.17). When different cut-offs were chosen to categorize objective and subjective sleep duration, the results did not change meaningfully (results available upon request). Other subjective sleep parameters were not related to mortality (Table 2).

### *Sensitivity analysis*

Because circadian rhythms can be influenced by undiagnosed morbidity, we also ran the analyses excluding deaths that occurred in the first year and in the first 2 years after the week of actigraphy. These exclusions reduce the possible effect of reversed causality. In these analyses, 148 and 128 deaths occurred during the remaining follow-up periods, respectively. In the fully adjusted model that excluded deaths in the first year, the observed hazard ratio was essentially unchanged compared with the previous analyses in which all participants were included (per SD of interday stability, HR = 0.83, 95% CI: 0.71, 0.97; per SD of intraday variability, HR = 1.23, 95% CI: 1.05, 1.45). Again, very similar results were observed when deaths that occurred in the first 2 years were excluded (per SD of interday stability, HR = 0.86, 95% CI: 0.73, 1.02; per SD of intraday variability, HR = 1.18, 95% CI: 0.98, 1.41).

## **Discussion**

In the present prospective, population-based cohort study, more fragmented and less stable 24-hour activity rhythms were associated with a 20% increase in all-cause mortality risk in a middle-aged and elderly population. These associations remained after adjustment for health parameters, possible sleep apnea, and napping. After adjustment for age and sex only, subjective sleep duration showed a U-shaped marginal association with mortality risk. However, after full adjustment, no sleep parameter, whether estimated objectively using an actigraph unit or subjectively using a sleep diary, predicted all-cause mortality. This suggests that although the circadian rhythm and sleep both change during aging, the circadian rhythm is independently related to mortality.

**Table 2.** Associations of 24-Hour Activity Rhythm and Sleep Parameters With All-Cause Mortality in 1,734 Men and Women, Rotterdam Study, the Netherlands, 2004-2013

	All-Cause Mortality			
	Age & gender adjusted		Fully adjusted <sup>a</sup>	
	HR	95% CI	HR	95% CI
<b>Activity rhythm</b>				
Interday stability (score)	0.75 <sup>c</sup>	0.65, 0.86	0.83 <sup>b</sup>	0.71, 0.96
Intraday variability (score)	1.37 <sup>c</sup>	1.20, 1.57	1.22 <sup>b</sup>	1.04, 1.44
<b>Objectively assessed sleep</b>				
Continuous sleep duration				
Sleep duration (hours)	0.42	0.11, 1.57	0.69	0.19, 2.53
Sleep duration squared (hours <sup>2</sup> )	1.06	0.95, 1.18	1.02	0.92, 1.13
Categorical sleep duration				
<6 hours	1.29	0.89, 1.87	1.12	0.77, 1.65
6-7.5 hours (reference)	0.00		0.00	
>7.5 hours	1.33	0.81, 2.18	1.18	0.70, 1.98
Sleep onset latency (minutes)	1.01 <sup>b</sup>	1.00, 1.02	1.01	1.00, 1.02
Wake after sleep onset (minutes)	1.01 <sup>b</sup>	1.00, 1.01	1.01	1.00, 1.01
<b>Subjectively assessed sleep</b>				
Continuous sleep duration				
Sleep duration (hours)	0.23 <sup>b</sup>	0.07, 0.74	0.40	0.11, 1.39
Sleep duration squared (hours <sup>2</sup> )	1.12 <sup>b</sup>	1.02, 1.22	1.07	0.97, 1.17
Categorical sleep duration				
<6 hours	1.45	0.97, 2.17	1.41	0.93, 2.13
6-7.5 hours (reference)	0.00		0.00	
>7.5 hours	1.13	0.77, 1.67	1.10	0.74, 1.64
Sleep onset latency (minutes)	0.99	0.98, 1.01	0.99	0.98, 1.01
Sleep quality sleep diary (score)	0.92	0.83, 1.02	0.97	0.87, 1.09
Sleep quality PSQI (score)	1.01	0.97, 1.06	0.99	0.94, 1.04
Poor sleep (PSQI score >5)	1.28	0.90, 1.83	1.13	0.76, 1.69

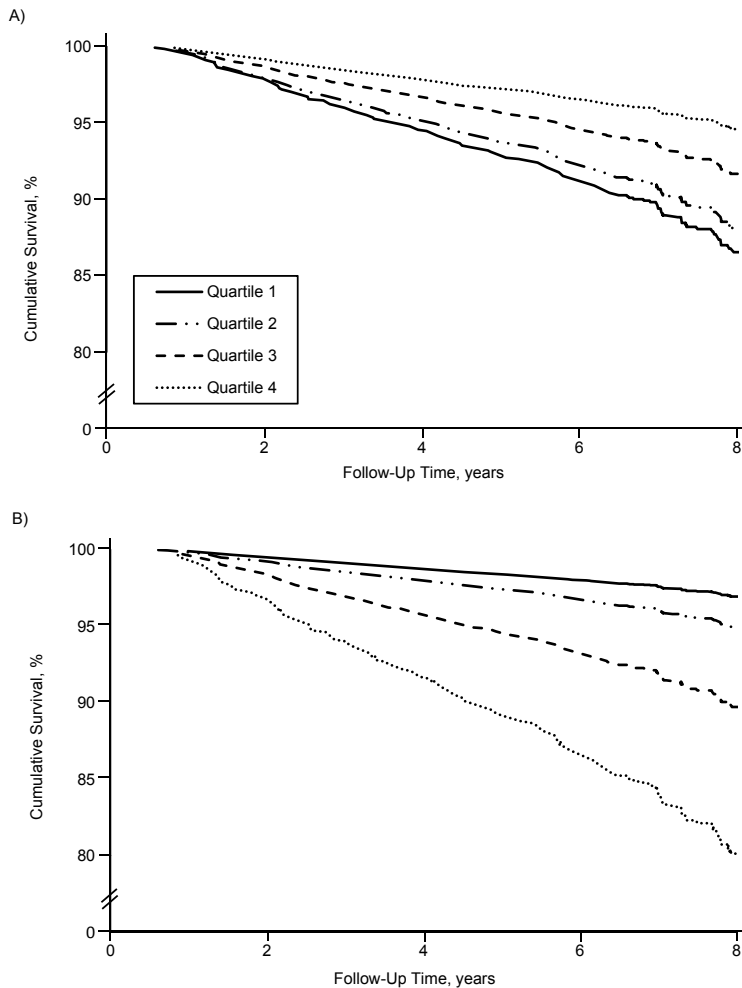
Abbreviations: CI, confidence interval; HR, hazard ratio; PSQI, Pittsburgh Sleep Quality Index.

<sup>a</sup> Adjusted for age, gender, activities of daily living, current smoking, diabetes, myocardial infarction, stroke, cognitive functioning, depressive symptoms, body mass index, sleep medication, napping and apnea.

<sup>b</sup>  $P < 0.05$ .

<sup>c</sup>  $P < 0.001$ .

Our finding that fragmentation and low stability of the 24-hour activity rhythm predict mortality has several possible explanations. First, the biological aging processes may be involved. Although our analyses were adjusted for age, age-related changes to the circadian organization are complex and might differ per level of circadian organization.<sup>12</sup> For example, changes may occur in the suprachiasmatic nucleus (the central clock of the brain), in peripheral oscillators, or in the ability of the suprachiasmatic nucleus to drive peripheral oscillators. In humans, postmortem studies demonstrated a reduction in the number of vasopressin-expressing neurons in the suprachiasmatic nucleus at old age (>80 years).<sup>30</sup>



**Figure 2** Crude cumulative survival plots per quartile of A) interday stability and B) intraday variability, the Rotterdam Study, the Netherlands, 2004–2013. Quartile 1 is the lowest quartile and quartile 4 is the highest. Survival was lower in participants with a low interday stability or a high intraday variability (fragmentation).

This may underlie a smaller amplitude of several circadian rhythms, a more fragmented 24-hour activity rhythm, and a temperature and melatonin phase that occur earlier than in younger people.<sup>12,30-35</sup> This loss of temporal organization between different rhythms can lead to suboptimal physiological functioning because physiological processes do not all take place at their optimal time of day.<sup>36</sup> As a result, people who suffer from temporal disorganization have a higher susceptibility to disease. Second, napping increases the fragmentation of the rhythm<sup>17</sup> and can also be an indicator of bad health.<sup>37</sup> However, previous literature on the association of napping with mortality were inconsistent. It was found that people who take

naps regularly might have a higher mortality rate,<sup>38,39</sup> especially those who sleep more than 9 hours per night.<sup>40</sup> On the other hand, another study found no significant benefit or harm of napping,<sup>41</sup> whereas yet another study found a protective association between napping and mortality for people with short sleep durations.<sup>40</sup> In these studies, circadian rhythm parameters were not taken into account. In our study, self-reported naps could not explain the association between the stability and fragmentation of the 24-hour activity rhythm and mortality. Third, the disturbed 24-hour activity rhythm might be an indicator of poor health. Occurrence of disease has been related to disrupted circadian rhythms, for example in persons with cardiovascular disease and Alzheimer's disease.<sup>21,42</sup> Also, more fragmented and less stable 24-hour activity rhythms have been related to sleepiness, depression, cognitive deficits, high body mass index, smoking, high blood pressure, and obesity.<sup>17,23,43-45</sup>

Although we controlled for several health measures, such as ADL, depressive symptoms, and diabetes, residual confounding by disease might explain part of the results. We also ran analyses in which we excluded deaths that occurred in the first 2 years to test for reverse causality. The observed hazard ratio was very similar to that from the analyses that included all participants. This suggests that the association between the 24-hour activity rhythm and mortality is not exclusively driven by short-term mortality.

Circadian rhythms and sleep patterns change as people age. For example, elderly people sleep less during the night and tend to fall asleep and wake up earlier.<sup>2-6</sup> Nevertheless, in the present study, the associations between sleep and 24-hour activity rhythm parameters were weak. We found that more fragmented and less stable circadian activity rhythms predicted mortality but none of the sleep variables, whether measured objectively or subjectively, predicted mortality in the fully adjusted model. During our follow-up period, 154 participants died, which implies that we had sufficient power to detect the moderate associations of 24-hour activity rhythm parameters on mortality. Arguably, our study might not have been powered to detect mild associations between sleep characteristics and mortality. Yet, the findings suggest that the circadian rhythm is more strongly and independently associated with mortality than is sleep duration.

There have been few studies on the association between activity rhythms and mortality.<sup>13-15,46,47</sup> Mortality risk was found to be higher in older men and women with less robust or abnormal 24-hour activity rhythms.<sup>13-15</sup> In patients with metastatic colorectal cancer, a higher mortality rate was associated with disturbed circadian rhythms; in dementia patients, it was associated with abnormal timing of the rhythm.<sup>46,47</sup> To our knowledge, the association of fragmentation and stability of the circadian activity rhythm with all-cause mortality has not been described before.

Our estimates showed a significant U-shaped relationship between continuously analyzed subjective sleep duration and mortality. However, these associations were nonsignificant after adjustment for health parameters. A U-shaped relationship between sleep duration and mortality was found in several previous studies (for 2 meta-analyses, see Cappuccio et al.<sup>9</sup> and Gallicchio et al.<sup>10</sup>). In general, stronger associations between long sleep duration and all-cause mortality were observed in the more extreme sleep categories ( $\geq 9$  hours). In our study, few persons were extreme short or long sleepers. Consequently, in our analyses, the power regarding extreme sleep duration was limited, which might explain the attenuation of the curvilinear relation between sleep duration and mortality after further adjustment. Previous studies in which sleep duration stratified on health status was examined were inconsistent.<sup>48,49</sup> In 1 study, Magee et al.<sup>48</sup> observed an association between sleep duration and mortality in persons with preexisting disease only; Mesas et al.<sup>49</sup> also found this association in healthy people. In addition, part of the association of short sleep duration with mortality can be explained by sleep apnea.<sup>50</sup> We adjusted for possible sleep apnea based on 2 questions from the PSQI.<sup>24,25</sup> Possible apnea was not a significant predictor of sleep duration or mortality in our fully adjusted model. However, the PSQI cannot be used to diagnose sleep apnea. We cannot rule out that sleep apnea, if assessed more in detail, might explain part of the observed associations.

In the present study, as in previous studies, perceived sleep quality was not related to all-cause mortality, whether it was measured using the sleep diary or with the PSQI.<sup>51</sup> Previously, sleep disturbances were associated with higher all-cause mortality risk only in men younger than 45 years of age and not in women or men older than 45 years.<sup>52</sup>

One strength of our study is that it is embedded in the Rotterdam Study, a prospective population-based cohort study. This increases the generalizability of our results, and we were able to adjust for many covariates. A second strength was our use of both subjective and objective measurements to estimate sleep duration and sleep onset latency. Because subjective and objective sleep variables are not strongly associated,<sup>18</sup> it is important to analyze both and to test whether they predict mortality independently. A third strength was the complete follow-up of the death date of all participants. Fourth, the 24-hour activity rhythms were analyzed nonparametrically, so no assumptions were made about the underlying shape of their circadian rhythms. This study also has some limitations. First, data collection on sleep disorders, such as restless leg syndrome and sleep apnea, was limited. Second, in our population-based study, 154 participants died. Therefore, we may not have been able to detect the mild associations of sleep parameters on mortality.

To conclude, in a representative middle-aged and elderly population, fragmentation and low stability of the 24-hour activity rhythm predicted all-cause mortality independent of and

better than sleep estimates. Changes in the regulation of circadian rhythms could indicate disease and reflect age-related alterations in the biological clock of the brain. Future research must show whether improving circadian activity rhythm disturbances can improve health and survival.



## Supplemental material

**Supplemental Table 1** Correlations Between 24-Hour Activity Rhythm and Sleep Parameters in 1,734 Men and Women, Rotterdam Study, the Netherlands, 2004-2007

	1	2	3	4	5	6	7	8
1 Interdaily stability	-							
2 Intradaily variability	-0.49 <sup>a</sup>	-						
3 Objective sleep duration	0.31 <sup>a</sup>	-0.26 <sup>a</sup>	-					
4 Objective sleep onset latency	-0.09 <sup>a</sup>	0.22 <sup>a</sup>	-0.11 <sup>a</sup>	-				
5 Wake after sleep onset	-0.19 <sup>a</sup>	0.28 <sup>a</sup>	-0.23 <sup>a</sup>	0.26 <sup>a</sup>	-			
6 Subjective sleep duration	0.09 <sup>a</sup>	-0.08 <sup>a</sup>	0.39 <sup>a</sup>	<0.01	0.07 <sup>a</sup>	-		
7 Subjective sleep onset latency	<0.01	0.03	0.11 <sup>a</sup>	0.14 <sup>a</sup>	0.13 <sup>a</sup>	-0.29 <sup>a</sup>	-	
8 Sleep quality sleep diary	0.11 <sup>a</sup>	-0.08 <sup>a</sup>	<0.01	<-0.01	-0.10 <sup>a</sup>	0.44 <sup>a</sup>	-0.27 <sup>a</sup>	-
9 Global PSQI score	-0.07 <sup>a</sup>	0.08 <sup>a</sup>	<0.01	0.12 <sup>a</sup>	0.12 <sup>a</sup>	-0.41 <sup>a</sup>	0.37 <sup>a</sup>	-0.45 <sup>a</sup>

<sup>a</sup>  $P < 0.05$ .

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# Chapter 2.2a

**Three Authors Reply: A comment on  
“The ongoing issue of how best to measure sleep  
in epidemiologic studies needs to be addressed”**

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In their letter to the editor<sup>1</sup> about our article,<sup>2</sup> Erren and Gross addressed the possibility that accumulated sleep disruption, that is, how much of an individual's sleep is not in phase with his or her biological clock, underlines the observed association between fragmentation or stability of the 24-hour activity rhythm and mortality. If available, chronotype data should be incorporated when investigating sleep and circadian rhythm organization.<sup>3,4</sup> Below, we share insights based on additional analyses of our data that address the importance of the issue.

Employment status as a proxy of social timing was tested as a potential confounder in the presented associations of sleep and circadian rhythm with mortality, but adjustment for that variable did not change effect estimates. Nevertheless, we observed some indication of circadian misalignment<sup>5</sup> among the employed participants. First, the absolute difference between time in bed on workdays and that on weekends was 66 (standard deviation, 48) minutes among the employed participants compared with 36 (standard deviation, 30) minutes in the nonworkers. Also, as an indicator of circadian misalignment,<sup>5</sup> the absolute difference between the midpoint of sleep on workdays and the midpoint of sleep on weekends was 59 (standard deviation, 43) minutes in the workers compared with only 29 (standard deviation, 26) minutes in the nonworkers.

Larger differences in time spent in bed on weekdays versus weekends and in the midpoint of sleep on weekdays versus weekends were associated with unstable activity rhythms (Table 1), which suggests that nonparametrically derived stability of the circadian rhythm assessed with actigraphy<sup>6</sup> can capture circadian misalignment. Table 1 also shows the importance of assessing fragmentation next to stability; fragmentation is barely related to circadian alignment (Table 1) and thus cannot be estimated from such questionnaire data.

Importantly, in contrast to stability and fragmentation, circadian misalignment indicators did not predict mortality, nor did including circadian misalignment in the survival models meaningfully attenuate the risk estimates (for stability, hazard ratio = 0.80, 95% confidence interval: 0.68, 0.93; for fragmentation, hazard ratio = 1.27, 95% confidence interval: 1.07, 1.51) compared with the unadjusted results (for stability, hazard ratio = 0.83, 95% confidence interval: 0.71, 0.96; for fragmentation, hazard ratio = 1.22, 95% confidence interval: 1.04, 1.44).<sup>2</sup>

In our elderly sample, 574 (33%) persons were still working at baseline, of whom only 16 (2.8%) died during the follow-up period (compared with the 8.9% mortality rate in the total study population). To reevaluate the validity of the reported results, we conducted the survival analysis in the nonworkers (n = 1,145, including 137 deaths), whose sleep habits



would be expected to more closely align with their biological clocks. The results were essentially unchanged (for stability, hazard ratio = 0.83, 95% confidence interval: 0.71, 0.97; for fragmentation, hazard ratio = 1.20, 95% confidence interval: 1.02, 1.42). We concluded that prevalent circadian alignment cannot easily explain our findings, possibly because we studied a group with an older mean age (mean age, 62.2 (standard deviation, 9.3) years), which is known to converge towards the early chronotype.<sup>7</sup>

**Table 1** Adjusted association between chronotype misalignment and circadian rhythm indices

Circadian misalignment	Interdaily Stability <sup>a</sup>			Fragmentation <sup>a</sup>		
	B	95% CI	p-value	B	95% CI	p-value
<b>Time in bed</b>						
Workday-weekend absolute difference	-0.34	-0.41 ; -0.27	<0.01	0.04	-0.02 ; 0.10	0.19
<b>Midpoint of sleep</b>						
Workday-weekend absolute difference	-0.60	-0.68 ; -0.52	<0.01	0.08	0.07 ; 0.14	0.04

<sup>a</sup>Outcomes were standardized and Winsorized at 4SD. Models are adjusted for age, sex, activities of daily living score, current smoking, diabetes, myocardial infarction, stroke, cognitive functioning, depressive symptoms, body mass index, sleep medication, napping and apnea.

Finally, a logical fallacy in the letter by Erren and Gross needs to be pointed out. In their hypothetical example, the associations of reported sleep disruption and Actigraphically derived circadian rhythm indices with mortality are compared based on risk effect estimates. They concluded that if effects were similar, the former could be used “because it is less cumbersome and provides more readily interpretable information” (1, p. 186). We argue that such inferences about the measured constructs must be made based on their mutual dependence and not by comparing the strengths of the associations with mortality.

In conclusion, chronotype data can help investigators in future studies estimate doses of accumulated sleep disruption, though the validity and precision must be empirically tested. The accuracy of sleep and circadian estimates derived from actigraphy might outweigh the simplicity of only administering sleep questionnaires.<sup>8</sup> Finally, the extent to which nonparametric circadian rhythm variables capture circadian misalignment should be systematically explored.

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# Chapter 2.3

## **Associations of heart failure with sleep quality:**

### **The Rotterdam Study**

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## Abstract

**Study Objectives:** The prevalence of sleep disturbances and heart failure increases with age. We aimed to evaluate the associations of incident heart failure and cardiac dysfunction with changes in sleep quality.

**Methods:** This prospective population-based study was conducted in the Rotterdam Study. Of the 3445 eligible persons (mean age  $72.0 \pm 7.1$  years) available for cross-sectional analyses, 8.9% ( $n = 307$ ) had prevalent clinical heart failure. In longitudinal analyses, 1989 eligible persons (mean age  $70.0 \pm 5.8$  years) were followed for an average of  $6.5 \pm 0.4$  years, of which 4.6% ( $n = 91$ ) had prevalent or incident clinical heart failure. Heart failure was assessed according to European Society of Cardiology criteria. To estimate cardiac function, we measured left ventricular fractional shortening, left ventricular systolic function, and E/A ratio by echocardiography. Heart failure and cardiac dysfunction were studied with linear regression in relation to sleep quality, assessed by the Pittsburgh Sleep Quality Index.

**Results:** No associations between clinical heart failure and sleep quality were observed in cross-sectional analyses. Clinical heart failure predicted a reduction of sleep quality ( $B = 1.00$  points on the Pittsburgh Sleep Quality Index; 95% CI 0.40, 1.60) in longitudinal assessment. This association was driven by the sleep onset latency and sleep quality components of the Pittsburgh Sleep Quality Index. Cardiac dysfunction was not related to sleep quality in cross-sectional or longitudinal analyses.

**Conclusions:** Clinical heart failure, but not cardiac dysfunction measured by echocardiography, increases the risk of poor sleep quality in the general population over time. These findings suggest that clinical manifestations of heart failure negatively affect sleep.

## Introduction

Sleep disturbances have been reported in around 60% of patients with heart failure (HF).<sup>1,2</sup> Despite improved treatment and an overall decline in mortality after a cardiovascular event, the number of HF patients is increasing among the elderly.<sup>3</sup> This is probably due to an aging population and to the improved chances of survival for HF patients.<sup>4</sup> Nevertheless, only 25% to 35% of patients with clinical HF survive up to 5 years after diagnosis, and HF poses a great burden in terms of treatment, hospitalization and quality of life.<sup>5-7</sup> HF is related to multiple physical and mental problems, including shortness of breath, depressive symptoms, cognitive impairment, and sleep problems.<sup>8,9</sup> Little is known about the changes in sleep occurring in these patients over time.

Previous studies investigating HF and sleep have been mostly cross-sectional and cannot establish a temporal relation. Prospective sleep studies have demonstrated that short and long sleep durations increase the risk of cardiovascular disease.<sup>10-13</sup> Furthermore, difficulty maintaining and initiating sleep are associated with incident HF.<sup>14</sup> However, there is evidence for a bi-directional relation between sleep and heart disease. For example, poor sleep quality and difficulty maintaining and initiating sleep are consequences of cardiovascular disease.<sup>15</sup> How sleep quality changes over time in patients with cardiac dysfunction, prevalent HF or new-onset HF is unclear.

We examined whether prevalent and incident HF and echocardiographic indicators of cardiac dysfunction are associated with sleep quality in a community-dwelling population using both cross-sectional and longitudinal designs. Also, the associations between HF and the separate component scores of the Pittsburgh Sleep Quality Index (PSQI) were analyzed.

## Methods

### *Participants*

This study is part of the Rotterdam Study, a prospective population-based cohort of the general population in Rotterdam, The Netherlands.<sup>16</sup> The study conforms to the principles outlined in the Declaration of Helsinki, and was approved by the Medical Ethics Committee according to the Wet Bevolkingsonderzoek ERGO (Population Study Act Rotterdam Study), executed by the Ministry of Health, Welfare and Sports of The Netherlands. All participants provided written informed consent.

Between 2002 and 2005, 3711 participants aged  $\geq 55$  years completed the PSQI and underwent echocardiography. Of these, 266 participants were excluded because of (1) diagnosis of HF that did not fulfill the criteria of the European Society of Cardiology; (2) poor cognitive function (Mini-mental state examination score  $\leq 23^{17}$ ); or (3)  $<6$  valid PSQI

component scores. This left 3445 participants for cross-sectional analyses. Five hundred forty-one participants died during follow-up. Between 2009 and 2012, 2105 participants completed the PSQI again. Of these, 116 participants were excluded because of (1) poor cognitive function at follow-up or (2) < 6 valid PSQI component scores at follow-up, leaving 1989 participants for the longitudinal analyses.

#### *Clinical Heart Failure*

Assessment of HF in the Rotterdam Study has been described previously.<sup>18</sup> In brief, HF was determined in accordance with the guidelines of the European Society of Cardiology, requiring objective evidence of cardiac dysfunction, together with typical symptoms of heart failure such as breathlessness, ankle swelling, pulmonary crepitation, or use of cardiovascular medication for HF.<sup>19</sup> In this study, HF was defined as prevalent if the date of diagnosis was before the date of the PSQI baseline measure. Incident HF was diagnosed if it occurred between the baseline and repeated PSQI assessment. Information on incident HF cases was obtained by digital linkage with medical records of general practitioners, which allowed continuous monitoring. Only definite and probable HF diagnoses were included in the analyses.<sup>20</sup>

#### *Echocardiography*

A resting echocardiogram was obtained from each participant at baseline to assess left ventricular systolic function (fractional shortening and visual assessment [normal, fair, moderate, or poor]) and diastolic function (E/A ratio [ $< 0.75$ ,  $0.75-1.50$ ,  $> 1.50$ ]) according to a standardized protocol.<sup>21</sup> Fractional shortening was computed as (left ventricular end diastolic dimension – left ventricular end systolic dimension) divided by left ventricular end diastolic dimension\*100%. E/A ratio was calculated as Doppler peak E filling velocity divided by Doppler peak A filling velocity. The echocardiograms were made with one of two commercially available systems. In the analyses, moderate and poor left ventricular systolic function were combined because of the low number of participants with poor function.

#### *Sleep Quality*

Sleep quality was measured with the PSQI, a self-rated 19-item questionnaire.<sup>22</sup> The global PSQI score comprises of sleep onset latency, sleep efficiency, sleep quality, sleep disturbances, sleep duration, daytime dysfunction, and use of sleeping medication in the past month (range 0-21). Higher scores represent a poorer sleep quality. Participants with < 6 valid PSQI component scores were excluded. The global PSQI was calculated as the sum of the component scores. If a participant had only 6 valid PSQI component scores, we calculated the global PSQI score, but weighted the summed score by multiplying with 7/6 ( $7 * \text{the sum of the 6 valid component scores} / 6$ ). The PSQI has a good test-retest reliability and validity.<sup>22</sup>

### *Statistical Analyses*

Clinical HF, fractional shortening, left ventricular systolic function, and E/A ratio were studied as determinants of sleep quality. Clinical HF was continuously monitored, the echocardiographic indicators of cardiac function and confounders were measured at baseline, and sleep quality was measured at baseline and at follow-up. We performed cross-sectional and longitudinal analyses to examine whether clinical HF and echocardiographic indicators of cardiac dysfunction are associated with sleep quality. To assess sleep quality changes in the longitudinal analyses, we adjusted for baseline sleep quality. Incident HF was also studied separately from prevalent HF. Additionally, the associations between HF and the separate component scores of the PSQI were analyzed longitudinally.

We adjusted the linear regressions for age and gender. Additional adjustments were made for education (low, intermediate, and high), possible sleep apnea, depressive symptoms (Center for Epidemiologic Studies-Depression scale<sup>23</sup>), diabetes mellitus, cognitive function (mini-mental state examination score), diuretic use, and the echocardiographic system used, as these variables changed effect estimates (> 5%) or were a priori confounders. Possible sleep apnea was based on 2 questions of the PSQI. Apnea was considered possible when participants reported that they snored loudly  $\geq 2$  nights per week and if they reported occasional respiratory pauses, or if they reported respiratory pauses  $\geq 1$ -2 nights per week.<sup>24</sup> Diuretic use (ATC code C03) was assessed with pharmacy records. Participants without a diuretic prescription were considered non-users, participants with a prescription up to the defined daily dose were considered low users, and participants with a prescription higher than the defined daily dose were considered high users. Systolic and diastolic blood pressure, smoking, alcohol intake, and body mass index were not entered as covariates, as they did not change effect estimates.

Missing values were handled by multiple imputation using 5 imputations. The maximum amount of missing data was for fractional shortening (4.9%). A p value < 0.05 was considered statistically significant. Analyses were performed in SPSS version 20 (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY).

A validation of PSQI measures was performed within 2 months in a random subsample of 173 participants with actigraphy and sleep diary measures conducted over the course of one week. The global PSQI score was correlated to sleep quality measured with three questions of the sleep diary ( $r = -0.41$ ,  $p < 0.001$ ), sleep duration correlated well with actigraphic assessment of average sleep duration ( $r = 0.33$ ,  $p < 0.001$ ).



## Results

### *Sample Characteristics*

The cross-sectional sample consisted of 3445 eligible participants (mean age 72.0±7.1 years; 43.2% male), of which 307 (8.9%) participants had prevalent HF at baseline (baseline characteristics can be found in Supplementary Table 1). The sample available for longitudinal analyses consisted of 1989 participants (mean age 70.0±5.8 years; 41.9% male), of which 40 (2.0%) participants had prevalent HF at baseline and 51 (2.6%) had incident HF during follow-up (mean follow-up 6.5±0.4 years). Therefore a total of 91 participants with HF were studied (baseline characteristics for the 1989 participants in the longitudinal analyses can be found in Table 1).

**Table 1** Baseline characteristics (n = 1989)

Measures	
Gender (male), n (%)	834 (41.9%)
Age, mean (SD)	70.0 (5.8)
Body mass index (kg/m <sup>2</sup> ), mean (SD)	27.7 (4.2)
Education, n (%): Low	316 (15.9%)
Intermediate	1358 (68.3%)
High	315 (15.8%)
Depressive symptoms <sup>a</sup> , mean (SD)	5.1 (6.7)
Possible sleep apnea, n (%)	180 (9.0%)
Systolic blood pressure (mm Hg), mean (SD)	147.8 (19.8)
Diastolic blood pressure (mm Hg), mean (SD)	80.8 (10.3)
Diabetes Mellitus, n (%)	226 (11.4%)
Current smoking, n (%)	242 (12.2%)
Alcohol intake (units per week), mean (SD)	8.0 (9.8)
Fractional shortening, mean (SD)	38.5 (6.7)
Left ventricular systolic function, n (%): Normal	1160 (58.3%)
Fair	750 (37.7%)
Moderate	58 (2.9%)
Poor	21 (1.1%)
E/A ratio, n (%): < 0.75	553 (27.8%)
0.75-1.50	1398 (70.3%)
> 1.50	38 (1.9%)
Diuretics use, n (%): No	1741 (87.5%)
Low	231 (11.6%)
High	17 (0.9%)
Global PSQI score <sup>b</sup> , mean (SD)	3.7 (3.4)

Baseline (imputed data), the maximum missing data was for fractional shortening (4.9%). <sup>a</sup> Measured with the Center for Epidemiologic Studies-Depression scale. <sup>b</sup>PSQI: Pittsburgh Sleep Quality Index. The baseline characteristics for the 3445 participants in the cross-sectional sample can be found in Supplementary Table 1.

### Linear Regression

No associations between clinical HF or cardiac dysfunction and sleep quality were found cross-sectionally (Table 2). The cross-sectional analyses only including the 1989 participants from the longitudinal analyses showed similar results (data available upon request). In Table 3, we only present the fully adjusted model, as the age- and gender-adjusted model had similar results. In longitudinal analyses, HF was related to a poorer sleep quality, i.e., an increase in global PSQI score between baseline and follow-up ( $B = 1.00$  points on the PSQI; 95% CI 0.40, 1.60;  $p < 0.01$ ; Table 3). This association was found in both prevalent ( $B = 1.04$  points on the PSQI; 95% CI 0.14, 1.94;  $p = 0.02$ ) and incident ( $B = 0.97$  points on the PSQI; 95% CI 0.20, 1.75;  $p = 0.01$ ) HF cases. None of the echocardiographic cardiac parameters were associated with changes in sleep quality.

**Table 2** The cross-sectional association of heart failure and echocardiographic parameters with sleep quality (n=3445)

	Global PSQI <sup>a</sup> B (CI)	p
<b>Heart failure</b>		
No heart failure (n=3138)	0 (ref)	
Prevalent heart failure (n=307)	-0.131 (-0.694, 0.432)	0.65
<b>Cardiac function</b>		
Fractional shortening (per %)	-0.001 (-0.017, 0.015)	0.88
Left ventricular systolic function		
Normal (n=1837)	0 (ref)	
Fair (n=1346)	-0.083 (-0.314, 0.148)	0.48
Moderate or poor (n=262)	0.056 (-0.369, 0.481)	0.80
E/A ratio		
< 0.75 (n=1079)	-0.118 (-0.359, 0.123)	0.34
0.75-1.50 (n=2292)	0 (ref)	
> 1.50 (n=74)	-0.231 (-1.000, 0.537)	0.56

Linear regression adjusted for gender, age, possible sleep apnea, depressive symptoms, diabetes mellitus, education, cognitive function, echo device used, and diuretic use. <sup>a</sup> PSQI: Pittsburgh Sleep Quality Index.

We analyzed the components of the global PSQI score separately with logistic regression. This enabled us to test how clinical HF affects the change of these aspects of sleep. HF was associated with changes in sleep quality (OR = 2.63; 95% CI 1.41, 4.93) and sleep onset latency (OR = 2.03; 95% CI 1.11, 3.72; component scores 0, 1 versus 2, 3; fully adjusted), but was not associated with changes in sleep duration, sleep efficiency, sleep disturbances, daytime dysfunction, or sleep medication (Table 4).

To disentangle the effect of sleep medication on the association between HF and change in global PSQI score, we omitted the sleep medication component from the global PSQI score

(at baseline and at follow-up) and added sleep medication use at baseline as extra covariate in the analysis. The association between HF and global PSQI score was only modestly attenuated ( $B = 0.86$ ; 95% CI 0.31, 1.40).

**Table 3** The longitudinal associations of heart failure and echocardiographic parameters with sleep quality (n=1989)

	Change in global PSQI <sup>a</sup>	
	B (CI)	p
<b>Heart failure</b>		
No heart failure (n=1898)	0 (ref)	
Clinical heart failure all (n=91)	<b>1.002 (0.402, 1.602)</b>	<b>&lt;0.01</b>
Prevalent heart failure (n=40)	<b>1.041 (0.141, 1.940)</b>	<b>0.02</b>
Incident heart failure (n=51)	<b>0.973 (0.197, 1.749)</b>	<b>0.01</b>
<b>Cardiac function</b>		
Fractional shortening (per %-point)	-0.008 (-0.028, 0.012)	0.44
Left ventricular systolic function		
Normal (n=1160)	0 (ref)	
Fair (n=750)	0.106 (-0.163, 0.375)	0.44
Moderate or poor (n=79)	-0.092 (-0.751, 0.567)	0.78
E/A ratio		
< 0.75 (n=553)	-0.019 (-0.311, 0.273)	0.90
0.75-1.50 (n=1398)	0 (ref)	
> 1.50 (n=38)	-0.490 (-1.390, 0.410)	0.29

Linear regression adjusted for gender, age, global PSQI<sup>a</sup> score at baseline, possible sleep apnea, depressive symptoms, diabetes mellitus, education, cognitive function, echo device used, and diuretic use. <sup>a</sup>PSQI: Pittsburgh Sleep Quality Index, change modelled by correction for baseline PSQI.

## Discussion

Clinical HF increased the risk of sleep problems in this sample of middle-aged and elderly persons. In our longitudinal analyses, we observed that sleep quality was reduced in participants with either prevalent or incident HF at follow-up. This relation could not be demonstrated with echocardiographic indicators of cardiac dysfunction. Therefore, findings suggest that it is clinical manifestations of HF that specifically affect sleep negatively.

Several studies have related poor sleep quality to prevalence of cardiovascular disease; however, these studies have not reported repeated sleep assessments.<sup>2,11,12,25</sup> Therefore, any effect of cardiovascular disease upon sleep must be inferred from case-control studies, which have been undertaken in a clinical setting. Only one of the studies focused specifically on HF and observed that HF patients had a lower sleep quality than controls.<sup>2</sup> In our cross-sectional analyses, we did not observe an association between HF and sleep. In clinical studies HF is probably more severe than in our study, which might explain the absence of the association in our cross-sectional analyses.

**Table 4** The longitudinal association of heart failure with the component scores of the Pittsburgh Sleep Quality Index (n=1989)

	OR (CI)	Sleep quality	OR (CI)	Sleep latency	OR (CI)	Sleep duration	OR (CI)	Sleep efficiency	OR (CI)	Sleep disturbances	OR (CI)	Sleep medication	OR (CI)	Daytime dysfunction
<b>Heart failure</b>														
No heart failure (n=1898)	0 (ref)	0 (ref)	0 (ref)	0 (ref)	0 (ref)	0 (ref)	0 (ref)	0 (ref)	0 (ref)	0 (ref)	0 (ref)	0 (ref)	0 (ref)	0 (ref)
Heart failure (n=91)	<b>2.63</b> <b>(1.41, 4.93)**</b>	<b>2.03</b> <b>(1.11, 3.72)*</b>	1.33 (0.72, 2.47)	1.67 (0.96, 2.91)	1.80 (0.66, 4.88)	1.77 (0.81, 3.84)	1.80 (0.66, 4.88)	1.80 (0.66, 4.88)	1.80 (0.66, 4.88)	1.80 (0.66, 4.88)	1.80 (0.66, 4.88)	1.80 (0.66, 4.88)	1.80 (0.66, 4.88)	1.80 (0.66, 4.88)

Logistic regression of the Pittsburgh Sleep Quality Index component scores on heart failure (component scores were dichotomized into 0,1 versus 2,3). \*<0.05, \*\*<0.01. All analyses are adjusted for the respective baseline sleep component scores to assess change. Further adjustments were made for gender, age, possible sleep apnea, depressive symptoms, diabetes mellitus, education, cognitive function, echo device used, and diuretic use.

2.3

For our longitudinal analyses we assessed sleep quality repeatedly. Our study suggests that clinical HF might lead to sleep problems. The reason why the association between HF and sleep quality was observed in longitudinal analyses, but not in cross-sectional analyses might be due to two reasons. First, in the longitudinal analyses, new-onset HF cases are included. Patients with new-onset HF probably experience the most substantial decline in sleep quality. Second, in the longitudinal analyses we tested the individual change in global PSQI score, while in cross-sectional analyses, the absolute level of this score was tested. Participants who develop HF might show a decline in global PSQI score compared to participants who do not develop HF, while their baseline global PSQI scores might not differ. There are several explanations for the observed association between clinical HF and sleep quality. HF symptoms such as restless legs, orthopnea, and nocturia due to redistribution of extravascular fluid in supine position, can cause disturbances in sleep and changes in the sleep-wake pattern. Another explanation is that HF and sleep problems share common etiological mechanisms. For example, vascular pathologies could independently explain HF and sleep problems.<sup>26,27</sup> Furthermore, sleep-disordered breathing is highly prevalent in people with HF.<sup>28</sup> We adjusted for possible sleep apnea to take this into account. In our study, possible sleep apnea was assessed by two questions from the PSQI. However, this is not a formal diagnosis of sleep apnea. We cannot rule out that if sleep apnea was measured by polysomnography, results could differ. It would be optimal to replicate results with sleep apnea assessed using polysomnography.

Only 25% to 35% of people with HF survive 5 years after first diagnosis.<sup>5,7</sup> In our study, the participants with prevalent HF completed the second PSQI assessment in the longitudinal analyses 10.2±2.8 years after onset of HF. The participants with recent-onset HF completed the PSQI assessment 3.5±1.8 years after onset of HF. Participants with HF in population-based studies are thus more likely to be long-term survivors with less severe HF, and this can lead to an underestimation of the impact of HF on sleep changes. However, in this study, the effect of incident HF upon sleep was only slightly different than the effect of prevalent HF. We could not find any effect of cardiac dysfunction measured with echocardiography, and sleep problems. This suggests that HF symptoms are on the causal pathway between cardiac dysfunction and sleep disturbances.

The major strengths of this study are the prospective data collection and the use of general practitioners' records to assess HF. HF was recorded early in the disease process, often before the patient was in specialist care and, hence, the chance of reverse causality was low. This study also has some limitations. First, healthy participants were more likely to complete the follow-up PSQI, as observed by the reduction in prevalent HF. Consequently, the longitudinal analyses included more relatively healthy older adults and could be less generalizable. Second, the number of participants with HF was relatively small. We had sufficient power to show consistent longitudinal effects, but including more participants with

HF might strengthen the findings and would have enabled us to evaluate specific subgroups. Third, information on sleep disorders such as sleep apnea was limited. In our study, possible sleep apnea was assessed with the PSQI. However, this is not a formal diagnosis of sleep apnea. We cannot rule out that formal diagnoses of sleep apnea could change the results.

To conclude, clinical HF, but not cardiac dysfunction as measured by echocardiography, increases the risk of poor sleep quality in the general population over time. Moreover, no cross-sectional associations were observed in this population-based study. These findings suggest that clinical manifestations of HF negatively affect sleep.

## Supplemental Material

**Supplementary Table 1** Baseline characteristics of the cross-sectional sample (n = 3445)

Measures		
Gender (male), n (%)		1487 (43.2%)
Age, mean (SD)		72.0 (7.1)
Body mass index (kg/m <sup>2</sup> ), mean (SD)		27.6 (4.2)
Education, n (%): Low		660 (19.2%)
Intermediate		2302 (66.8%)
High		483 (14.0%)
Depressive symptoms <sup>a</sup> , mean (SD)		5.9 (7.2)
Possible sleep apnea, n (%)		288 (8.4%)
Systolic blood pressure (mm Hg), mean (SD)		149.4 (22.1)
Diastolic blood pressure (mm Hg), mean (SD)		80.0 (11.4)
Diabetes Mellitus, n (%)		504 (14.6%)
Current smoking, n (%)		456 (13.2%)
Alcohol intake (units per week), mean (SD)		7.7 (10.1)
Fractional shortening, mean (SD)		37.9 (7.3)
Left ventricular systolic function, n (%): Normal		1837 (53.3%)
Fair		1346 (39.1%)
Moderate		150 (4.3%)
Poor		112 (3.3%)
E/A ratio, n (%): < 0.75		1079 (31.3%)
0.75-1.50		2292 (66.5%)
> 1.50		74 (2.2%)
Diuretics use, n (%): No		2932 (85.1%)
Low		455 (13.2%)
High		58 (1.7%)
Global PSQI score <sup>b</sup> , mean (SD)		3.9 (3.6)

Baseline (imputed data). <sup>a</sup>Measured with the Center for Epidemiologic Studies-Depression scale. <sup>b</sup>PSQI: Pittsburgh Sleep Quality Index.

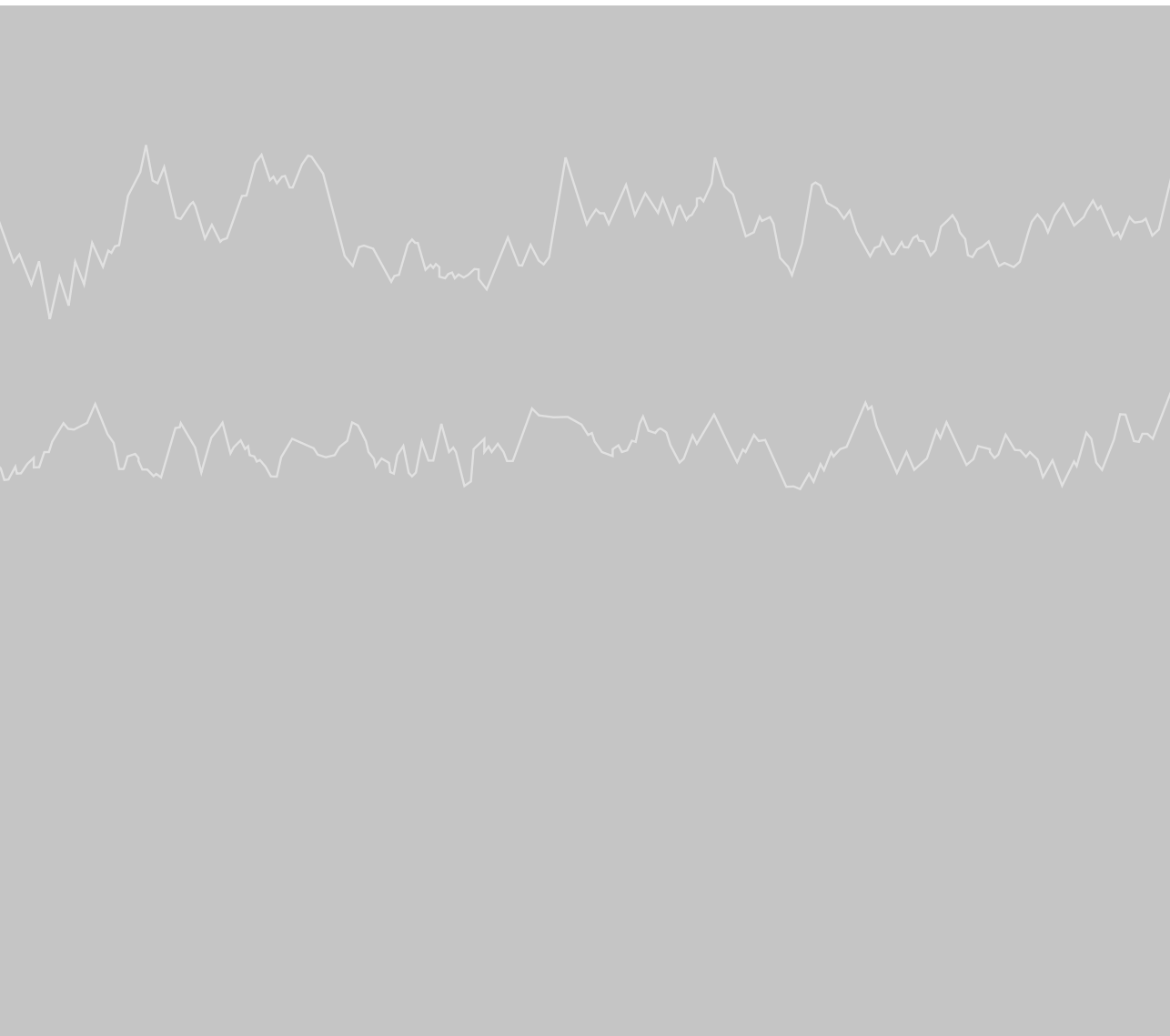
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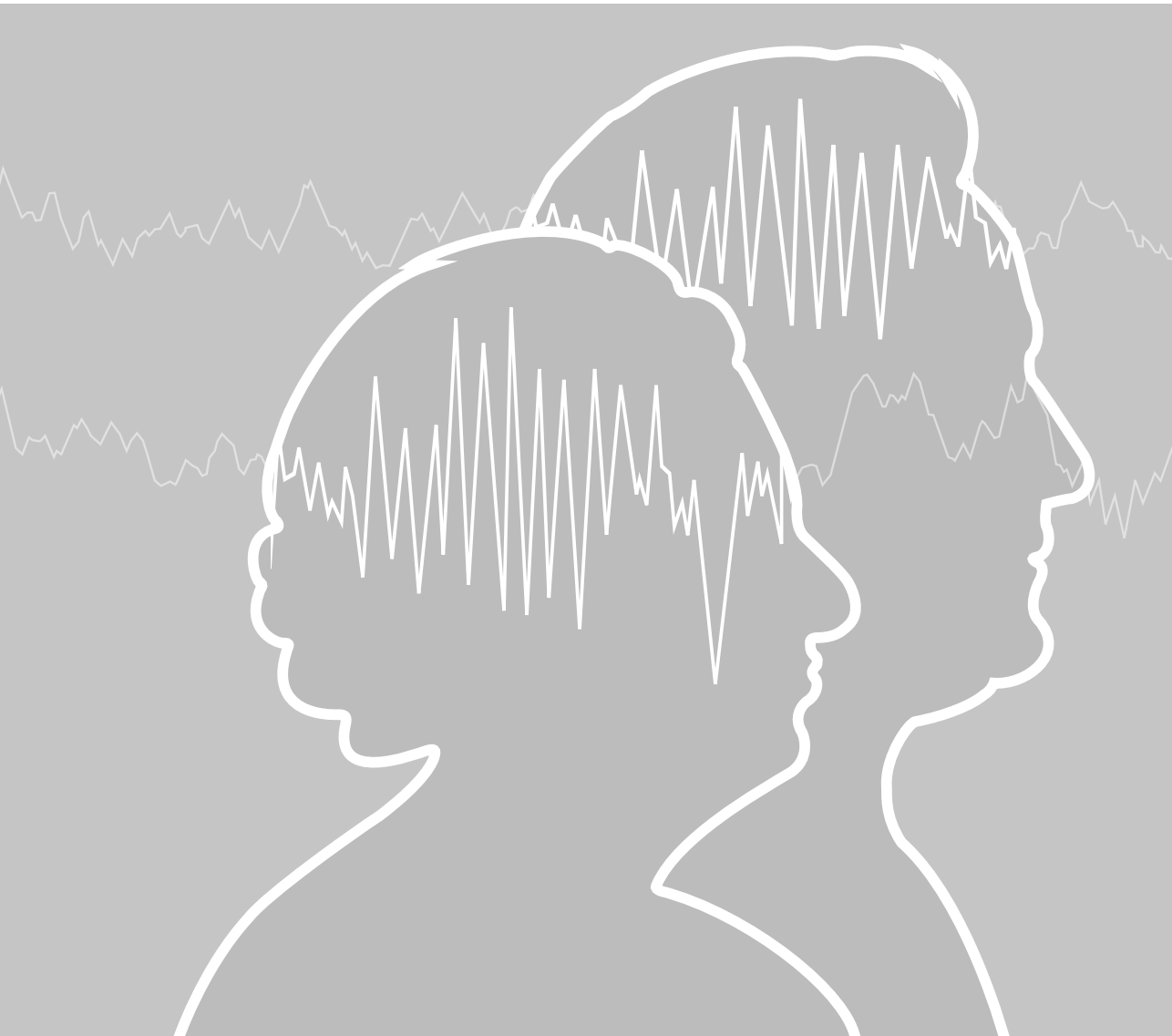
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# Chapter 3

## Brain structure and sleep







# Chapter 3.1

## **Cerebral small vessel disease is related to disturbed 24-h activity rhythms: a population-based study**

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## Abstract

**Background:** Cerebral small vessel disease is common in elderly persons. Patients with dementia or stroke frequently have cerebral small vessel disease and often experience disturbances in the sleep-wake rhythm. It is unknown whether cerebral small vessel disease is related to disturbances in sleep and 24-hour activity rhythms.

**Methods:** This study was conducted in the Rotterdam Study. A total of 970 community-dwelling persons (mean age 59.2 years) underwent brain MRI scanning and actigraphy. Cerebral small vessel disease was defined as white matter lesions (total volume in mL), presence of cerebral microbleeds and lacunar infarcts. 24-Hour activity rhythms and sleep were measured with actigraphy by estimating the instability and fragmentation of the activity rhythm and total sleep time. Sleep quality was assessed with the Pittsburgh Sleep Quality Index. White matter lesions, instability, fragmentation and sleep quality were standardized for analyses.

**Results:** Higher white matter lesion volume ( $B=0.09$  per standard deviation (SD), 95% CI=0.02; 0.15) and cerebral microbleeds ( $B=0.19$  per SD, 95% CI=0.02; 0.37) were significantly related to more fragmented 24-hour activity rhythms. None of the small vessel disease markers was related to total sleep time or sleep quality.

**Conclusions:** White matter lesion volume and presence of cerebral microbleeds are related to disturbed activity rhythms. This suggests that subclinical brain damage affects the 24-hour activity rhythm.

## Introduction

Circadian rhythms are involved in the control of many physiological processes, such as hormone secretion, body temperature and sleep-wake timing. Many elderly persons suffer from disturbances in their circadian rhythm and sleep. These disturbances are characterized by an increased sleep latency, a fragmented 24-hour activity rhythm, an earlier bed and wake up time, more napping, less sleep during the night and a lower sleep quality.<sup>1,2</sup> These disturbances could be explained by age-related changes occurring in the hypothalamic suprachiasmatic nucleus (SCN), the biological clock of the brain.<sup>3</sup> With aging, decreases in cell number and volume of the SCN are observed.<sup>4</sup> In persons with brain disease such as dementia and stroke disturbances in sleep and 24-hour activity rhythms are particularly profound,<sup>5,6</sup> suggesting that structural brain changes may play a role in these disturbances. Patients with dementia and stroke frequently have structural brain damage caused by cerebral small vessel disease, which may manifest as lacunar infarcts, white matter lesions (WMLs) or cerebral microbleeds (CMBs).<sup>7,8</sup> These markers of cerebral small vessel disease can often be detected in middle aged and elderly persons with magnetic resonance imaging.<sup>9-11</sup> Yet, it is unknown whether cerebral small vessel disease is related to disturbances in 24-hour activity rhythms and sleep.

The aim of this population-based study was to investigate the associations between cerebral small vessel disease and 24-hour activity rhythm and sleep parameters in a community-dwelling, stroke-free population. We hypothesize that WMLs, lacunar infarcts and CMBs are all associated with unstable and fragmented 24-hour activity rhythms, as well with worse sleep quality.

## Methods

### *Participants*

This study was conducted in the Rotterdam Study, a population-based cohort of middle-aged and elderly persons, living in a district of Rotterdam, the Netherlands.<sup>12</sup> The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC. All participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

Between September 2004 and March 2007 all participants were asked to participate in the actigraphy study, 2063 persons participated (response rate 78.4%). After exclusion of failed recordings due to technical problems and recordings containing less than 4 consecutive days and nights, 1734 (83.2%) recordings were included.<sup>13</sup> From these 1734 participants, 1179 persons without magnetic resonance imaging (MRI) contraindications (e.g. claustrophobia)



were invited for brain MRI scanning. Of these, 1031 agreed to participate (response rate 87.4%). We excluded scans with artefacts (n= 27) and participants with clinical stroke (n= 15) or cortical infarcts on MRI (n= 19) from analyses. Therefore 970 participants were included. The average time between the MRI scan and the week of actigraphy was 3 months (range 0 – 27 months).

#### *Magnetic resonance imaging*

Brain imaging was performed with a 1.5 Tesla MRI scanner (General Electric Healthcare, Milwaukee, USA, software version 11x) with an 8-channel head coil, and included T1-weighted, T2\*-weighted, proton density weighted and fluid-attenuated inversion recovery sequences, as described previously.<sup>14-16</sup> Cerebral small vessel disease was defined as WMLs (total volume in mL), presence of CMBs or lacunar infarcts (focal lesions  $\geq 3$  and  $< 15$  mm).

#### *Actigraphy*

Participants were asked to wear an actigraph (Actiwatch model AW4, Cambridge Technology Ltd, Cambridge, United Kingdom) for seven consecutive days and nights in order to obtain reliable estimates.<sup>17</sup> Actigraphs were obtained in 30-second epochs. We analyzed the 24-hour activity rhythms non-parametrically,<sup>5</sup> as described previously.<sup>13,18</sup> Fragmentation was assessed by the intradaily variability, indicating the rate of alternating between rest and activity. The stability was assessed by the interdaily stability, indicating how similar the day-night patterns are over days. We reverse coded interdaily stability to define interdaily *instability*. Therefore, both 24-hour activity rhythm variables represent a disturbed rhythm. We also used actigraphy to estimate total sleep time and wake after sleep onset, which have been described in more detail previously.<sup>19</sup>

#### *Pittsburgh Sleep Quality Index*

The Pittsburgh Sleep Quality Index (PSQI) was used to measure sleep quality and possible sleep apnea.<sup>20</sup> Sleep quality was measured with the global PSQI score (range 0-21). Higher scores represent a poorer sleep quality. Possible sleep apnea was considered when participants reported loud snoring at least 2 nights per week together with at least occasional respiratory pauses, or if they reported respiratory pauses during sleep at least 1-2 times per week.<sup>21</sup>

#### *Covariates*

Age, sex, body mass index (BMI), depressive symptoms, activities of daily living, possible sleep apnea, fasting blood glucose, total cholesterol, systolic blood pressure and use of antihypertensives, lipid lowering and sleep medication were considered as possible covariates. We assessed activities of daily living with the Stanford Health Assessment

Questionnaire,<sup>22</sup> depressive symptoms with the Center for Epidemiologic Studies-Depression scale<sup>23</sup> and cognitive function with the Mini Mental State Exam.<sup>24</sup> Serum glucose and total cholesterol were determined by an automated enzymatic procedure. Information on antihypertensive and lipid lowering medication was obtained from continuously monitored pharmacy records. Information on sleep medication was acquired by sleep diaries which participants kept during the week of actigraphy. Sleep medication use was analyzed as the total number of days a participant used sleep medication, taking into account the number of days the participant answered this question.

### *Statistical analyses*

We tested whether WMLs, the presence of CMBs or the presence of lacunar infarcts were related to 24-hour activity rhythm and sleep parameters with linear regression adjusted for age, sex, BMI, activities of daily living, depressive symptoms, systolic blood pressure, possible sleep apnea, glucose and cholesterol levels, and use of antihypertensives, sleep and lipid lowering medication. To correct for head size, analyses regarding WMLs were additionally adjusted for intracranial volume. The number of missing values of a variable never exceeded 1%. Missing categorical values were added as a separate missing category. WMLs, interdaily instability, fragmentation, sleep quality and wake after sleep onset were normalized and standardized. Correlations between 24-hour activity rhythm and sleep parameters were computed using Pearson correlation. Analyses were performed with IBM SPSS Statistics for Windows (Version 21. Armonk, NY:IBM Corp).

3.1

## **Results**

The mean age of the total population of 970 persons was 59.2 years ( $\pm 7.5$  standard deviation (SD)) and 52% were female. Other population characteristics can be found in Table 1. The 24-hour activity rhythm parameters interdaily instability and fragmentation were moderately correlated ( $r = 0.53$ ,  $P < 0.001$ ). These activity rhythm parameters were also moderately correlated with total sleep time ( $r = -0.34$ ,  $P < 0.001$ ;  $r = -0.31$ ,  $P < 0.001$ , respectively, Table 2).

Table 3 shows the associations of cerebral small vessel disease markers with the 24-hour activity rhythm parameters. WMLs were significantly related to more fragmentation of the 24-hour activity rhythm ( $B = 0.09$  per SD, 95% CI = 0.02; 0.15). Persons with CMBs were more likely to have more instability ( $B = 0.17$  per SD, 95% CI = -0.01; 0.34) and a higher fragmentation ( $B = 0.19$  per SD, 95% CI = 0.02; 0.37), although the association between CMBs and interdaily instability did not reach statistical significance.

**Table 1** Population characteristics (n=970)

Measures	Mean $\pm$ SD or n (%)
Demographics	
Age, years	59.2 $\pm$ 7.5
Gender, female	503 (51.9%)
Body mass index, kg/m <sup>2</sup>	27.6 $\pm$ 4.0
Activities of daily living, score	0.2 $\pm$ 0.3
Depressive symptoms, score	5.3 $\pm$ 7.1
Sleep medication, days	0.42 $\pm$ 1.4
Systolic blood pressure, mmHg	134.6 $\pm$ 20.1
Diastolic blood pressure, mmHg	80.7 $\pm$ 11.2
Antihypertensive medication, yes	271 (27.9%)
Total cholesterol, mmol/L	5.6 $\pm$ 1.0
Fasting blood glucose, mmol/L	5.6 $\pm$ 1.5
Diabetes mellitus, yes	99 (10.2%)
Lipid lowering medication, yes	195 (20.1%)
Possible sleep apnea, yes	137 (14.1%)
Cerebral small vessel disease	
White matter lesions, mL	3.8 $\pm$ 5.1
Lacunar infarcts, yes	43 (4.4%)
Cerebral microbleeds, yes	129 (13.3%)
24-Hour activity rhythms and sleep	
Interdaily instability, score	-0.8 $\pm$ 0.1
Fragmentation (intradaily variability), score	0.4 $\pm$ 0.1
Total sleep time, hours	6.3 $\pm$ 0.9
Wake after sleep onset, minutes	69.0 $\pm$ 25.3
Sleep quality, score	3.4 $\pm$ 3.3

**Table 2** Correlations between 24-hour activity rhythm and sleep parameters

	1	2	3	4	5
1. Interdaily instability	-				
2. Fragmentation (intradaily variability)	<b>0.53</b>	-			
3. Total sleep time	<b>-0.34</b>	<b>-0.31</b>	-		
4. Wake after sleep onset	<b>0.15</b>	<b>0.27</b>	<b>-0.18</b>	-	
5. Sleep quality <sup>a</sup>	0.06	<b>0.08</b>	-0.05	<b>0.10</b>	-

Bold indicates P<0.05.

<sup>a</sup>Higher scores represent a poorer sleep quality.

These results were only moderately changed after additional adjustment for total sleep time, wake after sleep onset and sleep quality. No associations were found between lacunar infarcts and 24-hour activity rhythm parameters. The associations of WMLs and CMBs with fragmentation were independent of each other. When both variables were studied as determinants of fragmentation in one multivariable adjusted model, they were each associated with fragmentation (B=0.08 per SD, 95% CI=0.01; 0.15 and B= 0.17 per SD, 95% CI= 0.00; 0.37, respectively).

WMLs, CMBs and lacunar infarcts were not related to total sleep time, wake after sleep onset and sleep quality (Supplemental Table 1).

**Table 3** Associations between cerebral small vessel disease and 24-hour activity rhythm parameters

	Interdaily instability <sup>a</sup>			Fragmentation (intradaily variability) <sup>a</sup>		
	B	95% CI	P value	B	95% CI	P value
White matter lesions <sup>a</sup>						
Age, sex adjusted	0.036	-0.033; 0.105	0.31	<b>0.098</b>	<b>0.030; 0.167</b>	<b>0.01</b>
Multivariable adjusted	0.028	-0.042; 0.100	0.43	<b>0.085</b>	<b>0.017; 0.154</b>	<b>0.02</b>
Lacunar infarcts (yes)						
Age, sex adjusted	-0.020	-0.311; 0.271	0.89	0.180	-0.108; 0.469	0.22
Multivariable adjusted	-0.100	-0.392; 0.194	0.51	0.103	-0.188; 0.393	0.49
Cerebral microbleeds (yes)						
Age, sex adjusted	<i>0.163</i>	<i>-0.013; 0.338</i>	<i>0.07</i>	<b>0.197</b>	<b>0.023; 0.371</b>	<b>0.03</b>
Multivariable adjusted	<i>0.167</i>	<i>-0.008; 0.341</i>	<i>0.06</i>	<b>0.192</b>	<b>0.019; 0.365</b>	<b>0.03</b>

Multivariable adjusted models are adjusted for age, sex, body mass index, activities of daily living, depressive symptoms, sleep apnea, total cholesterol, systolic blood pressure, blood glucose, antihypertensives, lipid lowering and sleep medication.

Analyses with white matter lesions are additionally adjusted for intracranial volume.

<sup>a</sup>Standardized variable.

## Discussion

In this population-based study, we found that WML volume and presence of CMBs were related to more unstable and fragmented 24-hour activity rhythms, independent of total sleep time, and sleep quality.

Unstable and fragmented 24-hour activity rhythms reflect disturbed activity rhythms. Previous studies of disrupted rhythms found that they predict morbidity, for example, dementia, cardiovascular disease and mortality, but also high BMI and depressive symptoms<sup>5,13,18,25</sup>. Little is known about cerebral small vessel disease and the associations with 24-hour activity rhythms and sleep in the general population. However, several potential mechanisms can be inferred from clinical studies. First, structural brain damage might disrupt 24-hour activity rhythms. Different pathological processes in cerebral blood vessels may directly cause structural brain damage, including those indicative of cerebral small vessel disease. WMLs, lacunar infarcts and CMBs can interrupt frontal-subcortical connections, including periventricular fibers, i.e. pathways to and from the hypothalamus, and thus interfere with the sleep-wake cycle.<sup>26,27</sup> Also, the volume and cell number in the SCN, could decrease and the function of this internal clock could decline,<sup>4</sup> possibly causing unstable 24-hour activity rhythms. In persons with brain disease such as Alzheimer's disease, who frequently have structural brain damage caused by cerebral small vessel disease,<sup>8</sup> such disturbances of the SCN are profound, and increase with the severity of Alzheimer's disease related brain

pathology.<sup>4,5</sup> In Alzheimer's disease patients disturbances in sleep and 24-hour activity rhythms are often observed.<sup>5,28</sup>

Second, because the relationship between cerebral small vessel disease with 24-hour activity rhythms and sleep likely are bi-directional, disturbances in sleep and 24-hour activity rhythms could contribute to the occurrence of cerebral small vessel disease. One possible effect mediator is changes in the neuroendocrine system. Disruptions in the sleep-wake cycle can impair the neuroendocrine system, including emotional and cognitive processes.<sup>28</sup> For example, sleep deprivation is related to an impaired ability to form new memories and to an inappropriate emotional response to negative aversive stimuli.<sup>29,30</sup> Functional impairment in the neuroendocrine system may precede long-term structural changes in the brain.

Third, an external factor may underlie the association, such as sleep disordered breathing or subclinical cardiovascular changes. Sleep disordered breathing causes disturbances in the sleep-wake pattern, such as fragmentation during sleep, and is related to stroke.<sup>31</sup> Also, subclinical cardiovascular risk factors, like hypertension or the absence of blood pressure dipping during sleep, are often seen in patients with sleep disordered breathing.<sup>32,33</sup> We accounted for subclinical cardiovascular disease by correcting for BMI, systolic blood pressure, total cholesterol, blood glucose, use of antihypertensive and lipid lowering medication, and for possible sleep apnea, as assessed by two questions from the PSQI. However, the PSQI cannot be used to diagnose sleep apnea.

As people age, their circadian rhythms and sleep change. Elderly fall asleep and wake up earlier, have increased fragmented nights, sleep less during the night and report a lower sleep quality.<sup>1,2</sup> In this study the 24-hour activity rhythm parameters interdaily instability and fragmentation were only moderately correlated to sleep parameters. This is in line with our findings that the associations of WMLs and CMBs with the fragmentation of the 24-hour activity rhythm were independent of total sleep time, wake after sleep onset and sleep quality.

To our knowledge this is the first study using both MRI scans and actigraphy in the general population to study associations between cerebral small vessel disease and 24-hour activity rhythms. The study has several strengths. First, it is embedded in a large population-based study. Second, we assessed three indicators of cerebral small vessel disease in combination with objective 24-hour activity rhythm parameters. The study also has some limitations. First, the information on the participant's clinical sleep disorders including sleep apnea was limited. To optimally control for the influence of sleep apnea, it needs to be assessed with polysomnography. Also, wrist actigraphy is only an indirect measurement of the circadian rhythm. Melatonin and core body temperature are more specific to study circadian rhythms. Finally, this study has a cross-sectional design, thus we cannot assess the causality of the relationship between cerebral small vessel disease and disturbed 24-hour activity rhythms. Our finding that cerebral small vessel disease is related to disturbed 24-hour activity

rhythms suggests that the disturbances seen in the sleep-wake rhythm in stroke and dementia patients arise early in the disease process before clinical signs are present. Future longitudinal studies are needed to study whether prevention of small vessel disease in the elderly (e.g. by antihypertensive treatment or exercise) protects against circadian rhythm disturbances. Alternatively, research can examine whether intervention supporting a stable and robust circadian rhythm protects against brain disease.

To conclude, in this population-based study of middle-aged and elderly persons, WMLs and the presence of CMBs were related to disturbed 24-hour activity rhythms, independent of sleep. Although this study cannot show the direction of the effects, these findings suggest that subclinical brain damage may affect circadian rhythms.

**Supplemental Table 1** Associations of cerebral small vessel disease with total sleep time, wake after sleep onset and sleep quality

	Objective total sleep time (hours)			Wake after sleep onset <sup>a</sup>			Sleep quality <sup>ab</sup>		
	B	95% CI	P value	B	95% CI	P value	B	95% CI	P value
<b>White matter lesions<sup>a</sup></b>									
Age, sex adjusted	-0.022	-0.084; 0.039	0.48	-0.004	-0.019; 0.011	0.61	0.033	-0.039; 0.104	0.37
Multivariable adjusted	-0.020	-0.083; 0.042	0.52	-0.004	-0.019; 0.011	0.60	0.014	-0.051; 0.080	0.67
<b>Lacunar infarcts (yes)</b>									
Age, sex adjusted	0.064	-0.194; 0.323	0.63	-0.060	-0.369; 0.248	0.70	0.215	-0.085; 0.515	0.16
Multivariable adjusted	0.120	-0.140; 0.380	0.37	-0.082	-0.393; 0.229	0.61	0.147	-0.127; 0.421	0.29
<b>Cerebral microbleeds (yes)</b>									
Age, sex adjusted	-0.054	-0.211; 0.103	0.50	-0.074	-0.261; 0.113	0.44	0.117	-0.065; 0.299	0.21
Multivariable adjusted	-0.056	-0.213; 0.100	0.48	-0.065	-0.252; 0.122	0.49	0.125	-0.040; 0.290	0.14

Multivariable adjusted models are adjusted for age, sex, body mass index, activities of daily living, depressive symptoms, sleep apnea, diabetes mellitus, total cholesterol, hypertension, lipid lowering and sleep medication.

Analyses with white matter lesions are additionally adjusted for intracranial volume.

<sup>a</sup>Standardized variable.

<sup>b</sup>Higher scores represent a poorer sleep quality

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# Chapter 3.2

**Apnea-hypopnea index, nocturnal arousals, oxygen  
desaturation and structural brain changes:  
A population-based study**

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## **Abstract**

Sleep apnea has been related to brain changes such as atrophy. However, which component of sleep apnea, the apnea-hypopnea index (AHI), nocturnal oxygen desaturation or arousals, can explain this association is unclear. In this large population-based study (n=681, mean age 62.1 years), we investigated the associations of AHI, nocturnal oxygen desaturation and arousals with cerebral gray matter, white matter and white matter lesion volumes. All participants underwent one night of polysomnography and MRI scanning of their brain. Gray matter, white matter and white matter lesion volumes adjusted for intracranial volume were studied as markers of brain atrophy. Nocturnal oxygen desaturation (per increase in events/min) was related to a smaller white matter volume (multivariable adjusted B= -8.3 ml, 95% CI= -16.7; -0.02). AHI and arousals (events/min) were not associated with gray matter, white matter or white matter lesion volumes. This suggests that oxygen desaturation during sleep relates to white matter brain atrophy, and that the amount of apneas or hypopneas or sleep apnea related arousals did not explain this association.

## Introduction

Sleep apnea is a common disorder; over 10% of the adult population (30-70 years old) have an apnea-hypopnea index (AHI)  $\geq 15$ , but the prevalence increases to about 20% in adults aged 60-70 years old.<sup>1</sup> It is characterized by repetitive respiratory events (apneas and hypopneas) during sleep, leading to sleep fragmentation, nocturnal intermittent hypoxia (or oxygen desaturation), arousals and daytime sleepiness. Sleep apnea has been associated with several clinical outcomes, such as hypertension, cardiovascular disease, cognitive decline and mortality.<sup>2-5</sup> Furthermore, in patients with stroke, sleep apnea (AHI  $\geq 10$ ) has a much higher prevalence than in the general population.<sup>6-8</sup> These findings suggest that sleep apnea may be related to structural brain changes in adults.

Previous studies of apnea severity and brain changes are inconsistent. Whereas several investigators found a relation of moderate to severe sleep apnea and the presence of white matter change and silent cerebrovascular lesions,<sup>9-11</sup> others reported no association.<sup>12-14</sup> The majority of these studies were not conducted in the general population or had a small sample size. Gray matter volumes were smaller in sleep apnea patients compared to controls, mainly in the hippocampus and frontal areas.<sup>15,16</sup> However, gray matter volume differences between sleep apnea patients and controls have not been found consistently.<sup>17</sup> It is yet unclear how several aspects of sleep apnea (the AHI, nocturnal oxygen desaturation and arousals) affects cerebral gray matter, white matter and white matter lesion volumes. In a large population-based sample of middle-aged and elderly persons, we studied the associations of AHI, nocturnal oxygen desaturation and arousals with white and gray matter brain atrophy and white matter lesion volumes. We hypothesized that the sleep apnea aspects are associated with gray and white matter brain atrophy and with larger white matter lesion volumes. We expected that the AHI would be the best predictive measure, because oxygen desaturation and arousal are included in its definition.

## Materials and Methods

### *Study population*

This study was conducted within the Rotterdam Study, a population-based cohort of persons aged 45 years and older, living in one district in Rotterdam, the Netherlands. The study targets neurological, psychiatric, cardiovascular and other chronic disorders.<sup>18</sup> The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the “Population Studies Act: Rotterdam Study”. All participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

From January 2012 until February 2014, 1434 persons were invited for the polysomnographic (PSG) sleep study; 811 participants (56.6%) agreed. Persons who participated in the PSG study did not significantly differ in age or sex from persons who refused participation. Of the 811 persons, we excluded 15 participants because the PSG was of insufficient quality. Of the included persons, 724 persons (91.0%) also had a usable MRI scan of the brain, acquired as part of the Rotterdam Scan Study.<sup>19</sup> Participants who used a continuous positive airway pressure mask, or who had a clinical stroke or MRI-defined cortical infarct were excluded (n=43). Therefore, the recordings of 681 persons were used in analyses. The time between the PSG and MRI scan was on average 10 months (standard deviation (SD) 17).

### *Polysomnography*

Ambulant PSG was recorded at the participant's home using the ambulatory Vitaport 4 (Temec Instruments, Kerkrade, the Netherlands). A trained research assistant placed all sensors. The PSG included electroencephalography (EEG: F3, F4, C3, C4, O1, O2, A1 and A2), bilateral electrooculography, electromyography, electrocardiography and respiration measurements.<sup>20</sup> Respiration was measured with respiratory belts, an oronasal thermocouple, a nasal pressure transducer and oximetry. Participants were instructed to spend the night as normal as possible. There were no restrictions on medication, alcohol and coffee use, or bedtimes. An experienced registered polysomnogram technologist (RPSGT) scored all recordings for apneas and hypopneas. Apneas were defined as a continuous reduction of airflow of at least 90% from baseline for at least 10 seconds. Hypopneas were defined as a continuous reduction of airflow of at least 30% from baseline for at least 10 seconds, together with oxygen desaturation of at least 3% from pre-event baseline, or an arousal.<sup>21</sup> We calculated the AHI as the total number of apneas and hypopneas per hour of sleep using Prana software (PhiTools, Strasbourg, France). Nocturnal peripheral oxygen desaturation was defined as the number of times per minute the participant's oxygen saturation dropped by at least 3% during sleep, regardless of presence of an apnea or hypopnea.<sup>21</sup> Arousals were measured in events per minute. Of all participants, 21 persons did not have information on oxygen desaturation and 51 persons did not have information on arousals.

### *Magnetic resonance imaging*

Brain imaging was performed with a 1.5-Tesla scanner (General Electric Healthcare, Milwaukee, USA, software version 11x) with an eight-channel head coil and included T1-weighted, T2\*-weighted, proton-density-weighted and fluid-attenuated inversion recovery sequences.<sup>19</sup> Gray matter, white matter and white matter lesion volumes were quantified by a validated automatic tissue classification technique based on a k-nearest neighbor classifying algorithm using T1-weighted, proton density weighted and FLAIR scans.<sup>22,23</sup> Intracranial volume was calculated by summing gray matter, white matter, white matter lesions and cerebrospinal fluid volumes.

### *Covariates*

Age, sex, educational level, body mass index, smoking, alcohol use, depressive symptoms, diabetes mellitus, myocardial infarction and use of sleep medication were analyzed as possible confounders based on established risk factors for brain changes.<sup>9</sup> Additionally, systolic blood pressure and total cholesterol were considered as possible intermediates and entered in additional analyses. Information on educational level (low, intermediate, high), smoking (no, previous, current) and depressive symptoms (assessed using the Center for Epidemiologic Studies Depression scale)<sup>24</sup> was collected during a home interview. During a research center visit, height and weight were measured to calculate the body mass index (kg/m<sup>2</sup>) and sitting blood pressure was measured twice using a random-zero sphygmomanometer. Serum total cholesterol was measured in mmol/L using an automated enzymatic procedure. History of myocardial infarction and diabetes were determined by medical records and self-report. Participants were asked whether they used sleep medication on the night of the PSG.

### *Statistical analysis*

We studied whether AHI, nocturnal oxygen desaturation and arousals were associated with gray and white matter brain atrophy and white matter lesion volumes using multivariable linear regression analyses. All analyses were adjusted for intracranial volume to correct for head size. Furthermore, white matter lesion volumes were normalized by natural logarithmic transformation and standardized. All sleep apnea related determinants were studied continuously and categorically. For these categorical analyses, we defined low AHI (AHI <15), moderate AHI (15-<30) and high AHI (≥30); oxygen desaturation was transformed into tertiles; and arousals were analyzed dichotomously.

We specified two etiological models and a mediator model. The first model was adjusted for age, sex and intracranial volume. The second model, the multivariable adjusted model, was additionally adjusted for body mass index, education, smoking, alcohol use, diabetes, myocardial infarction and the interval between brain scan and polysomnography study. The mediator model was additionally adjusted for systolic blood pressure and total cholesterol. We conducted two sensitivity analyses. First, AHI, oxygen desaturation and arousals were all included in one mutually adjusted model to study their independent effects. In this analysis, parameters were standardized to facilitate interpretation. Second, we tested whether sex-specific differences were present for the associations.

The proportion of missing values of the covariates never exceeded 3%. Missing values in quantitative covariates were replaced by the mean. For missing values in qualitative covariates a separate missing category was used. Analyses were performed using SPSS Statistics (version 21; SPSS, Chicago, IL, USA).

## Results

Descriptive statistics of the participants (n=681) can be found in Table 1. Participants were on average 62.1 (range 51 – 95) years, 56% was female and the average body mass index was 27.2 (SD 4.4). The average AHI was 13.5 (SD 12.5); n= 447 had a low AHI (<15), n= 153 had a moderate AHI (15-<30) and n= 81 had a high AHI (≥30). The average oxygen desaturation was 0.3 per minute (range 0 – 1.9) and the average arousal index was 0.05 per minute (range 0 – 2.6). The AHI correlated strongly with the number of oxygen desaturations ( $r= 0.79$ ,  $p<0.001$ ), but did not correlate with the number of arousals ( $r= -0.001$ ,  $p=0.98$ ). The number of oxygen desaturations and arousals correlated weakly ( $r= -0.17$ ,  $p<0.001$ ).

**Table 1** Descriptive statistics (n=681)

	Mean (SD), n (%)
Age, years	62.1 (5.4)
Sex, female	380 (55.8%)
Education: Low	53 (7.8%)
Intermediate	409 (60.1%)
High	217 (31.9%)
Body mass index, kg/m <sup>2</sup>	27.2 (4.4)
Smoking: Never	199 (29.2%)
Current	113 (16.6%)
Previous	368 (54.0%)
Alcohol use at night of PSG, units	0.5 (1.0)
Depressive symptoms, score	5.4 (6.7)
Systolic blood pressure, mmHg	132.9 (17.9)
Total cholesterol, mmol/l	5.6 (1.1)
Diabetes mellitus, yes	41 (6.0%)
Myocardial infarction, yes	11 (1.6%)
Sleep medication, yes	55 (8.1%)
AHI	13.5 (12.5)
Low AHI (<15)	447 (65.6%)
Moderate AHI (15-<30)	153 (22.5%)
High AHI (≥30)	81 (11.9%)
Nocturnal oxygen desaturation, n/min	0.3 (0.3)
Arousals, n/min	0.05 (0.15)

Abbreviations: AHI, apnea-hypopnea index; PSG, polysomnography; SD, standard deviation.

We found no associations of AHI with gray matter, white matter and white matter lesion volumes, whether modeled continuously or categorically (Table 2). No sex-specific differences were present in the associations of AHI and brain volumes.

**Table 2** Associations of AHI and brain structural measurements (n= 681)

	Gray matter (ml) B (95% CI), p	White matter (ml) B (95% CI), p	White matter lesions (SD)* B (95% CI), p
AHI continuous			
Age, sex adjusted	-0.06 (-0.24; 0.11), 0.48	-0.13 (-0.33; 0.07), 0.19	0.002 (-0.003; 0.01), 0.49
Multivariable adjusted†	-0.06 (-0.25; 0.12), 0.49	-0.06 (-0.28; 0.15), 0.55	0.001 (-0.004; 0.01), 0.72
Low AHI (<15), n= 447	0 (reference)	0 (reference)	0 (reference)
Moderate AHI (15-<30), n= 153			
Age, sex adjusted	3.4 (-1.7; 8.6), 0.19	-2.8 (-8.7; 3.2), 0.36	-0.03 (-0.17; 0.11), 0.69
Multivariable adjusted†	3.9 (-1.4; 9.2), 0.16	-1.9 (-7.9; 4.2), 0.55	-0.05 (-0.19; 0.10), 0.53
High AHI (≥30), n= 81			
Age, sex adjusted	-3.5 (-10.1; 3.2), 0.31	-5.6 (-13.3; 2.0), 0.15	0.04 (-0.13; 0.22), 0.62
Multivariable adjusted†	-3.4 (-10.3; 3.5), 0.33	-4.0 (-11.9; 3.9), 0.32	0.03 (-0.16; 0.21), 0.78

Abbreviations: AHI, apnea-hypopnea index; CI, confidence interval; SD, standard deviation. Values represent difference in brain tissue volume per unit increase in AHI. Linear regression analyses adjusted for intracranial volume. \*Normalized and standardized; †Additionally adjusted for body mass index, education, smoking, alcohol use, diabetes, myocardial infarction and interval between brain scan and polysomnography study.

More oxygen desaturations (events/minute) during sleep was related to a smaller white matter volume (B= -10.4 ml, 95% CI= -18.0; -2.8, Table 3). In the multivariable adjusted model, the effect size was attenuated (B= -8.3 ml, 95% CI= -16.7; -0.02). Further adjustment for the possible mediators systolic blood pressure and total cholesterol did not change the effect (B= -8.4, 95% CI= -16.7; -0.03). Categorical analyses confirmed a dose-dependent effect. No associations were found between oxygen desaturation and gray matter or white matter lesion volumes. We found no associations between the numbers of arousals with gray and white matter brain atrophy or white matter lesion volumes, whether modeled continuously or dichotomously (Table 4).

In a mutually adjusted analysis, oxygen desaturation remained associated with white matter (B= -5.5 ml per SD, 95% CI= -9.9; -1.0) whereas the AHI and arousals were not (B= 3.4 ml per SD, 95% CI= -1.0; 7.8; B= -1.0 ml per SD, 95% CI= -3.7; 1.6 respectively. Parameters were standardized to facilitate interpretation).

## Discussion

In this large population-based study, we assessed whether aspects of sleep apnea were related to brain changes. The AHI, indicating the frequency of breathing pauses, and arousals were not related to gray and white matter brain atrophy and white matter lesion volumes. In contrast, nocturnal oxygen desaturation was related to white matter atrophy independent of covariates.



**Table 3** Associations of nocturnal oxygen desaturation with brain structural measurements (n= 660)

	Gray matter (ml) B (95% CI), p	White matter (ml) B (95% CI), p	White matter lesions (SD)* B (95% CI), p
Oxygen desaturation (n/min)			
Age, sex adjusted	-4.3 (-11.0; 2.3), 0.20	<b>-10.4 (-18.0; -2.8), 0.01</b>	0.04 (-0.14; 0.21), 0.67
Multivariable adjusted <sup>†</sup>	-4.6 (-11.7; 2.7), 0.22	<b>-8.3 (-16.7; -0.02), 0.049</b>	-0.004 (-0.20; 0.19), 0.97
Mediator model <sup>‡</sup>	-4.6 (-11.8; 2.7), 0.22	<b>-8.4 (-16.7; -0.03), 0.049</b>	-0.004 (-0.20; 0.19), 0.97
Low oxygen desaturation, n= 220			
	0 (reference)	0 (reference)	0 (reference)
Moderate oxygen desaturation, n= 221			
Age, sex adjusted	-1.4 (-6.6; 3.8), 0.60	-1.5 (-7.5; 4.6), 0.63	-0.09 (-0.23; 0.05), 0.19
Multivariable adjusted <sup>†</sup>	-1.1 (-6.5; 4.2), 0.67	-0.7 (-6.8; 5.4), 0.83	-0.11 (-0.25; 0.04), 0.14
High oxygen desaturation, n= 219			
Age, sex adjusted	-2.8 (-8.1; 2.4), 0.29	<b>-6.1 (-12.2; -0.1), 0.047</b>	-0.00 (-0.14; 0.14), 0.99
Multivariable adjusted <sup>†</sup>	-2.6 (-8.2; 3.1), 0.37	-4.2 (-10.7; 2.3), 0.20	-0.03 (-0.18; 0.12), 0.70

Abbreviations: AHI, apnea-hypopnea index; CI, confidence interval; SD, standard deviation. Values represent difference in brain tissue volume per unit increase in oxygen desaturation. Linear regression analyses adjusted for intracranial volume. \*Normalized and standardized; <sup>†</sup>Additionally adjusted for body mass index, education, smoking, alcohol use, diabetes, myocardial infarction and interval between brain scan and polysomnography study; <sup>‡</sup>Additionally adjusted for systolic blood pressure and total cholesterol.

**Table 4** Associations of nocturnal arousals and brain structural measurements (n= 630)

	Gray matter (ml) B (95% CI), p	White matter (ml) B (95% CI), p	White matter lesions (SD)* B (95% CI), p
Arousals continuous			
Age, sex adjusted	5.7 (-8.9; 20.3), 0.44	0.11 (-16.8; 17.0), 0.99	0.21 (-0.17; 0.60), 0.28
Multivariable adjusted <sup>†</sup>	4.4 (-10.3; 19.2), 0.44	-0.85 (-17.8; 16.1), 0.92	0.24 (-0.15; 0.63), 0.22
No arousals, n= 396			
	0 (reference)	0 (reference)	0 (reference)
Arousals, n= 234			
Age, sex adjusted	2.8 (-1.7; 7.3), 0.22	1.6 (-3.6; 6.8), 0.54	0.02 (-0.10; 0.14), 0.73
Multivariable adjusted <sup>†</sup>	2.0 (-2.7; 6.6), 0.41	2.1 (-3.2; 7.4), 0.44	0.04 (-0.09; 0.16), 0.57

Abbreviations: CI, confidence interval; SD, standard deviation. Values represent difference in brain tissue volume per unit increase in arousals. Linear regression analyses adjusted for intracranial volume. \*Normalized and standardized; <sup>†</sup>Additionally adjusted for body mass index, education, smoking, alcohol use, diabetes, myocardial infarction and interval between brain scan and polysomnography study.

Nocturnal intermittent oxygen desaturation is related to an increase in sympathetic vasoconstriction, which can increase blood pressure and change the structure and function of blood vessels, including blood vessels in the brain.<sup>25</sup> We found a relation between nocturnal oxygen desaturation and white matter atrophy, which was not accounted for by systolic blood pressure or total cholesterol. Furthermore, the association between oxygen desaturation and white matter atrophy was independent of myocardial infarction, another possible cause of oxygen desaturation. This suggests that oxygen desaturation is directly causing white matter atrophy or a different factor, such as anemia or arteriosclerosis, might

underlie this association. Anemia and arteriosclerosis have both been associated with white matter loss and stroke.<sup>26,27</sup> In contrast, white matter atrophy might also cause oxygen desaturation. Patients with stroke have a higher prevalence of sleep disordered breathing than persons with the same age without stroke.<sup>6</sup> It has been found that after the acute phase of stroke the AHI decreases in some patients.<sup>7,28</sup> However, it also has been reported that only central respiratory events (not obstructive events) decrease after this acute phase.<sup>29</sup>

Hypoxia and hypotension have smaller effects on gray matter than on white matter volume.<sup>30,31</sup> This might explain why we found no association of oxygen desaturation and gray matter atrophy.

Unexpectedly as white matter atrophy and white matter lesions often co-occur, oxygen desaturation was not associated with more white matter lesions. Vernooij et al. (2008) found that white matter atrophy and white matter lesions are both related to a loss of microstructural integrity of white matter, but in distinct brain regions.<sup>32</sup> This indicates that these processes are independent, and have a different pathophysiology. The oxygen desaturation measured in this population-based sample might not be severe enough to cause white matter lesions, but can cause subtle white matter atrophy.

Previous studies of the association of apnea severity and white matter change are inconsistent. Several studies found that moderate to severe sleep apnea is associated with the presence of white matter lesions, impaired white matter integrity, silent cerebrovascular lesions and stroke.<sup>7,9-11,33,34</sup> Also, smaller gray matter volumes have been found in sleep apnea patients, especially in hippocampal and frontal areas.<sup>15,16</sup> However, other studies observed no association of sleep apnea severity with gray matter, white matter or white matter lesion volumes.<sup>12-14,17</sup> We found no associations between AHI and white matter lesions or brain volumes. In general, previous studies had small sample sizes and were conducted in case-control designs, whereas we assessed sleep apnea components in a large study from the general population. Our results suggest a less prominent association of sleep apnea with brain damage in the general population than in clinical samples. Many persons with mild or moderate sleep apnea in the general population do not seek treatment. This suggests that some community-dwelling persons suffer less from the consequences of sleep apnea than those seeking treatment with the same degree of apnea. It is also possible that sleep apnea patients with white matter atrophy are more impacted in their daily functioning and more likely to be referred than those without lesions. Therefore the results in clinical samples might be stronger. Another reason for the discrepancies in results is that many studies of sleep apnea assess sleep apnea severity only by the AHI index. The AHI is a crude measure of measuring sleep apnea. For example, the definitions for hypopneas used to calculate the AHI have varied in the field historically, calculating the AHI ignores the temporal distribution of the apneas and hypopneas and it does not take the duration of events into account.<sup>35</sup>

This study has several strengths. First, it is embedded in a population-based cohort. Therefore, results are generalizable and we could assess many different covariates. Furthermore, to our knowledge this is the largest study of sleep apnea and brain changes in the cerebrum in the general population. However, this study also has some limitations. First, it is a cross-sectional study. Therefore we cannot rule out a long-term effect of sleep apnea. Second, brain imaging and PSG were not conducted in the same day.

## **Conclusion**

To conclude, this population-based study showed that nocturnal oxygen desaturation is related to white matter atrophy, but that the AHI or sleep apnea related arousals do not explain this association. This suggests that oxygen desaturation is a particularly sensitive predictor of white matter atrophy. Future research is needed to study whether different causes of oxygen desaturation, such as anemia or arteriosclerosis, explain the association of oxygen desaturation and white matter atrophy.

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# Chapter 3.3

## **Brain structure, EEG activity during sleep and sleep quality: A population-based study of middle-aged and elderly persons**

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*Submitted*

## Abstract

**Study objectives:** Poor sleep, accompanied by changes in the EEG, is commonly seen at older age. In parallel, variations in the brain, such as atrophy, vascular lesions, and brain connectivity commonly occur. We hypothesized that variations in brain morphology underlie poor sleep in middle-aged and elderly persons.

**Design:** Cross-sectional study.

**Setting:** Population-based, Rotterdam Study, the Netherlands.

**Participants:** 643 participants (mean age 62 years (range 52 – 95), 54.1% women).

**Interventions:** None.

**Measurements and Results:** All participants underwent MRI and polysomnography to assess brain tissue volumes, vascular lesions and connectivity between brain areas, and absolute delta and beta power, total sleep time, sleep onset latency and wake after sleep onset. More beta power related to shorter sleep time ( $\beta = -0.18$ , 95% CI= -0.27; -0.10), longer wake after sleep onset ( $\beta = 0.25$ , 95% CI= 0.17; 0.34) and poor subjective sleep quality ( $\beta = 0.11$ , 95% CI= 0.03; 0.19). Image-derived brain measures were related to beta power during NREM sleep: smaller gray matter volume was associated with more beta power ( $\beta = -0.10$ , 95% CI= -0.18; -0.03), whereas both smaller white matter volume and less white matter integrity were related to less beta power ( $\beta = 0.22$ , 95% CI= 0.15; 0.29,  $\beta = 0.15$ , 95% CI= 0.06; 0.24, respectively). We did not find direct or indirect associations of image-derived brain measures with delta power or other sleep parameters.

**Conclusions:** Brain structural variations relate to beta power. However, structural brain variations did not account for the associations of beta power with sleep time, sleep latency, wake after sleep onset, and sleep quality.

## Introduction

The electroencephalogram (EEG) is key in identifying poor sleep during polysomnography (PSG), the gold-standard for sleep research. Poor sleep quality has been associated with physical disabilities, depressive symptoms, cognitive impairment, morbidity and mortality.<sup>1-3</sup> Poor sleepers are characterized by lower levels of all frequencies below the beta frequency (delta, theta, alpha and sigma), especially delta power, during non-rapid eye movement (NREM) sleep, whereas beta power is higher during NREM sleep.<sup>4</sup> Delta power (or slow wave activity) is an indicator of homeostatic sleep pressure, and in healthy persons gets stronger the longer they are awake.<sup>5</sup> On the other hand, elevated beta power during sleep indicates an increased cortical activation, or hyperarousal, during sleep,<sup>4</sup> which marks sleep disruption. These EEG patterns during sleep are often person specific and invariant over the night.<sup>6</sup> Therefore, it has been postulated that EEG patterns may mainly relate to other stable characteristics, such as brain structure, and to a lesser extent to other day-by-day varying sleep influencing mechanisms.

About 80% of elderly persons report at least one sleep complaint, such as problems falling asleep, short sleep, waking up frequently, and a lower sleep quality.<sup>1, 7, 8</sup> These changes can also be observed in the EEG; in older persons, lower EEG power frequencies, such as delta, decrease, whereas higher EEG frequencies, such as beta, increase during sleep.<sup>9-11</sup> However, with aging structural brain changes also occur, for example atrophy, vascular lesions, and reduced connectivity between brain areas.<sup>12</sup> Studies performed in middle-aged and elderly patients with insomnia found smaller gray matter volumes in insomnia patients,<sup>13, 14</sup> suggesting an association between brain structure and sleep quality. Possibly these structural changes in the brain underlie the alteration of the sleep EEG at old age.

In a population-based setting of middle aged and elderly persons, we studied the associations between image-derived brain measures and sleep parameters, measured both subjectively and objectively. Brain morphology, connectivity and brain vascular pathology were assessed by brain magnetic resonance imaging (MRI) and beta and delta power during sleep were assessed with PSG. Also, the sleep parameters total sleep time, sleep onset latency and wake after sleep onset were measured with PSG, whereas subjective sleep quality was assessed by a validated questionnaire. We hypothesized that age-related structural brain variation (smaller tissue volumes, vascular lesions, and reduced connectivity between brain areas) relates to poor sleep, reflected both in EEG activity during sleep and other objective and subjective sleep parameters.



## Methods

### *Study population*

This study was conducted within the Rotterdam Study, a population-based cohort among middle-aged and elderly adults that started in 1990 in Rotterdam, the Netherlands. The study aims to investigate causes and consequences of age-related diseases. Since 2005, brain imaging is part of the core examination in the Rotterdam Study and since 2012 PSG has been introduced.<sup>15</sup> The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus University Medical Center and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the “Population Studies Act: Rotterdam Study”. All participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

From January 2012 until February 2014, 1434 persons were invited to participate in a PSG sleep study; of whom 811 participants (56.6%) agreed. Persons who participated in the PSG study did not significantly differ in age and sex from persons who refused to participate in this study. Of the PSG sleep study participants, 727 persons (90.0%) had a good quality MRI scan of the brain, acquired as part of the Rotterdam Scan Study.<sup>16</sup> Participants with an MRI-defined cortical infarct were excluded from the present study (n=22). Of the remaining 702 persons, 643 persons (91%) had a sufficient quality EEG for quantitative analyses. The average time between MRI scan and PSG was 10 months (standard deviation (SD) 17).

### *Sleep*

PSG recordings were performed at participants' home using the ambulatory Vitaport 4 (Temec Instruments, Kerkrade, the Netherlands). A trained research assistant placed all sensors. The PSG included EEG (F3, F4, C3, C4, O1, O2, A1 and A2), bilateral electrooculography, electromyography, electrocardiography and respiration measurements.<sup>17</sup> Respiration was measured with respiratory belts, an oronasal thermocouple, a nasal pressure transducer and oximetry. Participants were instructed to spend the night as normal as usual with no restrictions on bedtimes and the use of medication, alcohol and coffee. An registered polysomnogram technologist scored all PSG-recordings in 30-second epochs according to standard American Academy of Sleep Medicine scoring criteria.<sup>18</sup> Each epoch was scored as wake, N1, N2, N3 or REM sleep.

Quantitative EEG analyses were performed with PRANA Software Suite (PhiTools, Strasbourg, France). First, artifacts were automatically detected. Spectral analyses were then performed using 4-second windows on the same central EEG lead (C3/A2) and output data were averaged over 30-second epochs. This single channel was used for continuity. Spectral powers during NREM were finally summed in the delta (0.75-4.5 Hz) and beta (15.5-22.5 Hz) frequency bands. Delta power and beta power were normalized by logarithmic transformation.

The PSG was also used to estimate total sleep time, sleep onset latency, and wake after sleep onset. Sleep onset latency and wake after sleep onset were normalized by logarithmic transformation.

Subjective sleep quality was assessed with the global score of the Pittsburgh Sleep Quality Index (PSQI), which has been demonstrated to have a good test-retest reliability and validity.<sup>19</sup> Higher scores represent a poorer sleep quality. The global quality score was normalized by logarithmic transformation. Information on global sleep quality was available for 622 persons (97% of total sample).

### *Brain structure*

Brain imaging was performed with a 1.5-Tesla scanner (General Electric Healthcare, Milwaukee, USA, software version 11x) with an eight-channel head coil and included T1-weighted, T2\*-weighted, proton-density-weighted and fluid-attenuated inversion recovery (FLAIR) sequences.<sup>16</sup> Using automated post-processing tissue classification, we obtained global volumes of total brain, gray matter, white matter and white matter lesions (ml).<sup>20, 21</sup> Volumes were expressed as percentages of the intracranial volume (ICV). Furthermore, white matter lesion volume was expressed as percentage of ICV, and normalized by logarithmic transformation. In addition, diffusion tensor imaging (DTI) was used in a subset of our sample (n= 408, 63.5%) to measure white matter microstructure. For DTI, we performed a single-shot, diffusion-weighted, spin-echo, echoplanar imaging sequence. Maximum b value was 1,000 s/mm<sup>2</sup> in 25 non-collinear directions.<sup>22</sup> All diffusion data were pre-processed using a standardized pipeline, including correction for motion and eddy currents, estimation of the diffusion tensor, and registration to tissue segmentation to obtain global mean DTI measures in the normal-appearing white matter.<sup>22</sup> These measures included mean diffusivity (MD) and fractional anisotropy (FA). In general, higher values of MD and lower values of FA are indicative of poor microstructural integrity of the white matter.

### *Covariates*

We selected age, sex, educational level, body mass index, smoking, blood pressure, diabetes, myocardial infarction, stroke, depressive symptoms, cognitive function, coffee and alcohol use, sleep apnea and use of sleep medications as possible confounders based on prior knowledge. Information on educational level (low, intermediate, high), smoking (no, previous, current) and depressive symptoms (assessed using the Center for Epidemiologic Studies Depression scale<sup>23</sup>) was collected during a home interview. During a visit to the research center, height and weight were measured to calculate the body mass index (kg/m<sup>2</sup>); sitting blood pressure was measured twice using a random-zero sphygmomanometer; and cognitive function was measured by the Mini-Mental State Examination.<sup>24</sup> Stroke, myocardial infarction and diabetes mellitus were determined by medical records and self-

report. To measure coffee, alcohol and sleep medication use, we asked the participants whether they used any one of these on the night of the PSG. Sleep apnea was assessed with the apnea-hypopnea index (AHI). Apneas were defined as a continuous reduction of airflow of at least 90% from baseline for at least 10 seconds. Hypopneas were defined as a continuous reduction of airflow of at least 30% from baseline for at least 10 seconds, together with oxygen desaturation of at least 3% from pre-event baseline or an arousal.<sup>25</sup> We calculated the AHI as the total number of apneas and hypopneas per hour of sleep.

### *Statistical analysis*

First, we tested the associations of delta and beta activity during sleep with total sleep time, sleep onset latency and wake after sleep onset from the PSG, as well as subjective sleep quality assessed by questionnaire. Second, we studied whether smaller brain volumes, vascular lesions and microstructural integrity were associated with delta and beta power. Last, we tested whether these structural brain measures were related to total sleep time, sleep onset latency, wake after sleep onset and subjective sleep quality.

All analyses were performed using multiple linear regressions adjusted for age, sex, time between MRI scan and PSG study, body mass index, smoking, diabetes, depressive symptoms, sleep apnea and coffee, alcohol and sleep medication use at the PSG night. We did not adjust for the other covariates because these did not change the effect estimates by more than 10% nor were significant predictors of the outcome. All determinants and outcomes were standardized.

In a sensitivity analyses, we only included persons that slept at least four hours during the PSG night.

Post hoc, we analyzed whether beta activity had a negative mediation effect (also called suppression) in the relation between white matter volume and total sleep time,<sup>26</sup> since we found inverse associations in the presumed mediation pathway (i.e. a positive association between white matter volume and beta power and negative association between beta power and total sleep time).

Only covariates had missing values. The number of missing values per parameter never exceeded 3%. Missing values in quantitative predictors were replaced by the mean. Missing values in qualitative predictors were accounted for in a separate missing category. A P-value <0.05 was considered statistically significant. Analyses were performed using SPSS Statistics (version 21; SPSS, Chicago, IL, USA).

## Results

Population characteristics are listed in Table 1. The mean age of this study sample (n= 643) was 62.2 years old (range 52 – 95 years) and 54% was female. Participants slept on average 380 minutes (6.3 hours) at the PSG night (range 98 – 598 minutes).

**Table 1.** Population characteristics (n=643)

Measures	Mean $\pm$ SD or n (%)
Demographics	
Age, years	62.2 $\pm$ 5.4
Female	348 (54.1)
Education: Low	49 (7.6)
Intermediate	385 (59.9)
High	207 (32.2)
Health indicators	
Body mass index, kg/m <sup>2</sup>	27.3 $\pm$ 4.4
Smoking: No	185 (28.8)
Current	111 (17.3)
Previous	347 (54.0)
Systolic blood pressure, mmHg	133.2 $\pm$ 18.1
Diastolic blood pressure, mmHg	81.3 $\pm$ 10.7
Diabetes mellitus	41 (6.4)
Myocardial infarction	12 (1.9)
Stroke	11 (1.7)
Depressive symptoms, score	5.4 $\pm$ 6.8
Cognitive functioning, score	28.6 $\pm$ 1.3
PSG related parameters	
Alcohol use at night of PSG, units	0.5 $\pm$ 0.9
Coffee use at night of PSG, cups	0.9 $\pm$ 0.8
Sleep medication at night of PSG	57 (8.9)
Apnea Hypopnea Index	13.9 $\pm$ 12.9
Brain structure	
Total brain volume	959.3 (98.0)
Gray matter volume	538.2 (53.2)
White matter volume	417.5 (56.6)
White matter lesion volume	3.6 (4.1)
Fractional anisotropy	0.3 (0.01)
Mean diffusivity	0.7 (0.02)
Objective sleep assessed by PSG	
Delta power, $\mu$ V <sup>2</sup>	145.0 (100.9)
Beta power, $\mu$ V <sup>2</sup>	3.1 (2.0)
Total sleep time, minutes	379.8 $\pm$ 64.1
Sleep onset latency, minutes	21.0 $\pm$ 24.6
Wake after sleep onset, minutes	69.2 $\pm$ 44.8
Subjective sleep	
Global PSQI, score	3.4 $\pm$ 3.3

Abbreviations: PSG, polysomnography; PSQI, Pittsburgh Sleep Quality Index; SD, standard deviation

**Table 2.** Associations between EEG activity during NREM sleep and other objective and subjective sleep parameters (n=643)

EEG power	Total sleep time (per SD)		Sleep onset latency (per SD)		Wake after sleep onset (per SD)		Subjective sleep quality (per SD) <sup>a</sup>	
	$\beta$ (95% CI)	P	$\beta$ (95% CI)	P	$\beta$ (95% CI)	P	$\beta$ (95% CI)	P
Delta power (per SD)	0.02 (-0.07; 0.11)	0.65	-0.01 (-0.09; 0.08)	0.91	<b>-0.10 (-0.19; -0.01)</b>	<b>0.03</b>	0.08 (-0.01; 0.16)	0.08
Beta power (per SD)	<b>-0.18 (-0.27; -0.10)</b>	<b>&lt;0.001</b>	0.08 (-0.003; 0.17)	0.06	<b>0.25 (0.17; 0.34)</b>	<b>&lt;0.001</b>	<b>0.11 (0.03; 0.19)</b>	<b>0.01</b>

Abbreviations: CI, confidence interval; NREM non-rapid eye movement; SD, standard deviation. Values are amount of change (95% CI) in normalized and standardized objective and subjective sleep parameter per SD increase in normalized and standardized delta or beta power, adjusted for age, sex, interval between brain scan and polysomnography study, body mass index, smoking, diabetes, depressive symptoms, coffee and alcohol use, apnea hypopnea index, and sleep medication use at polysomnography night. <sup>a</sup>Assessed with the Pittsburgh Sleep Quality Index, a higher score represents lower sleep quality, n=622.

In Table 2 the fully adjusted associations of NREM sleep delta and beta power with total sleep time, sleep onset latency, wake after sleep onset and sleep quality are presented. A higher beta power was related to a shorter total sleep time ( $\beta = -0.18$ , 95% CI= -0.27; -0.10), a longer wake after sleep onset ( $\beta = 0.25$ , 95% CI= 0.17; 0.34) and a lower sleep quality ( $\beta = 0.11$ , 95% CI= 0.03; 0.19, a high sleep quality score corresponds to a poor sleep quality). Higher delta power was related to shorter wake after sleep onset ( $\beta = -0.10$ , 95% CI= -0.19; -0.01).

Table 3 shows the associations between brain tissue volumes and delta and beta activity during NREM sleep. A smaller gray matter volume was associated with more beta power ( $\beta = -0.10$ , 95% CI= -0.18; -0.03), whereas smaller total brain and white matter volumes were associated with less beta power ( $\beta = 0.15$ , 95% CI= 0.07; 0.23,  $\beta = 0.22$ , 95% CI= 0.15; 0.29, respectively). White matter lesion volume was related to more beta power ( $\beta = 0.08$ , 95% CI= 0.01; 0.16). When we analyzed white matter microstructure, we found that higher FA, indicating better microstructural integrity, related to more beta power ( $\beta = 0.15$ , 95% CI= 0.06; 0.24). The brain tissue volumes were not associated with delta power.

**Table 3.** Associations between brain structures and EEG activity during NREM sleep (n=643)

Brain structure	Delta (per SD)		Beta (per SD)	
	$\beta$ (95% CI)	P	$\beta$ (95% CI)	P
<b>Tissue volumes</b>				
Total brain volume (per SD)	0.04 (-0.04; 0.12)	0.35	<b>0.15 (0.07; 0.23)</b>	<b>&lt;0.001</b>
Gray matter volume (per SD)	-0.03 (-0.10; 0.04)	0.38	<b>-0.10 (-0.18; -0.03)</b>	<b>0.004</b>
White matter volume (per SD)	0.06 (-0.01; 0.13)	0.10	<b>0.22 (0.15; 0.29)</b>	<b>&lt;0.001</b>
<b>Vascular lesions</b>				
White matter lesions volume (per SD)	0.02 (-0.06; 0.09)	0.67	<b>0.08 (0.01; 0.16)</b>	<b>0.03</b>
<b>Microstructural integrity (n=408)</b>				
MD <sup>a</sup> (per SD)	0.04 (-0.06; 0.14)	0.41	-0.04 (-0.13; 0.06)	0.44
FA <sup>a</sup> (per SD)	0.02 (-0.07; 0.11)	0.70	<b>0.15 (0.06; 0.24)</b>	<b>0.001</b>

Abbreviations: CI, confidence interval; FA, fractional anisotropy; MD, mean diffusivity; NREM non-rapid eye movement; SD, standard deviation. Brain tissue volumes are expressed in % of intracranial volume. Values are amount of change (95% CI) in normalized and standardized brain power (per SD) per SD increase in brain tissue volumes, adjusted for age, sex, interval between brain scan and polysomnography study, body mass index, smoking, diabetes, depressive symptoms, coffee and alcohol use, apnea hypopnea index, and sleep medication use at polysomnography night. <sup>a</sup>Additionally adjusted for intracranial volume, white matter and white matter lesion volume.

We did not find associations between structural brain variations and total sleep time, sleep onset latency, wake after sleep onset and subjective sleep quality (Supplemental Table 1). Results did not change when we only included persons who slept at least four hours during the PSG night (n=628, results available upon request).

In post hoc analysis, we analyzed whether beta activity had a negative mediation (suppression) effect in the relation between white matter volume and total sleep time.<sup>26</sup> We found a significant suppression effect of beta power in the relation between white matter volume and total sleep time (indirect effect= -0.04, 95% CI= -0.07; -0.02). This revealed a suggestive, although not significant, direct association between a larger white matter volume and larger total sleep time (direct effect per SD increase in white matter volume= 0.08, 95% CI= -0.001; 0.16). This suppression effect is depicted in supplementary Figure 1.

## Discussion

Among community-dwelling middle-aged and elderly subjects, we found an association between structural brain variation and beta power during NREM sleep: a smaller gray matter volume was accompanied by more beta power, whereas a smaller white matter volume and poor white matter microstructure were related to less beta power. Furthermore, more absolute beta power related to a shorter total sleep time, a longer wake after sleep onset and poor subjective sleep quality. However, structural brain variation did not relate to poor sleep, objectified by total sleep time, sleep onset latency and wake after sleep onset, nor to subjective sleep quality.

EEG patterns during sleep are person specific and invariant over the night.<sup>6</sup> Therefore, it has been postulated that EEG patterns may mainly relate to other stable characteristics, such as brain structure, and to a lesser extent to other day-by-day varying sleep influencing mechanisms. Because with aging both structural brain variation and sleep changes occur, we hypothesized that smaller brain volumes, vascular lesions, and reduced connectivity between brain areas relate to poor sleep.

In line with our hypothesis, we found that a smaller gray matter volume related to more beta power. A previous study of gray matter volume and EEG frequencies found a positive relation between gray matter volume and alpha, sigma and beta frequencies, the opposite of what we found.<sup>27</sup> However, a key difference between this study and ours is the age range (18 – 35 years versus 52 – 95 years). Several studies have found smaller gray matter volumes in insomnia patients.<sup>13, 14</sup> This suggests that smaller gray matter volume is related to poor sleep. However, in our study a smaller gray matter volume was only related to more beta power and not directly related to poor sleep. This might imply that other factors than gray matter structure, for example gray matter functioning or genetic factors, may be more important in the association with these sleep parameters.

We also demonstrated an association of larger white matter volume and better white matter structure with more beta power. More beta power during the night indicates increased cortical activation (hyperarousal), causing problems falling asleep, fragmented sleep and, as

a consequence, short sleep duration. With aging, hyperarousal could be caused by impaired functioning of the circadian clock.<sup>28, 29</sup> This is supported by our findings that more beta activity during NREM sleep was related to a shorter total sleep time, a shorter wake after sleep onset and poor subjective sleep quality. Though more beta activity during sleep is considered unfavourable, during wake, more beta power is associated with better vigilance and cognition.<sup>30-32</sup> This may partly explain the association we found between a larger white matter volume and more beta power. It may be hypothesized that the important role of white matter in connectivity of brain regions during the day, is also reflected in more beta power at night. More research is needed to confirm this hypothesis.

Brain variation occurs with age, including brain atrophy, more vascular lesions, and poorer brain connectivity. In this study, brain atrophy was represented by expressing brain tissue volumes as percentage of the ICV, with lower tissue volumes indicating more brain atrophy. Next to this macrostructural brain change, also microstructural changes take place with aging. With older age poorer microstructural integrity is seen, reflected in decreases in FA and increases in MD.<sup>33</sup> In our study the association between more white matter volume and more beta power was supported by the observation that lower FA is associated with less beta power.

We did not find any associations between structural brain variations and objective total sleep time, sleep onset latency, wake after sleep onset or subjective sleep quality. There are several possible explanations for this lack of associations. First, we should consider the possibility that structural brain variations do not underlie the association between more beta power and poor sleep. It could be that other factors, for example genetic factors or general health, affect sleep quality more directly. Second, we found indications that the effect between white matter and total sleep time is suppressed by beta power. This suggests that the association between brain structure, specifically white matter, and total sleep time is obscured by the relation of white matter with beta power. White matter might be essential for the transportation of beta power. It may be hypothesized that the important role of white matter in connectivity of brain regions during the day, is also reflected in more beta power at night.

White matter and beta power are inversely related to total sleep time. Third, structural brain variation is possibly only in specific brain regions related to total sleep time, sleep onset latency, wake after sleep onset and subjective sleep quality. We measured global gray and white matter volumes, together with global microstructural white matter, but we did not investigate specific lobes or smaller brain regions.

In contrast to our hypothesis, smaller gray and white matter volumes were not related to the slow wave delta activity. A relation has been found between gray and white matter volumes and slower EEG frequencies during NREM sleep.<sup>34, 35</sup> However, in line with our study, Buchmann et al. (2011) did not find a relation between global gray matter volume and



slow wave activity.<sup>27</sup> It is difficult to compare these sleep studies to our study, because they were performed in small groups of healthy participants and, except for the study of Mander et al. (2013), in young participants. Also, these studies used different imaging techniques. Possibly, only specific brain areas relate to delta activity.

Our study was embedded in a population-based cohort which gives rise to several strengths. First, results are generalizable and a large number of covariates was available. In addition, the current study consists of a large sample with both ambulant PSG and a brain-MRI generating more power. Also, we estimated white matter microstructural integrity. Most studies including these measurements are small. However, this study also has some limitations. First, it is a cross-sectional study. Therefore we cannot say anything about the direction of effects or study brain atrophy over time. Although we hypothesized that brain structure underlies sleep quality, sleep seems to be important for brain health as well.<sup>36</sup> Xie et al. (2013) found that during sleep potentially neurotoxic waste products are removed from the brain interstitial space. Problems in this sleep-process could lead to brain damage. Second, we looked at the delta and beta power frequencies derived from C3/A2, we are therefore not able to comment on differences between regions of the brain. Third, there was a time-lag between the MRI and sleep measures, we have tried to minimize any confounding effects of this time-lag by controlling for it in all analyses.

To conclude, in this study in a middle-aged and elderly general population, we confirmed that more beta power during NREM sleep were related to a shorter total sleep time, a longer wake after sleep onset and poor reported sleep quality. In addition to this, we found that structural brain variation related to beta power during NREM sleep: a smaller gray matter volume was associated with more beta power, whereas a smaller white matter volume and poorer white matter microstructure were associated with less beta power. However, this brain structural variation did not affect other aspects of poor sleep directly, which suggests that brain structural variations do not account for the association between more beta power and poor sleep. Future longitudinal studies are needed to investigate whether age-related structural brain variation in specific brain areas does relate to poor sleep.

## Supplemental Material

Supplemental Table 1. Associations between brain structures and sleep parameters (n=643)

Brain structure	Total sleep time (per SD)		Sleep onset latency (per SD)		Wake after sleep onset (per SD) <sup>a</sup>		Sleep quality (per SD) <sup>a</sup>	
	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P
<b>Tissue volumes</b>								
Total brain volume (per SD)	0.05 (-0.04; 0.14)	0.28	-0.04 (-0.13; 0.05)	0.34	-0.01 (-0.10; 0.08)	0.79	0.03 (-0.06; 0.11)	0.56
Gray matter volume (per SD)	0.004 (-0.08; 0.08)	0.92	-0.01 (-0.09; 0.07)	0.84	-0.04 (-0.12; 0.04)	0.31	0.07 (-0.01; 0.14)	0.09
White matter volume (per SD)	0.03 (-0.05; 0.12)	0.42	-0.03 (-0.11; 0.05)	0.45	0.03 (-0.05; 0.11)	0.45	-0.04 (-0.12; 0.04)	0.29
<b>Vascular lesions</b>								
White matter lesions volume (per SD)	0.03 (-0.06; 0.11)	0.54	-0.03 (-0.12; 0.05)	0.45	-0.04 (-0.12; 0.05)	0.36	-0.07 (-0.15; 0.01)	0.11
<b>Microstructural integrity (n=408)</b>								
MD <sup>b</sup> (per SD)	-0.01 (-0.12; 0.10)	0.91	0.02 (-0.09; 0.12)	0.77	-0.08 (-0.19; 0.03)	0.14	-0.05 (-0.15; 0.05)	0.34
FA <sup>b</sup> (per SD)	-0.01 (-0.11; 0.10)	0.87	-0.06 (-0.16; 0.05)	0.28	0.08 (-0.02; 0.19)	0.11	0.03 (-0.07; 0.13)	0.52

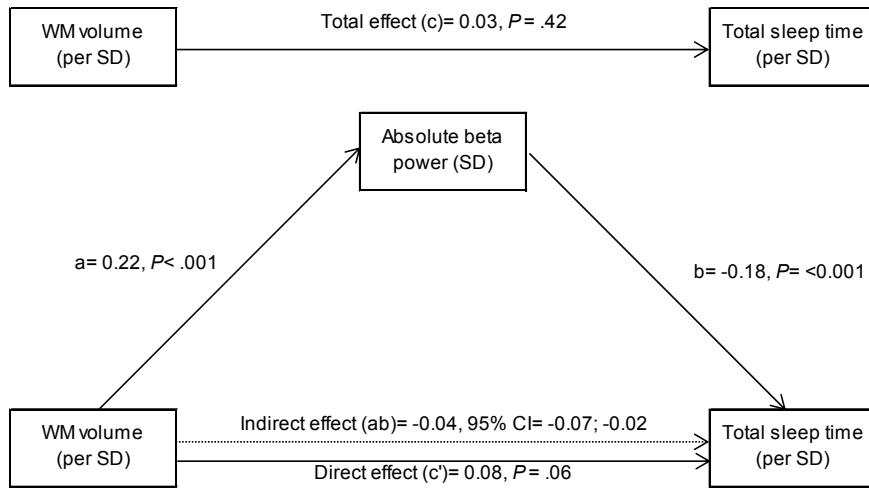
Abbreviations: CI, confidence interval; FA, fractional anisotropy; MD, mean diffusivity; SD, standard deviation

Brain tissue volumes are expressed in % of intracranial volume

Values are amount of change (95% CI) in normalized delta or beta power (per SD) per SD increase in brain tissue volumes, adjusted for age, sex, interval between brain scan and polysomnography study, body mass index, smoking, diabetes, depressive symptoms, coffee and alcohol use, apnea hypopnea index, and sleep medication use at polysomnography night

<sup>a</sup>Assessed with the Pittsburgh Sleep Quality Index, a higher score represents lower sleep quality, n=622

<sup>b</sup>Additionally adjusted for intracranial volume, white matter and white matter lesion volume



**Supplemental Figure 1** Suppression effect of beta power on the relation of white matter volume and total sleep time. Analyses are adjusted for age, sex, interval between brain scan and polysomnography study, body mass index, smoking, diabetes, depressive symptoms, coffee and alcohol use, apnea hypopnea index and, sleep medication use at polysomnography night. Values represent unstandardized betas and the associated *P* values. The 95% CI for the indirect effect is obtained from Preacher and Hayes’ bootstrapping method, based on 10000 samples.<sup>26</sup> Abbreviations: a, effect of predictor on suppressor; b, effect of suppressor on outcome; ab, indirect effect (effect a multiplied by effect b) of predictor on outcome; c, total effect (including direct effect *c*’ and indirect effect ab) of predictor on outcome; *c*’, direct effect of predictor on outcome; SD, standard deviation; WM, white matter.

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# Chapter 3.4

**Gray matter, white matter and hippocampal  
volume in middle-aged and older adults with insomnia:  
A population-based study**

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*To be submitted*

## Abstract

**Study objectives:** We investigated whether gray matter, white matter and hippocampal volumes are related to insomnia. Furthermore, within persons with insomnia, we studied whether brain volumes were related to the degree of sleep onset latency, sleep duration, sleep efficiency and sleep quality.

**Design:** Cross-sectional study.

**Setting:** This study was performed within the Rotterdam Study, the Netherlands.

**Participants:** 248 persons with insomnia (mean age 63 years) and 2729 persons without insomnia (mean age 62 years).

**Interventions:** None.

**Measurements and Results:** Insomnia was defined by a weighted set of individual Pittsburgh Sleep Quality Index items. All participants had an MRI scan of the brain to measure total brain, gray matter, white matter and hippocampal volumes. Insomnia was associated with a 10 ml smaller white matter volume (380 ml versus 390 ml, 95% CI= -18.9; -1.5). In persons with insomnia, a smaller white matter volume was related to a lower sleep efficiency (B per 10 ml= 0.65, 95% CI= 0.09; 1.20) and a smaller gray matter volume was related to a poorer sleep quality (OR per 10 ml= 0.93, 95% CI= 0.87; 0.99). Furthermore, a smaller hippocampal volume was related to a shorter sleep duration (B per ml= 0.35, 95% CI= 0.02; 0.68) and to a poorer sleep quality (OR per ml= 0.50, 95% CI= 0.29; 0.87).

**Conclusions:** In this population-based study, persons with insomnia had smaller white matter volumes, suggesting that structural brain variations underlie insomnia or that insomnia causes structural brain variations. Moreover, the size of brain structures, such as the hippocampus, corresponded to the severity of insomnia.

## Introduction

Insomnia is a highly prevalent sleep disorder, especially in older persons. Over 10% of the adult population suffers from insomnia.<sup>1</sup> Insomnia is characterized by problems in falling asleep or staying asleep, leading to daytime dysfunction because of fatigue during the day.<sup>2</sup> Insomnia affects physical, mental and social functioning and is related to a poor health-related quality of life.<sup>3</sup> The prevalence of insomnia is higher in elderly persons than in young or middle-aged persons; this increase in prevalence in the elderly is attributed to age-related sleep changes. Sleep in elderly persons is more fragmented, with less deep sleep and more early morning awakenings.<sup>1,4</sup> Furthermore, elderly persons have less physical activity, less exposure to light and more somatic disorders, which increases the risk of insomnia.<sup>2,5</sup> The sleep disturbances in insomnia are thought to partly derive from hyperarousal, as evidenced by studies of the stress system and brain waves of persons with insomnia.<sup>2</sup> Insomnia is associated with an elevated activation of the hypothalamic-pituitary-adrenal axis and with cortical arousal during NREM sleep.<sup>6,7</sup> However, hyperarousal of the stress system is mainly found in insomnia patients with an objectively measured short sleep duration and to a lesser extent in insomnia patients with a “normal” sleep duration (defined by Vgontzas et al. (2013) as  $\geq 6$  hours of sleep).<sup>8</sup> Vgontzas et al. (2013) suggest that different arousal levels of the stress system between short sleeping and “normal” sleeping insomnia patients underlie two subtypes of insomnia; one ‘biological’ type in which insomnia patients have hyperarousal of the stress system and a short sleep duration, and one ‘psychological’ phenotype, including patients with a “normal” sleep duration and sleep misperception.<sup>8</sup> Previous studies found differences in brain volumes between insomnia patients and healthy controls.<sup>9</sup> However, these studies were generally performed in small clinical samples and results are not consistent.<sup>10-18</sup> Several studies have associated insomnia with smaller gray matter volumes in different brain areas, including the frontal lobe.<sup>10,11</sup> Other researchers, however, found an increase in gray matter volume in patients with insomnia,<sup>12</sup> while some reported no differences between persons with and without insomnia in white and gray matter.<sup>13</sup> Spiegelhalter et al. (2014) reported reduced integrity of fronto-subcortical white matter tracts.<sup>17</sup> Furthermore, two studies reported a difference in hippocampal volume between persons with insomnia and controls<sup>14,18</sup>; a finding several studies failed to replicate.<sup>12,15,16</sup> Interestingly, in the studies that did not find a difference in hippocampal volume between insomnia patients and healthy controls, subanalyses in insomnia patients showed that the hippocampal volume was associated with insomnia characteristics such as poor sleep maintenance and the duration of insomnia.<sup>12,15,16</sup>

In the present population-based study we examined differences in total brain, gray matter, white matter and hippocampal volumes between a large group of insomnia cases and controls. Furthermore, we investigated, in persons with insomnia, the associations of gray



matter, white matter and hippocampal volumes with the sleep parameters sleep onset latency, sleep duration, sleep efficiency and sleep quality. We hypothesized that smaller gray matter and white matter volumes can be found in persons with insomnia. Second, we hypothesized that, in persons with insomnia, smaller gray matter, white matter and hippocampal volumes are associated with a longer sleep onset latency, shorter sleep duration, a lower sleep efficiency and a poorer sleep quality.

## Methods

### *Setting*

The present study was embedded in the Rotterdam Study, a prospective population-based cohort of persons aged 45 years or older, living in the Ommoord district in Rotterdam, the Netherlands.<sup>19</sup> The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus Medical Center and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the “Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study)”. All participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

### *Participants*

Participants with a home interview between January 2002 to December 2008 and who underwent a MRI scan of the brain were eligible for this study (n=5800). This home interview included the Pittsburgh Sleep Quality Index (PSQI)<sup>20</sup> and the Center for Epidemiologic Studies-Depression scale (CES-D).<sup>21</sup> Participants missing more than two items on the PSQI (n=148), participants with missing data on the CES-D (n=20), or participants with artifacts on the MRI scan (n=327) were excluded, leaving 5305 eligible persons.

### *Insomnia*

We defined insomnia cases using the PSQI. Missing items on the PSQI were imputed by single imputation for participants missing one or two items. An insomnia score was made from a weighted set of individual PSQI items, that correlated highly (0.84) with the Insomnia Severity Index (ISI)<sup>22</sup> in a previous analysis of web-based data.<sup>(van Someren, manuscript in preparation, 2015)</sup>

See Supplemental Table 1 for the individual PSQI items with their weights. The insomnia severity score ranged from -1.7 to 29.1 in the present study, comparable to the ISI range (0-28). We defined persons with an insomnia severity score  $\geq 15$  as insomnia cases, in line with the ISI that uses a cut-off  $\geq 15$  to define clinical insomnia. In total, 248 (4.7% of 5305) participants scored  $\geq 15$  and were considered insomnia cases. Controls were defined as participants with a PSQI score  $\leq 5$ , a CES-D score  $< 16$ , and a score of 0 on the sleep question

of the CES-D (n=2729). Participants with intermediate scores, i.e. those that did not meet the criteria for insomnia cases or controls (n=1569), and those participants that had no sleep complaints and skipped several PSQI items (n=759), were not included as controls.

### *Brain imaging*

Brain imaging was performed with a 1.5 Tesla MRI scanner (General Electric Healthcare, Milwaukee, USA, software version 11x) with an 8-channel head coil, and included T1-weighted, T2\*-weighted, proton density weighted and fluid-attenuated inversion recovery (FLAIR) sequences.<sup>23</sup> Gray matter, white matter and white matter lesion volumes were quantified by a validated automatic tissue classification technique based on a k-nearest neighbor classifying algorithm, complemented with intensity based white matter lesion detection on the FLAIR sequence.<sup>24,25</sup> Total brain volume was calculated as the sum of gray matter, white matter, and white matter lesion volumes. More information about the neuroimaging protocol and the visual and automated rating of brain images in the Rotterdam Study can be found elsewhere.<sup>23</sup> The hippocampus was segmented using an automated method described previously.<sup>26</sup> This segmentation was not performed in all participants, because this structure did not pass the data processing quality control. 165 insomnia cases and 1932 controls were included in the analyses of hippocampal volumes.

### *Covariates*

Age, sex, educational level, body mass index, cognitive functioning, depressive symptoms, hypertension, possible sleep apnea, myocardial infarction, stroke, diabetes and use of sleep medication were tested as potential confounders based on prior knowledge.<sup>2,27</sup> Educational level, depressive symptoms (CES-D score), sleep medication use (anatomical therapeutic chemical (ATC) codes N05 and N06)<sup>28</sup> and possible sleep apnea were assessed during the home interview. Possible sleep apnea was assessed with the PSQI.<sup>20</sup> Sleep apnea was considered possible when participants reported loud snoring at least 2 nights per week together with at least occasional respiratory pauses, or if they reported respiratory pauses during sleep at least 1-2 times per week.<sup>29</sup> At a visit to the research center, height and weight were measured without heavy clothing and shoes, to calculate the body mass index (kg/m<sup>2</sup>); cognitive functioning was measured using the Mini Mental State Examination<sup>30</sup>; and sitting blood pressure was measured twice on the right upper arm using a random-zero sphygmomanometer. Hypertension was defined as a systolic blood pressure  $\geq 140$  mm Hg, a diastolic blood pressure  $\geq 90$  mm Hg or use of antihypertensive medication. Myocardial infarction, diabetes, and stroke were determined using medical records.

*Statistical analyses*

We analyzed whether persons with insomnia differed from controls in total brain, gray matter, white matter and hippocampal volumes, using an analyses of covariance (ANCOVA). Model 1 was adjusted for age and sex. Model 2 was adjusted for age, sex, educational level, depressive symptoms, use of sleep medication, stroke and diabetes. We included a covariate in the model if the adjustment changed the effect estimate of the main determinants by more than 10%. Consequently, body mass index, cognitive functioning, hypertension, possible sleep apnea and myocardial infarction were not included in the models presented. Next, we tested whether findings reflect atrophy by testing whether the effect was independent of head size. To achieve this, model 3 was adjusted for the same covariates as model 2 and additionally for intracranial volume. However, collinearity of measures precluded this test as indicated by high variance inflation factors (VIF) in analyses with total brain (VIF 8.6), gray matter (VIF 3.7) and white matter volumes (VIF 3.4). Consequently, model 3 was only performed for hippocampal volumes (VIF 1.8).

In addition, we performed two sensitivity analyses. First, significant associations in model 2 were further analyzed to study whether white matter volumes in the frontal, parietal, temporal and occipital brain lobes underlay the observed associations. Second, to test whether the associations of total brain, gray matter, white matter and hippocampal volumes with insomnia differed in elderly and middle-aged persons, we added the interaction term  $\text{age} \times \text{brain volume of interest}$  to the model.

Next, we tested in the 248 insomnia cases whether total brain, gray matter, white matter and hippocampal volumes associated with sleep onset latency, sleep duration, sleep efficiency and sleep quality. These sleep parameters were assessed using the PSQI.<sup>20</sup> Sleep onset latency, sleep duration and sleep efficiency were analyzed as continuous variables using a multivariable linear regression analyses. Sleep onset latency was normalized by logarithmic transformation and standardized. Sleep quality was analyzed as a dichotomous variable using logistic regression analyses. Sleep quality was dichotomized into a moderately poor and a very poor sleep quality. Since these analyses were performed in persons with insomnia, none of these persons reported a good sleep quality.

Because of our large sample size, we were also able to differentiate insomnia cases with a short and those with a relatively long sleep duration using three different cut-offs (<4 hours versus  $\geq 4$  hours, <5 hours versus  $\geq 5$  hours and <6 hours versus  $\geq 6$  hours) in a series of sensitivity analyses. We did not include analyses comparing insomnia cases sleeping <7 hours to insomnia cases sleeping  $\geq 7$  hours, because only 21 persons reported sleeping longer than or equal to 7 hours. We investigated whether volumes of the total brain, gray matter, white matter or hippocampus of insomnia cases with a relatively short sleep duration differed from those of insomnia cases with a relatively long sleep duration and from controls. To test these stratified associations, we used an ANCOVA. Again, model 1 was adjusted for age and

sex and model 2 was adjusted for age, sex, educational level, depressive symptoms, use of sleep medication, stroke and diabetes. Seven participants did not answer the sleep duration question, therefore 241 persons with insomnia were included in these stratified analyses. All statistical tests were two-sided, and a P-value <0.05 was considered statistically significant. Given the low prevalence of missing data (maximum of 2%), missing values for continuous covariates were replaced by the mean, and for missing values of categorical covariates a separate category was defined. Analyses were performed in SPSS version 21 (SPSS Inc., Chicago, Illinois).

**Table 1** Characteristics of the study population

Mean (SD), n (%)	Controls n=2729	Insomnia cases n=248	Statistical test <sup>a</sup> insomnia cases - controls
Age, years	62.4 (8.7)	63.0 (9.8)	-0.9
Sex, female	1201 (44.0%)	203 (81.9%)	130.7***
Education: Low	259 (9.5%)	32 (12.9%)	
Intermediate	1765 (64.7%)	175 (70.6%)	9.8**
High	668 (24.5%)	41 (16.5%)	
Body mass index, kg/m <sup>2</sup>	27.5 (4.0)	28.2 (4.7)	-2.4*
Depressive symptoms, score	2.2 (3.1)	15.2 (10.6)	-19.2***
Cognitive functioning, score	28.0 (1.7)	27.8 (2.1)	2.1*
Global PSQI, score	1.6 (1.4)	12.0 (2.8)	-56.3***
Possible sleep apnea, yes	218 (8.0%)	23 (9.3%)	1.3
Sleep medication, yes	194 (7.1%)	108 (43.5%)	327.9***
Hypertension, yes	1626 (59.6%)	151 (60.9%)	1.1
Myocardial infarction, yes	112 (4.1%)	12 (4.8%)	0.3
Stroke, yes	64 (2.3%)	4 (1.6%)	0.5
Diabetes mellitus, yes	256 (9.4%)	37 (14.9%)	7.9**

Abbreviations: PSQI, Pittsburgh Sleep Quality Index; SD, standard deviation

\*p< 0.05; \*\*p< 0.01; \*\*\*p< 0.001

<sup>a</sup>Statistical test: Student's *t* test for continuous variables and  $\chi^2$  for categorical variables

## Results

This population-based study included 248 insomnia cases and 2729 controls. Population characteristics can be found in Table 1. Insomnia patients were more often female and less educated and had a higher body mass index, more depressive symptoms, a lower cognitive function, more diabetes and more often used sleep medication.

Table 2 shows the results of the associations of insomnia with total brain, gray matter, white matter and hippocampal volumes, as tested with ANCOVA. Persons with insomnia had 15 ml smaller total brain (909 ml versus 924 ml, 95% CI= -29.1; -1.1) and 10 ml smaller white matter volumes (380 ml versus 390 ml, 95% CI= -18.9; -1.5), in the multivariable adjusted

model. The association between white matter volumes and insomnia was observed in the frontal, temporal and occipital brain lobes (Supplemental Table 2.) Persons with insomnia did not differ significantly from controls on gray matter (515 ml versus 520 ml, 95% CI= -13.1; 2.3) and hippocampal volume (6.0 ml versus 6.0 ml, 95% CI= -0.13; 0.11). The addition of intracranial volume in the association of insomnia with hippocampal volume did not meaningfully change the results (6.1 ml versus 6.1 ml, 95% CI= -0.05; 0.18). The interaction term age\*brain structure of interest was not significant in any analysis.

**Table 2** Brain volume differences in participants without insomnia and persons with insomnia

	No insomnia (n=2729)	Insomnia (n=248)	Case - Control		
			Comparison of insomnia with no insomnia	Overall comparison	
	Mean <sup>a</sup>	Mean <sup>a</sup>	Difference (95% CI)	F	P
Total brain volume (ml)					
Age, sex adjusted	946	931	<b>-14.1 (-24.9; -3.4)</b>	<b>6.6</b>	<b>0.01</b>
Multivariable adjusted <sup>b</sup>	924	909	<b>-15.1 (-29.1; -1.1)</b>	<b>4.4</b>	<b>0.04</b>
Gray matter volume (ml)					
Age, sex adjusted	530	524	-5.9 (-11.8; 0.05)	3.8	0.05
Multivariable adjusted <sup>b</sup>	520	515	-5.4 (-13.1; 2.3)	1.9	0.17
White matter volume (ml)					
Age, sex adjusted	409	400	<b>-9.1 (-15.8; -2.4)</b>	<b>7.1</b>	<b>0.01</b>
Multivariable adjusted <sup>b</sup>	390	380	<b>-10.2 (-18.9; -1.5)</b>	<b>5.3</b>	<b>0.02</b>
Hippocampal volume (ml) <sup>c</sup>					
Age, sex adjusted	6.0	5.9	-0.03 (-0.12; 0.07)	0.28	0.60
Multivariable adjusted <sup>b</sup>	6.0	6.0	-0.01 (-0.13; 0.11)	0.05	0.83

Abbreviations: CI, confidence interval; ICV, intracranial volume

<sup>a</sup>Estimated means differ because of different adjustments

<sup>b</sup>Adjusted for age, sex, educational level, depressive symptoms, cognitive functioning, use of sleep medication and myocardial infarction

<sup>c</sup>No insomnia, n=1926; Insomnia, n=165

Next, we examined whether in persons with insomnia the total brain, gray matter, white matter and hippocampal volumes were associated with sleep onset latency, sleep duration, sleep efficiency and sleep quality (Table 3). A smaller total brain and white matter volume were each related to a lower sleep efficiency (total brain, B per 10 ml= 0.36, 95% CI= 0.02; 0.69; white matter, B per 10 ml= 0.65, 95% CI= 0.09; 1.20). Also, a smaller gray matter volume was associated with a poorer sleep quality (OR per 10 ml= 0.93, 95% CI= 0.87; 0.99). Furthermore, a smaller hippocampal volume was related to a shorter sleep duration (B per ml= 0.35, 95% CI= 0.02; 0.68) and a poorer sleep quality (OR= 0.50, 95% CI= 0.29; 0.87). Additional adjustment for intracranial volume in the associations of hippocampal volume and sleep characteristics mildly attenuated these results (sleep duration, B per ml= 0.32, 95% CI= -0.04; 0.68; sleep quality, OR= 0.53, 95% CI= 0.29; 0.97).

**Table 3** Associations of brain volumes and subjective sleep parameters within the group of insomnia cases (n=248)

	Sleep onset latency (SD) <sup>a</sup> n= 224		Sleep duration (hours) n= 241		Sleep efficiency (%) n= 217		Sleep Quality (score) <sup>b</sup> n= 248	
	B (95% CI)	P	B (95% CI)	P	B (95% CI)	P	OR (95% CI)	P
<b>Brain volumes</b>								
Total brain volume (10 ml)								
Age, sex adjusted	-0.01 (-0.03; 0.01)	0.29	<b>0.02 (0.00; 0.04)</b>	<b>0.05</b>	<b>0.42 (0.09; 0.74)</b>	<b>0.01</b>	0.97 (0.94; 1.00)	0.07
Multivariable adjusted <sup>c</sup>	-0.01 (-0.03; 0.01)	0.43	0.02 (0.00; 0.04)	0.10	<b>0.36 (0.02; 0.69)</b>	<b>0.04</b>	0.98 (0.94; 1.01)	0.19
Gray matter volume (10 ml)								
Age, sex adjusted	-0.02 (-0.05; 0.01)	0.22	<b>0.04 (0.00; 0.08)</b>	<b>0.03</b>	0.53 (-0.06; 1.12)	0.08	<b>0.92 (0.87; 0.98)</b>	<b>0.01</b>
Multivariable adjusted <sup>c</sup>	-0.02 (-0.05; 0.02)	0.34	0.04 (0.00; 0.07)	0.08	0.44 (-0.17; 1.05)	0.16	<b>0.93 (0.87; 0.99)</b>	<b>0.03</b>
White matter volume (10 ml)								
Age, sex adjusted	-0.01 (-0.04; 0.02)	0.40	0.03 (-0.01; 0.06)	0.13	<b>0.75 (0.21; 1.29)</b>	<b>0.01</b>	0.98 (0.92; 1.03)	0.39
Multivariable adjusted <sup>c</sup>	-0.01 (-0.04; 0.02)	0.54	0.02 (-0.01; 0.06)	0.26	<b>0.65 (0.09; 1.20)</b>	<b>0.02</b>	0.98 (0.93; 1.05)	0.66
Hippocampal volume (ml) <sup>d</sup>								
Age, sex adjusted	-0.03 (-0.30; 0.25)	0.85	<b>0.34 (0.02; 0.67)</b>	<b>0.04</b>	1.54 (-3.78; 6.86)	0.57	<b>0.51 (0.30; 0.87)</b>	<b>0.01</b>
Multivariable adjusted <sup>c</sup>	-0.01 (-0.29; 0.27)	0.96	<b>0.35 (0.02; 0.68)</b>	<b>0.04</b>	1.42 (-3.95; 6.80)	0.60	<b>0.50 (0.29; 0.87)</b>	<b>0.01</b>

Abbreviations: CI, confidence interval; ICV, intracranial volume; SD, standard deviation

Linear regression analyses of brain volumes with subjective sleep parameters, except for a logistic regression analysis of brain volumes with sleep quality

<sup>a</sup>normalized and standardized

<sup>b</sup>moderately poor sleep quality (reference) versus very poor sleep quality

<sup>c</sup>Adjusted for age, sex, educational level, depressive symptoms, cognitive functioning, use of sleep medication and myocardial infarction

<sup>d</sup>sleep onset latency, n = 148; sleep duration, n = 162; sleep efficiency, n = 145, sleep quality, n = 165

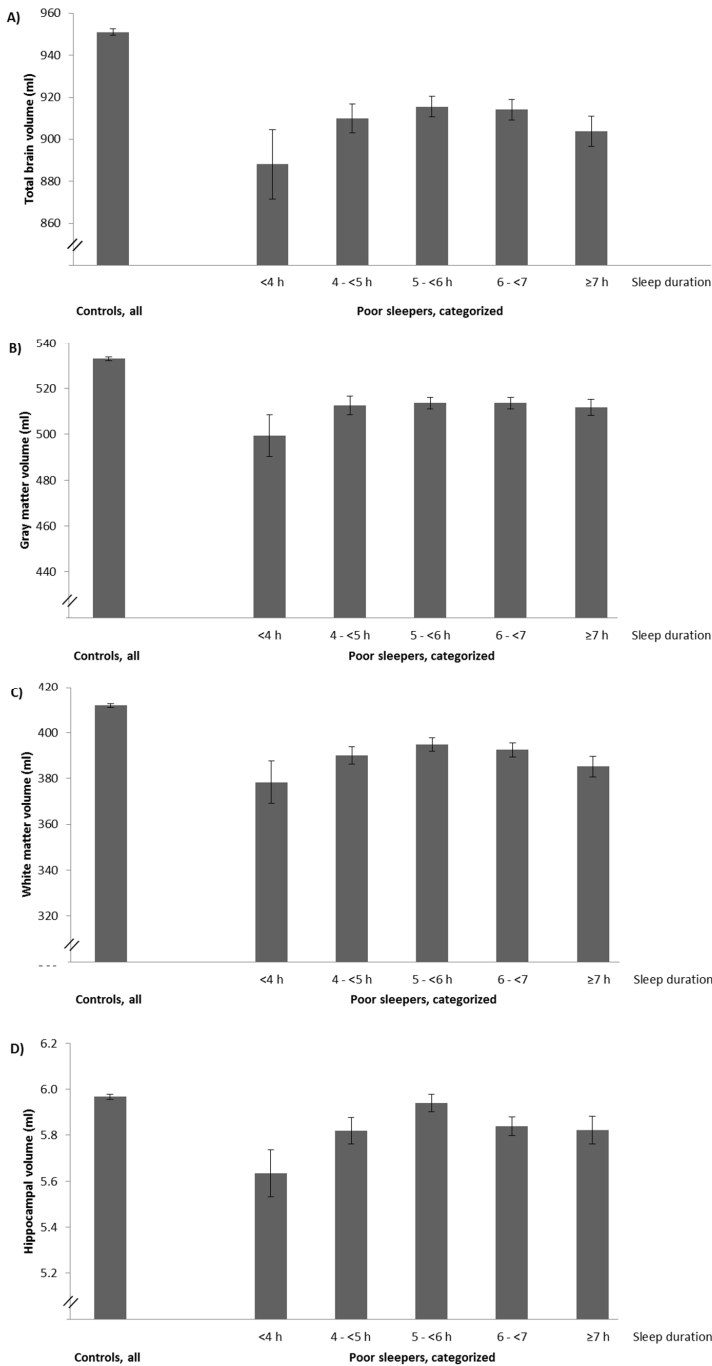
To illustrate the associations between brain volumes and sleep duration in persons with insomnia, we categorized persons with insomnia by sleep duration (Figure 1). This figure shows that in persons with insomnia, total brain, gray matter, white matter and hippocampal volumes tend to be smaller compared to controls. Furthermore, in persons with insomnia, a shorter sleep duration is related to smaller brain volumes; the clearest association is seen with the hippocampus.

In a series of sensitivity analyses, we compared the brain volumes of insomnia cases with a short sleep duration to those with a sleep duration above the cut-off ( $\geq 4$  hours,  $\geq 5$  hours or  $\geq 6$  hours), and to those of controls (Table 4, Supplementary Tables 3 and 4). Results of the analyses with different cut-offs are very consistent. In Table 4 we present the analyses of insomnia cases sleeping  $< 5$  hours versus insomnia cases sleeping  $\geq 5$  hours and controls. A smaller total brain (901 ml versus 924 ml, 95% CI= -40.7; -3.9) and white matter volume (375 ml versus 390 ml, 95% CI= -26.4; -3.5) were found in short sleeping insomnia cases (sleeping  $< 5$  hours) compared to controls. In persons with insomnia, short sleeping insomnia cases had smaller hippocampal volumes than the relatively longer sleeping insomnia cases (5.9 ml versus 6.1 ml, 95% CI= -0.38; -0.01).

## Discussion

In this large population-based study, we investigated whether total brain, white matter, gray matter and hippocampal volumes differ between persons with insomnia and controls. A smaller white matter volume was related to insomnia. In persons with insomnia, smaller total brain and white matter volumes were associated with a lower sleep efficiency. A smaller gray matter volume was associated with poorer sleep quality. Furthermore, a smaller hippocampus was related to a shorter sleep duration and poorer sleep quality. This suggests that structural brain correlates underlie the severity of insomnia or that insomnia causes structural brain changes.

In this population-based study, persons with insomnia were more often female, lower educated and had more depressive symptoms, in line with previous studies.<sup>27</sup> In persons with insomnia, we observed smaller total brain and white matter volumes than in controls, especially in the frontal, temporal and occipital lobes. In line with these results is a study of the integrity of white matter tracts in insomnia patients, reporting reduced integrity of fronto-subcortical white matter tracts.<sup>17</sup> In contrast, Winkelman et al. (2010) could not demonstrate differences in whole brain volume between insomnia patients and controls.<sup>15</sup>



**Figure 1.** Mean total brain (A), gray matter (B), white matter (C) and hippocampal (D) volumes in persons without insomnia and persons with insomnia. Persons with insomnia are categorized into different sleep duration groups



**Table 4** Brain volume differences in participants without insomnia, persons with insomnia sleeping ≥5 hours and persons with insomnia sleeping <5 hours per night

	No insomnia (n=2729)		Insomnia Sleep duration ≥5 hours (n=142)		Insomnia Sleep duration <5 hours (n=99)		Case - Control Comparison of insomnia (≥5 hours) with no insomnia		Case - Control Comparison of insomnia (<5 hours) with no insomnia		Within cases Comparison of insomnia (<5 hours) with insomnia (≥5 hours)		Overall comparison
	Mean <sup>a</sup>	Mean <sup>a</sup>	Mean <sup>a</sup>	Mean <sup>a</sup>	Difference (95% CI)	Difference (95% CI)	Difference (95% CI)	Difference (95% CI)	Difference (95% CI)	Difference (95% CI)	F	P	
Total brain volume (ml)	946	936	924	901	-9.1 (-23.0; 4.7)	-21.6 (-37.9; -5.2)	-12.4 (-33.2; 8.3)	4.0	0.02				
Age, sex adjusted	924	912	522	512	-11.4 (-28.2; 5.3)	-22.3 (-40.7; -3.9)	-10.9 (-31.5; 9.8)	3.0	0.05				
Multivariable adjusted <sup>b</sup>													
Gray matter volume (ml)	530	526	512	512	-4.1 (-11.7; 3.5)	-8.1 (-17.1; 0.9)	-4.0 (-15.4; 7.5)	2.0	0.14				
Age, sex adjusted	520	515	404	395	-4.6 (-13.9; 4.6)	-8.0 (-18.1; 2.2)	-3.3 (-14.7; 8.1)	1.3	0.27				
Multivariable adjusted <sup>b</sup>													
White matter volume (ml)	409	404	375	375	-5.2 (-13.8; 3.4)	-14.2 (-24.4; -4.0)	-9.0 (-21.9; 3.9)	4.3	0.01				
Age, sex adjusted	390	383	6.0	5.8	-6.8 (-17.2; 3.7)	-14.9 (-26.4; -3.5)	-8.2 (-21.0; 4.7)	3.4	0.03				
Multivariable adjusted <sup>b</sup>													
Hippocampal volume (ml) <sup>c</sup>	6.0	6.0	6.1	5.9	0.06 (-0.06; 0.18)	-0.14 (-0.29; 0.01)	-0.20 (-0.38; -0.01)	2.3	0.10				
Age, sex adjusted	6.0	6.1			0.07 (-0.08; 0.21)	-0.13 (-0.29; 0.04)	-0.19 (-0.38; -0.01)	2.2	0.12				
Multivariable adjusted <sup>b</sup>													

Abbreviations: CI, confidence interval; ICV, intracranial volume

Bold indicates significance at p&lt;0.05 level

<sup>a</sup>Estimated means differ because of different adjustments<sup>b</sup>Adjusted for age, sex, educational level, depressive symptoms, cognitive functioning, use of sleep medication and myocardial infarction.<sup>c</sup>No insomnia, n=1926; Insomnia with a sleep duration of ≥5 hours, n=100; Insomnia with a sleep duration <5 hours, n=62

For analyses using different cut-offs to define short sleep duration see Supplemental Tables 3 and 4

This discrepancy in results might be due to the difference in sample size between the studies; Winkelman et al. (2010) used a case-control design with 20 well defined insomnia patients, aged 25 – 55 years, whereas we studied 248 persons with insomnia, aged 45 – 92 years old. Differences in brain structure between insomnia cases and controls might be partly explained by brain functional differences. Several studies point to an altered brain function as a possible substrate of insomnia.<sup>2,6,9,11,16,31-33</sup> Persons with insomnia suffer from hyperarousal during sleep, reflected by a greater brain metabolism and more beta activity during sleep.<sup>6,31</sup> Hyperarousal represents enhanced sensory processing during sleep. This leads to a smaller difference in cortical arousal between the sleep and wake states, and to more interference of sleep onset or sleep maintenance by environmental stimuli.<sup>32</sup> In addition, insomnia patients showed cognitive deficits, reduced attention and a decreased capability to recognize the optimal sleep temperature, also pointing towards brain changes in these individuals.<sup>11,16,33</sup> A review by Vgontzas et al. (2013) suggests that insomnia with an objective short sleep duration has a stronger biological basis than insomnia with a relatively long sleep duration.<sup>8</sup> Insomnia with a sleep duration  $\geq 6$  hours is thought to be a more psychological condition, without an apparent biological substrate.<sup>8</sup> It is associated to a state of sleep misperception. Because of our large sample size, we were able to study structural brain correlates of several sleep parameters in persons with insomnia. Smaller total brain, white matter, gray matter and hippocampal volumes were associated with a shorter sleep duration, a lower sleep efficiency and a poorer sleep quality. This suggests that structural brain differences in persons with insomnia underlie the severity of insomnia. This might be difficult to find in small studies. In line with the theory that insomnia with a short sleep duration is the biological most severe phenotype,<sup>8</sup> we found the smallest total brain, gray matter, white matter and hippocampal volumes in the very short sleeping persons with insomnia (Figure 1). Except for two studies<sup>14,18</sup> previous studies did not find a difference in hippocampal volumes between insomnia patients and controls.<sup>12,15,16</sup> However, in the primary insomnia patients, the hippocampal volume was negatively related to objectively measured poorer sleep maintenance and to insomnia duration in these studies.<sup>12,15,16</sup> More apparent differences in brain volume between insomnia patients and good sleepers might be found if the sleep duration of insomnia patients is taken into account or if larger sample sizes are investigated. Our finding that a smaller brain volume related to insomnia, and that among persons with insomnia structural brain correlates seem to underlie the severity of insomnia, could be explained by several potential mechanisms. First, age is associated with brain degeneration and more hyperarousal during sleep.<sup>34,35</sup> In insomnia patients, brain degeneration might underlie the hyperarousal during sleep. In this study participants were on average 63 years old. Because of multicollinearity, we were not able to adjust the total brain, gray matter and white matter analyses for intracranial volume. This suggests that the amount of brain atrophy of the persons was small. Therefore, the size of the brain, not brain atrophy, seem

to underlie these results. Second, insomnia may precede brain degeneration. During sleep neurotoxic waste products are removed from the brain.<sup>36</sup> Since persons with insomnia have sleep problems, this process might be impaired, causing brain damage. Third, genes associated with insomnia might also be associated to brain health. A genome-wide association study found alleles associated with a long sleep duration, that previously were associated to a better metabolic profile.<sup>37</sup>

This study has several strengths. First, it has a large sample size, the current study is much larger than previous studies in this field. This enabled us to study the associations of brain volumes and several sleep parameters within the group of insomnia cases. Furthermore, this study is embedded in a population-based cohort. Therefore, we could assess many different covariates and the results are generalizable. However, some limitations should also be discussed. We did not formally diagnose insomnia, but assessed insomnia using a weighted set of individual PSQI items, that correlated highly (0.84) with the Insomnia Severity Index (ISI)<sup>22</sup>. Second, this study has a cross-sectional design, and therefore we were not able to study causality and a possible age effect in insomnia. However, we tested the interaction of age with brain volumes on the risk of insomnia, and this interaction was not significant. This suggests that our findings do not merely reflect an aging process. We could also show that atrophy does not explain the results. Third, we did not measure the sleep parameters sleep onset latency, sleep duration and sleep efficiency objectively. Objectively measured sleep parameters will minimize the influence of the subjective sleep complaints. However, the use of subjectively measured sleep parameters probably leads to an underestimation of the effects.

To conclude, in this population-based study we found structural brain differences between insomnia cases and controls. This difference was mainly driven by persons with severe insomnia, as indexed by short sleep. Furthermore, in persons with insomnia, smaller total brain, white matter, gray matter and hippocampal volumes were associated with sleep complaints such as short sleep duration, low sleep efficiency and poorer sleep quality. These results suggest that structural brain correlates underlie the severity of insomnia or that insomnia causes structural brain changes. However, group differences between poor sleepers and controls are small. This probably explains the inconsistent results in studies with a small number of participants. Understanding the underlying neurobiology of insomnia, including the importance of sleep duration in persons with insomnia, might aid in understanding insomnia and in development of more specific therapies for insomnia patients. Future research is needed to validate the structural brain correlates of insomnia severity and to elucidate the direction of causality.

## Supplemental Material

**Supplemental Table 1** Pittsburgh Sleep Quality Index Items with their weight

Item	Weight
(Intercept)	4.933
Sleep latency (minutes)	0.008
Actual sleep (hours)	-0.181
No sleep within 30 minutes	0.624
Night-time wake	0.963
Too hot	0.138
Bad dreams	0.328
Other reason for bad sleep	0.176
Bad sleep quality	3.091
Medication use	0.505
Difficulty staying awake during the day	0.596
Problems getting things done	1.901
Sleep efficiency	-4.540

Weights are derived from “van Someren, manuscript in preparation, 2015”

**Supplemental Table 2** White matter brain lobe differences in participants without insomnia and persons with insomnia

	No insomnia (n=2729)	Insomnia (n=248)	Case - Control	Overall comparison	
			Comparison of insomnia with no insomnia	F	P
	Mean	Mean	Difference (95% CI)		
White matter volume (ml)					
Frontal lobe	142	136	<b>-4.0 (-7.2; -0.8)</b>	<b>5.9</b>	<b>0.02</b>
Parietal lobe	87	85	-2.0 (-4.1; 0.1)	3.6	0.06
Temporal lobe	70	68	<b>-1.6 (-3.2; -0.0)</b>	<b>4.1</b>	<b>0.04</b>
Occipital lobe	44	43	<b>-1.5 (-2.6; -0.4)</b>	<b>6.7</b>	<b>0.01</b>

Abbreviations: CI, confidence interval

Analyses are adjusted for age, sex, educational level, depressive symptoms, cognitive functioning, use of sleep medication and myocardial infarction

**Supplementary Table 3** Brain volume differences in participants without insomnia, persons with insomnia sleeping ≥4 hours and persons with insomnia sleeping <4 hours per night

	No insomnia (n=2729)		Insomnia Sleep duration ≥4 hours (n=215)		Insomnia Sleep duration <4 hours (n=26)		Case - Control Comparison of insomnia (≥4 hours) with no insomnia		Case - Control Comparison of insomnia (<4 hours) with no insomnia		Within cases Comparison of insomnia (<4 hours) with insomnia (≥4 hours)		F	P
	Mean		Mean		Mean		Difference (95% CI)		Difference (95% CI)		Difference (95% CI)			
Total brain volume (ml)	924		911		888		-13.2 (-28.0; 1.6)		-36.4 (-68.3; -4.4)		-23.2 (-56.1; 9.6)		<b>3.5</b>	<b>0.03</b>
Gray matter volume (ml)	520		515		504		-4.7 (-12.8; 3.5)		-15.9 (-33.5; 1.7)		-11.2 (-29.4; 6.9)		1.9	0.15
White matter volume (ml)	390		382		367		-8.8 (-18.0; 0.4)		-20.4 (-40.4; -0.5)		-11.6 (-32.1; 8.8)		<b>3.2</b>	<b>0.04</b>
Hippocampal volume (ml) <sup>a</sup>	6.0		6.1		5.7		0.04 (-0.09; 0.16)		-0.29 (-0.56; -0.02)		-0.33 (-0.60; -0.05)		2.6	0.07

Abbreviations: CI, confidence interval

All analyses are adjusted for age, sex, educational level, depressive symptoms, use of sleep medication, stroke and diabetes. Bold indicates significance at p<0.05 level

<sup>a</sup>No insomnia, n=1926; Insomnia with a sleep duration of ≥4 hours, n=143; Insomnia with a sleep duration <4 hours, n=19

**Supplementary Table 4** Brain volume differences in participants without insomnia, persons with insomnia sleeping ≥6 hours and persons with insomnia sleeping <6 hours per night

	No insomnia (n=2729)		Insomnia Sleep duration ≥6 hours (n=69)		Insomnia Sleep duration <6 hours (n=172)		Case - Control Comparison of insomnia (≥6 hours) to no insomnia		Case - Control Comparison of insomnia (<6 hours) to no insomnia		Within cases Comparison of insomnia (≥6 hours) to (<6 hours) to insomnia comparison		Overall comparison	F	P
	Mean	Mean	Mean	Mean	Difference (95% CI)	Difference (95% CI)	Difference (95% CI)	Difference (95% CI)	Difference (95% CI)	Difference (95% CI)					
Total brain volume (ml)	924	921.1	903	-3.2 (-24.6; 18.2)	<b>-21.4 (-37.0; -5.8)</b>	-18.2 (-40.7; 4.3)	<b>3.8</b>	<b>0.02</b>							
Gray matter volume (ml)	520	521	511	0.92 (-10.8; 12.7)	<b>-8.9 (-17.5; -0.3)</b>	-9.9 (-22.3; 2.6)	2.4	0.09							
White matter volume (ml)	390	387	378	-3.5 (-16.8; 9.8)	<b>-13.1 (-22.8; -3.4)</b>	-9.6 (-23.6; 4.4)	<b>3.5</b>	<b>0.02</b>							
Hippocampal volume (ml) <sup>a</sup>	6.0	6.1	6.0	0.04 (-0.14; 0.23)	-0.03 (-0.17; 0.10)	-0.08 (-0.27; 0.12)	0.3	0.74							

Abbreviations: CI, confidence interval

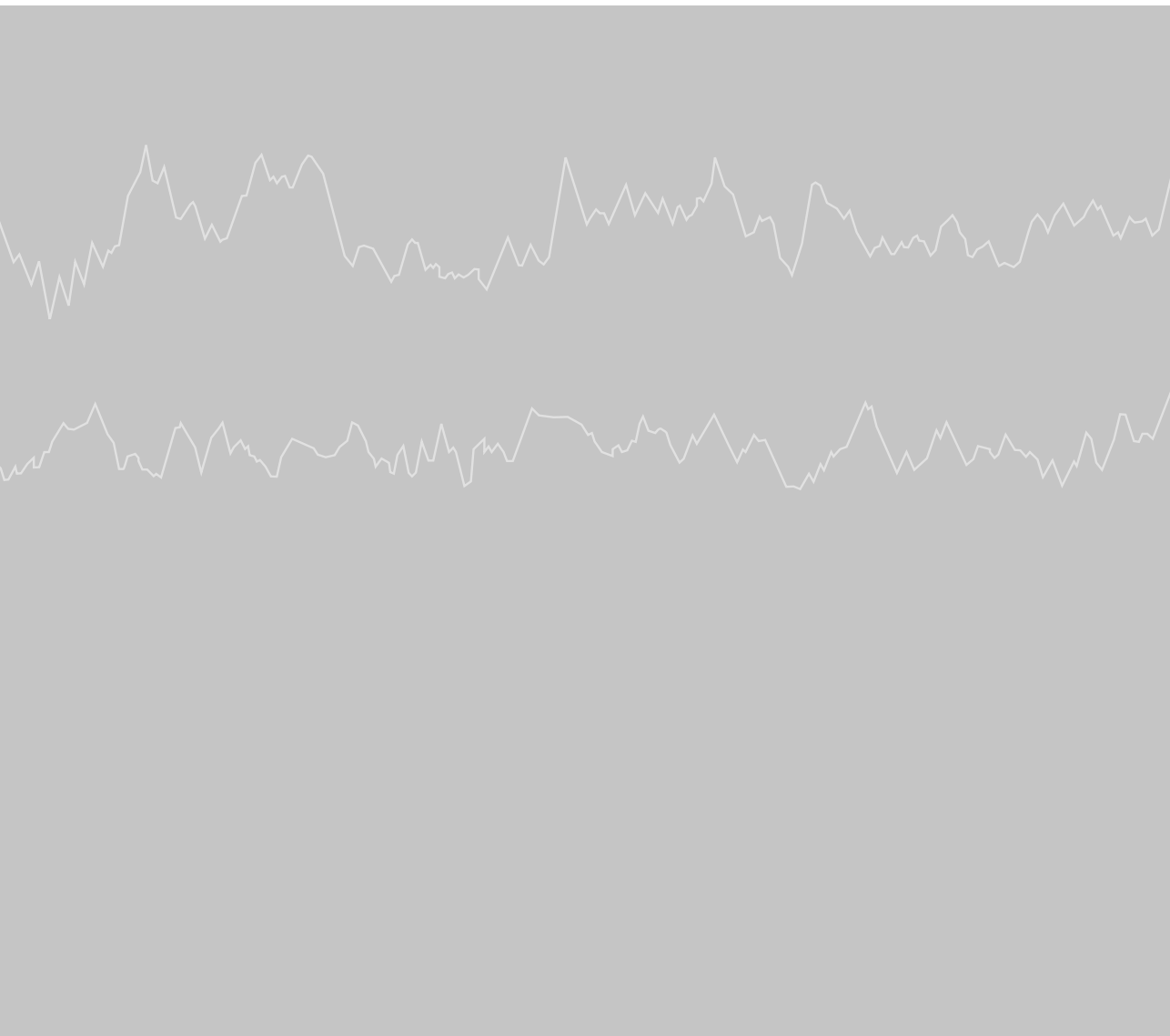
All analyses are adjusted for age, sex, educational level, depressive symptoms, use of sleep medication, stroke and diabetes. Bold indicates significance at p<0.05 level.  
<sup>a</sup>No insomnia, n=1926; Insomnia with a sleep duration of ≥6 hours, n=48; Insomnia with a sleep duration <6 hours, n=114.

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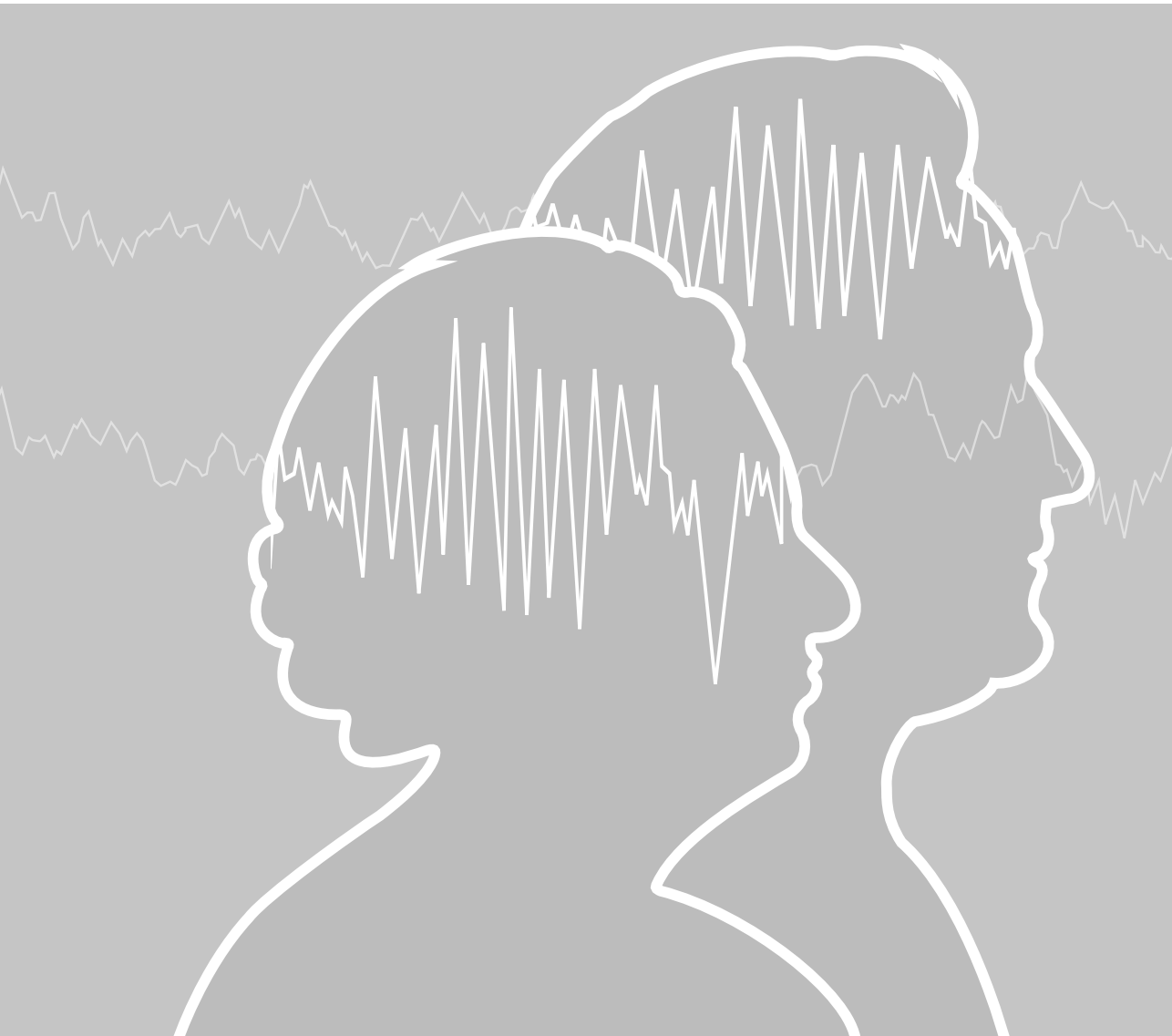
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# Chapter 4

## General Discussion





In this thesis I investigated the associations between sleep, 24-hour activity rhythms, and health in a middle-aged and elderly population, with a particular focus on the relation between sleep and structural brain variation. In this chapter I will review the main findings of the studies. Next, I will address some methodological considerations, clinical implications, and make suggestions for future research.

## Main findings

### *Sleep, 24-hour activity rhythms and health parameters*

Sleep and 24-hour rhythm disturbances are common in older age. In the elderly about 80% of persons report at least 1 sleep complaint.<sup>1</sup> Furthermore, elderly persons have problems falling asleep, have fragmented sleep, wake up too early, nap - involuntarily - during the day, do not get enough sleep at night, and report a low sleep quality.<sup>2,3</sup> This decrease in sleep quality is related to several health problems, such as depression, cardiovascular disease and poor cognitive performance.<sup>4-6</sup> We demonstrated that the 24-hour activity rhythm disturbances are related to several demographic and lifestyle factors (Chapter 2.1). Older age was characterized by a more stable, but also to a more fragmented activity rhythm. Furthermore, a high body mass index and smoking were related to unstable and fragmented activity rhythms. This last finding was in line with the association between 24-hour rhythm disturbances and mortality (Chapter 2.2); persons with an unstable and/or fragmented rhythm had a higher risk of mortality.

Fragmentation of the 24-hour activity rhythm may be a general health indicator. In this thesis I showed that fragmentation is related to more depressive symptoms, a high body mass index, cerebral small vessel disease and mortality. This is in line with other studies that related fragmented rhythms to health problems, including cognitive deficits, high blood pressure, cardiovascular disease and Alzheimer's disease.<sup>7-10</sup>

The association between a higher age and a more stable rhythm was not in line with the finding of an unstable rhythm and a higher mortality risk. We had hypothesized that a higher age would be associated to a more unstable rhythm. This association between age and a more stable rhythm might be explained by changes in the life style of older persons with health problems; they live a more structured life compared to younger persons. This structured life compensates for the disturbed functioning of the suprachiasmatic nucleus, the biological clock of the brain. Post-mortem studies in very old persons demonstrated a reduction in volume and number of vasopressin-expressing neurons of the suprachiasmatic nucleus.<sup>11</sup> This reduction may underlie the ability to drive the circadian rhythm, a smaller amplitude of several circadian rhythms and a more fragmented 24-hour activity rhythm.<sup>12</sup> The loss of temporal organization between different rhythms can lead to suboptimal physiological functioning, because physiological processes do not take place at their optimal time of day.

As a result people have a higher disease susceptibility. The structured life of elderly may - partly - compensate for the disturbances in their circadian rhythms. However, taking age, several demographic and health factors into account, an unstable rhythm independently predicted mortality in our study. This suggests that change of behavior cannot accommodate all circadian rhythm disturbances.

In Chapter 2.2a we discussed the importance of a different 24-hour rhythm variable: circadian misalignment. Circadian misalignment represents a form of 'social jetlag'; the mismatch between a person's biological clock and social clock (i.e. the clock driven by the schedules of daily life, such as work). This results in chronic sleep loss.<sup>13</sup> The circadian misalignment was measured as the absolute difference in time spent in bed on weekdays and on weekends. Also it was measured as the absolute difference in midpoints of sleep between weekdays and weekends. Whereas fragmentation of the 24-hour activity rhythm was only very modestly related to circadian misalignment, the stability of the rhythm was related to circadian misalignment. However, an unstable 24-hour activity rhythm was related to mortality independently of circadian misalignment. Therefore, information about circadian misalignment can add to the understanding of the interrelation of sleep, 24-hour rhythms and health.

The mechanism behind sleep is thought to consist of two interacting processes.<sup>14</sup> The first process, Process S, represents the homeostatic sleep drive, or sleep pressure. The longer you are awake, the stronger the sleep pressure. The sleep pressure declines during sleep. The second process, Process C, represents the circadian rhythm, driven by the suprachiasmatic nucleus or biological clock of the brain. This process is independent of prior sleep, and regulates the 24-hour rhythm of the sleep-wake pattern. In the studies described in this thesis, sleep parameters and 24-hour rhythm measures were only weakly to moderately related. This is reflected in the results. For example, unstable and fragmented rhythms related to mortality independent of sleep (Chapter 2.2). Also, brain pathology in the form of cerebral small vessel disease related to disturbed 24-hour activity rhythms, independent of sleep (Chapter 3.1).

In Chapter 2.2 I describe a significant U-shaped relationship between continuously analysed subjective sleep duration and mortality. However, this association was non-significant when adjusted for health parameters. U-shaped relationships between sleep duration and mortality have been described in several previous studies. Two meta-analyses found that both short and long self-reported sleep durations were predictors of mortality in cohort studies.<sup>15,16</sup> In general, more profound associations between long sleep and all-cause mortality were observed in the more extreme categories ( $\geq 9$  hours). In our study, only few persons were extreme short or long sleepers. Consequently, we were limited in testing extreme sleep durations in our analyses. This might explain the attenuation of the curvilinear relation between sleep duration and mortality after further adjustment.

With age, not only the number of sleep problems increase, also the prevalence of heart failure increases. Sleep disturbances have been reported in almost 70% of patients with heart failure.<sup>17</sup> Prospective sleep studies demonstrated that short and long sleep durations increase the risk of cardiovascular disease.<sup>18-21</sup> Furthermore, difficulty maintaining and initiating sleep are associated with incident heart failure.<sup>22</sup> However, it was not known whether sleep quality in patients with heart failure changes over time (Chapter 2.3). We investigated the effect of clinical heart failure and subclinical cardiac dysfunction on sleep quality. We found that clinical heart failure, but not echocardiographic indicators of subclinical cardiac dysfunction, increased the risk of poor sleep quality in the general population during follow-up. This suggests that a poor sleep quality is only caused by severe heart failure, or that heart failure symptoms are on the causal pathway between cardiac dysfunction and sleep disturbances. Heart failure symptoms, such as restless legs or shortness of breath while lying flat, could cause disturbances in sleep and changes in the sleep-wake pattern. Heart failure and sleep problems could share common etiological mechanisms. For example, vascular pathologies could independently explain heart failure and sleep problems.<sup>23,24</sup> In this thesis, I also discussed the association between cerebral small vessel disease, a form of vascular brain pathology, and sleep (Chapter 3.1). In this study, we did not find a relation between cerebral small vessel disease and total sleep time or sleep quality.

#### *Brain structure and sleep*

With increasing age, the brain undergoes several structural alterations, including loss of brain tissue and accumulation of vascular pathology. When mild, many of these changes may go unnoticed or do not cause overt clinical symptoms. However, even in the subclinical stage, such (small) brain changes can have an effect on health and behavior. In Chapter 3.1 we studied the association between cerebral small vessel disease and 24-hour activity rhythm and sleep parameters. Cerebral small vessel disease causes structural brain damage, which may manifest as lacunar infarcts, white matter lesions or cerebral microbleeds.<sup>25,26</sup> These markers of cerebral small vessel disease are common in middle-aged and elderly persons, although they often occur unnoticed.<sup>27-29</sup> We found that white matter lesion volume and presence of cerebral microbleeds were related to more unstable and fragmented 24-hour activity rhythms, independent of total sleep time, and sleep quality. This indicates that subclinical brain damage affects circadian rhythms. Also, it indicates that sleep-wake disturbances commonly found in patients with stroke or Alzheimer's disease may have arisen already early in the disease process before clinical signs are present.

A factor possibly underlying the association between cerebral small vessel disease and 24-hour activity rhythm disturbances is sleep apnea. Sleep apnea - characterized by repetitive respiratory events during sleep – can lead to reduced airflow, oxygen desaturation (hypoxia), arousals, and daytime sleepiness. If these respiratory events (apneas and hypopneas) occur

several times per hour, they can underlie the development of hypertension, cardiovascular disease and stroke.<sup>30-32</sup> In Chapter 3.2 we assessed whether aspects of sleep apnea (the apnea-hypopnea index (AHI), nocturnal oxygen desaturation and arousals), were related to global structural brain changes. We found that the AHI - indicating the frequency of breathing pauses - and arousals were not related to gray and white matter brain atrophy or white matter lesion volumes. In contrast, nocturnal oxygen desaturation related to white matter atrophy. Because the association of oxygen desaturation and white matter atrophy was independent of blood pressure, total cholesterol and myocardial infarction, oxygen desaturation probably causes white matter atrophy directly. The association between cerebral small vessel disease and disturbed 24-hour activity rhythms (Chapter 3.1) could possibly be partly explained by oxygenation problems and fragmentation of sleep due to sleep apnea. In the study described in Chapter 3.1 we were only able to adjust the associations for possible sleep apnea. Possible sleep apnea was assessed by two questions from the Pittsburgh Sleep Quality Index.<sup>33,34</sup> Adjustment for possible sleep apnea did not change the associations between cerebral small vessel disease and disturbed 24-hour activity rhythms. However, we could not account for oxygen desaturation as measured by polysomnography (PSG).

Some previous studies found an association between sleep apnea severity and structural brain changes, although the reports are not consistent.<sup>35,36</sup> In general, these studies had smaller sample sizes and were conducted in case-control designs using clinical sleep apnea patients versus healthy controls. Usually, associations described between sleep apnea and structural brain variation were less strong in the general population than in clinical samples. Persons with mild or moderate sleep apnea probably do not seek treatment and suffer less from the consequences of sleep apnea than those seeking treatment despite the same symptoms of sleep apnea. The advantage of studying diseases in a population-based setting is studying a broader range of the disease, including persons with mild signs of the disease and persons with severe signs.

In Chapter 3.3 we studied the associations between brain structure and different sleep parameters, including sleep waves. Brain structure variations were related to beta power during NREM sleep: more gray matter volume was associated with more beta power. More beta power during the night is an indicator of hyperarousal. Hyperarousal causes problems falling asleep, fragmented sleep and short sleep duration. This is supported by our findings that more beta waves during NREM sleep were related to poor objective and subjective sleep quality, (i.e. a shorter total sleep time, less sleep efficiency, and poor sleep quality). In contrast to what we hypothesized, these associations were independent of reduced gray matter volumes. This suggests that gray matter brain structure does not account for the association between more beta power and poor sleep.

In contrast, more white matter volume was related to more beta power. In line with this finding, higher fractional anisotropy - indicating better white matter microstructural integrity – also related to more beta power. Although beta power during sleep – indicating hyperarousal – is considered unfavourable, during the awake state, beta power is a meaningful signal. For example, beta power during wake is associated with better vigilance and cognition.<sup>37,38</sup> It may be hypothesized that the important role of white matter in connectivity of brain regions during the day, is also reflected in more beta power at night. White matter volume was not directly related to sleep quality, including total sleep time. One possible explanation is that the effect between white matter and total sleep time is suppressed by beta power. In post-hoc analyses, we found indications for a suppression effect of beta power between white matter and total sleep time. This suggests that the association between brain structure, specifically white matter, and total sleep time is obscured by the relation of white matter with beta waves. White matter and beta waves are inversely related to total sleep time. We found that persons with insomnia had smaller white matter volumes than good sleepers (Chapter 3.4). Furthermore, in persons with insomnia, smaller white and gray matter and hippocampal volumes were related to a shorter sleep duration and a poorer sleep quality. Vgontzas et al. (2013) suggested that there are two phenotypes of insomnia: one ‘biological’ phenotype in which insomnia patients have hyperarousal of the stress system and a short sleep duration, and one ‘psychological’ phenotype, including patients with a “normal” sleep duration and sleep misperception.<sup>39</sup> Because of our large sample size, we were able to study the structural brain associations of several sleep parameters in persons with insomnia. Smaller total brain, white matter, gray matter and hippocampal volumes were associated with a shorter sleep duration, a lower sleep efficiency and a poorer sleep quality. This suggests that structural brain differences in persons with insomnia may underlie the severity of insomnia or that insomnia causes structural brain changes. Indeed, several studies point to an altered brain function as a possible substrate of insomnia.<sup>40</sup> Persons with insomnia have more hyperarousal during sleep, as reflected by a greater brain metabolism and more beta activity.<sup>41,42</sup> Furthermore, insomnia is characterized by problems in falling asleep or staying asleep, leading to daytime dysfunction because of fatigue during the day. This is in line with our finding that that higher beta waves during NREM sleep related to poorer objective and subjective sleep quality, represented by a shorter total sleep time, less sleep efficiency and poor sleep quality (Chapter 3.3).



## Methodological considerations

### *Analysing brain waves during sleep: Absolute or relative?*

Measuring sleep using PSG makes it possible to study brain waves during sleep; electroencephalography (EEG) is central to PSG. In 1968 Rechtschaffen and Kales wrote “A Manual of Standardized Terminology, Techniques and Scoring for Sleep Stages of Human Subjects”. This manual contains a set of rules used for the scoring of sleep stages, also known as the R&K rules.<sup>43</sup> In these rules EEG information was divided into 30-second epochs and the EEG information was used to categorize sleep into one of four sleep stages (stage 1-4, non-REM sleep) and REM sleep. The R&K rules were the most widely used sleep scoring rules until 2007, when the American Academy of Sleep Medicine updated the R&K scoring rules.<sup>44</sup> A major change was the combination of sleep stages 3 and 4 to form stage N3. Five different brain waves can be observed with EEG: delta (<4 Hz), theta (4 – 8 Hz), alpha (8 – 12 Hz), beta (12 – 40 Hz) and gamma (40 – 100 Hz). Delta waves are the slowest brain waves with the highest amplitude, and are associated with the deepest sleep (N3), also called slow wave sleep. The lighter sleep phases N1 and N2 are characterized by theta waves. Furthermore, sleep stage N2 is characterized by the occurrence of spindles and K-complexes. Alpha waves are most prominent when relaxing with eyes closed. Beta and gamma waves are associated with wakefulness. Too much beta and gamma power at night is associated with short total sleep time, low sleep efficiency and poor sleep quality (Chapter 3.3).

Although the scoring of sleep stages is standardized according to the R&K scoring rules,<sup>43</sup> or the updated rules of the AASM,<sup>44</sup> such standardization is not used when studying brain waves during sleep directly. Some studies report absolute EEG power (Hz) and some report relative power (% of total power). This inconsistency causes difficulty in comparing different studies, but also causes differences in interpretation of the results reported. In the remaining part of this paragraph I will discuss the advantages and disadvantages of reporting about absolute and relative EEG power.

The advantage of analysing brain waves with relative EEG power is that the effects of the different brain waves within one study are easily comparable; each brain wave is expressed as percentage of the total EEG power. In other words, the brain waves are standardized by using relative EEG power. Furthermore, EEG patterns during sleep are highly person-specific.<sup>45</sup> Persons can be distinguished by their particular EEG topographic distribution. Relative EEG power measures can account for these individual differences in total absolute EEG power and thereby making comparisons between individuals easier. Relative EEG power is typically used as a measure when patient and control groups are compared. For example, Perlis et al. (2001) compared delta and beta patterns during sleep in patients with primary insomnia, patients with major depression (secondary insomnia) and good sleeping controls.<sup>46</sup> The authors found that each of these groups have characteristic temporal delta and beta

patterns during sleep. For example, primary insomniacs have more relative beta during the night compared to secondary insomniacs and good sleepers. Secondary insomniacs on the other hand have less relative delta power compared to primary insomniacs and good sleepers. The disadvantage of such outcome reports based on relative EEG power, is that the reader is left in doubt about what happens with the absolute EEG power. For example, if over time the total EEG power is higher, and absolute delta and beta power did not change, relative delta and beta power is decreased. Therefore, interpreting relative EEG power can be difficult.

Absolute EEG power measures are easier to interpret. By definition, a higher absolute level of a specific wave can only be interpreted as more power of that specific wave. Relative EEG power can be used to standardize absolute EEG power, but z-scores can also be used. Z-scores make the effect size of estimates of different waves better interpretable. However, this comes at a price, the effect estimate is more difficult to interpret. In our studies, we analysed absolute delta and beta power in relation to brain structures in Chapter 3.3. However, the disadvantages of absolute EEG power measures are: 1) that this measure can be influenced by the equipment used; 2) groups of individuals differ in the total amount of absolute EEG power due to the specific set-up; and 3) the results can only be interpreted against the background of total sleep time: the longer someone sleeps, the more absolute sleep-EEG power occurs and is recorded. To account for the total sleep time in the association of white matter brain volume and white matter microstructure with beta power (Chapter 3.3), we performed two sensitivity analyses (results available upon request). First, we additionally adjusted the association between white matter volume and beta power during sleep for total sleep time. Second, we repeated these analyses limiting the assessment to the first four hours of sleep. In this way almost every participant had the same total sleep time. Importantly, our results did not change.

To make comparison between different studies easier, I suggest a standardized method for the reporting of EEG power. To make the biological interpretation the most straightforward, it is important to report the absolute EEG power. However, to additionally report the relative EEG power makes the comparison between studies feasible.

### *Multicollinearity*

Multivariate regression models comprise many variables; most covariates are included to adjust the association of interest for possible confounding. However, multicollinearity (also called collinearity) can occur if two predictors in one multiple regression model are highly correlated. This can lead to unreliable effect estimates. For example, length and weight both are measures of body proportions. Therefore, these variables are highly correlated. Let's assume the following hypothetical analysis. The length of a person predicts the income of this person. When weight would be added as covariate in the model of length and income,

it will most probably partly explain the same variance of income. Therefore, when both length and weight are included in this model, the model could render them both statistically insignificant, even though they are individually related to the outcome.

A method often used to detect multicollinearity is the variance inflation factor (VIF). The VIF is calculated as  $1/(1-R^2)$ , where  $R^2$  (coefficient of determination) is obtained by regressing the predictor of interest on all the other predictors.<sup>47</sup>  $R^2$  indicates the percentage of the explained variance. The VIF estimates the inflation of the variance of an effect estimate due to the linear correlation with other covariate(s). Therefore, the VIF is dependent on the complete model tested, and can differ for two variables depending on the association of interest. For example, a VIF of 3 reflects a three times larger variance of a specific variable (the square root of the variance is the standard deviation), that stems from the correlation with other variables. This can result in a large effect estimate, disappearance of the effect, or even to a change in the direction of the estimated association. In epidemiological and statistical literature, no standard recommendation is provided for the maximum acceptable VIF value. Published recommendations for VIF values range from 2.5 – 10.<sup>47,48</sup> Note that the VIF can be calculated for each covariate; it is however most important for the determinant of interest, because only the variance of the relevant effect estimate is increased. In other words, if a high VIF occurs between two confounders this typically does not influence the main association studied.<sup>47</sup>

In neuroscience and epidemiological research of brain morphology, correction for differences in individual head sizes are often conducted by adjusting for intracranial volume (ICV) or by expressing brain volumes as percentage of the ICV. The ICV comprises total brain volume (white matter, gray matter and white matter lesion volumes) and cerebral spinal fluid, and is thought to remain stable over time as it reflects maximum attained head size. Especially the total brain volume is highly correlated with the ICV (In the Rotterdam Study  $r = 0.9$ ,  $p < 0.001$ ). This can potentially introduce the problem of multicollinearity. I will illustrate this with an example of my own research (Chapter 3.4).

The Rotterdam Study includes middle-aged and elderly persons. We observe brain atrophy in older persons, i.e. gray and white matter volumes decrease in age. This process is often accompanied by an increase in white matter lesion volume, as a marker of accumulation of vascular pathology with age. However, the ICV generally does not change with ageing.<sup>49</sup> Therefore, the correlation between ICV and white and gray matter volumes may change with age. In an older population, adjusting for ICV and likewise expressing brain volumes as percentage of ICV can be used to estimate brain atrophy, i.e. the current brain size is adjusted for the maximum size attained during life. In the insomnia study described in this thesis (Chapter 3.4), we found a high correlation ( $r = 0.9$ ) and a high VIF (8.6) between total brain volume and intracranial volume. Therefore, we decided not to adjust total brain volume for ICV. However, many studies that express brain structural volumes as percentage

of ICV, do not take multicollinearity into account. The high correlation between ICV and total brain volume in this study suggests that the amount of brain atrophy was small. On the other hand, in our study of sleep apnea and brain structure (Chapter 3.2) we deliberately adjusted analyses for ICV. In that study the VIF was always smaller than 2 for the determinant of interest.

To get more insight into the issue of multicollinearity in epidemiologic brain research, I suggest that studies report the VIF of their determinant of interest. With a moderate to high VIF (for example a  $VIF > 5$ ), studies should report the analyses both adjusted and unadjusted for ICV to get more insight in the possible effects of multicollinearity.

#### *Fragmentation of the circadian rhythm and chronotype*

Different persons have different chronotypes. So-called ‘morning types’ prefer to rise up and sleep in early, ‘evening types’ prefer to rise up and sleep in late. This preference probably depends on the functioning of the biological clock, located in the suprachiasmatic nucleus of the brain, and on the synchronisation of this biological clock by external cues of time (“The Zeitgebers”), such as light, temperature and exercise. The process of synchronisation by the environment is termed entrainment.

A person’s chronotype impacts daily functioning, especially because most people are bound to work, school or social life. Most work or school times are scheduled between 9.00 and 17.00. In general, whereas morning types will not experience problems with this schedule, evening types certainly may. For example, an evening type that prefers to go to bed at 3.00, has problems waking up at 7.00, especially when this happens 5 times a week. As a consequence, many persons have to sleep in on weekends, to compensate for the sleep deprivation during the week. This misalignment is called ‘social jetlag’.

Most sleep and circadian rhythm researchers investigate circadian rhythms as determinants without taking chronotype into account. However, chronotype information can add specificity to the associations studied, or chronotype can confound the association between circadian rhythms and the outcome of interest. For example, if the consequences of working night-shifts are studied, the consequences might be underestimated for morning types since this subgroup of exposed persons experiences more problems working night-shifts than evening types. In general, persons around age 20 have the latest chronotypes, whereas older persons usually have earlier chronotypes.<sup>50</sup> Therefore, older persons will in general have more problems with night-shifts compared to younger persons. On the other hand, evening types might perform poorer at school or during early working hours, because of daytime sleepiness and attention problems.<sup>51</sup> Furthermore, the association between chronotype and sleep duration depends on time of week. When studying the average sleep duration per week, chronotype and sleep duration are not related. However, when sleep duration is analysed separately for free days and work days, sleep duration depends on

chronotype: the later a person's chronotype, the longer he or she sleeps at free days and shorter at work days.<sup>50</sup>

Erren and Gross (2015) wrote a letter to the editor of *American Journal of Epidemiology* about our article of circadian activity rhythms and mortality (Chapter 2.2).<sup>52</sup> The authors suggested that chronotype data should have been incorporated in our study.

In our response to this letter to the editor (Chapter 2.2a), we investigated the influence of chronotype information on the association between disturbed circadian activity rhythms and all-cause mortality. First, we added employment status as a proxy of social timing as potential confounder in the association of circadian activity rhythms and mortality. Cohort studies, such as the Rotterdam Study, usually cover a large age range, including workers and non-workers (for example persons who are retired). Usually, non-workers are less bound by schedules, and therefore can live to their biological clock accordingly. Second, we calculated the difference in time in bed and midpoint of sleep between weekend and week days as measures of circadian misalignment in workers and non-workers.<sup>13</sup> Circadian misalignment is subject to the interaction of chronotype and social or working times: earlier chronotypes show more circadian misalignment than late chronotypes when working evening or nights shifts, whereas late chronotypes show more circadian misalignment when working morning shifts. Adjustment for employment status did not influence our results: unstable and fragmented circadian activity rhythms predicted mortality independent of employment status. Yet, workers showed more circadian misalignment than non-workers. Workers spent over an hour more in bed during weekends than on weekdays and went to bed later. Their average midpoint of sleep varied between 3:27 (weekdays) and 4:20 (weekends). In contrast, non-workers spent only half an hour more in bed during weekends, and their average midpoint of sleep varied between 4:00 (weekdays) and 4:16 (weekends).

Employment status, chronotype, bedtimes and wake up times can all be assessed by questionnaire, although bed and wake up times are more precisely estimated when measured objectively. However, the question arises: Can the nonparametric circadian activity rhythms parameters stability and fragmentation add additional information to typical chronotype measures? Unstable activity rhythms are related to larger differences in time spent in bed and to larger differences in the midpoint of sleep on weekdays versus weekends. This suggests that chronotype data or circadian misalignment can be partly captured with actigraphy. However, fragmentation was not related to the circadian misalignment. Thus, I argue that fragmentation cannot be estimated subjectively. Therefore, when investigating circadian activity rhythms, both chronotype data and fragmentation should be incorporated into study protocol and assessments.

## Clinical implications

In this thesis I studied the importance of sleep and circadian activity rhythms in relation to several health parameters. Although the participants of the studies in this thesis were derived from the general population and not from patient groups with a clinical diagnosis of sleep or circadian rhythm problems, I think the results of these studies have implications for clinical practice.

### *Circadian activity rhythm as a marker of general health*

Sleep problems are common in the elderly population. Poor sleep quality has been associated with physical disabilities, depressive symptoms, cognitive impairment and morbidity.<sup>1,53</sup> In this thesis, I showed that not only sleep parameters, but also the circadian activity rhythm is important for health. In fact, in my studies of sleep and circadian activity rhythms with mortality and cerebral small vessel disease, the circadian activity rhythm had a stronger and independent association with mortality and cerebral small vessel disease than the sleep parameters (Chapters 2.2 and 3.1). Furthermore, disturbances in the circadian activity rhythm were also related to poor cognitive functioning and more depressive symptoms (Chapter 2.1). I hypothesize that the circadian activity rhythm is a marker of general health. This implicates that changes in the circadian activity rhythm reflect or indicate changes in different domains of health. I would like to distinguish two pathways, although they are certainly not exclusive. First, it could be that this general health marker is etiologically involved in general health; it is an intermediate on the causal pathway. This means, that problems in the circadian rhythm cause health problems such as cardiovascular disease or neurological disorders. It also implies that treatment of circadian rhythm disturbances could improve the health of a person. Second, the circadian rhythm could change because of underlying health problems. The circadian rhythm is only a marker signaling disease or disease processes. In this case treatment of the circadian rhythm disturbances might, if anything, relieve some symptoms, but will not truly improve general health and prognosis. In any case, information on the circadian rhythm of a patient should aid healthcare professionals to better monitor the effectivity of interventions. More longitudinal epidemiological studies with repeated assessments of the circadian rhythm are needed to improve our insight in whether circadian rhythms are cause and/or consequence of pathophysiological processes.

Further, there is initial evidence that the circadian rhythm influences the effect of taking medications at different time points.<sup>54</sup> Some drugs may have an optimal time of day to be administered and their effect may be decreased when taken at a different time point. This implies that a disturbed circadian rhythm will not only be unfavorable for general health, but it might directly reduce the effectiveness of pharmacotherapy.

### *Circadian activity rhythm as a marker of early disease*

Disturbances in circadian activity rhythms might also be an early sign of disease. Patients with Alzheimer's disease and other types of dementias suffer from circadian rhythm disturbances, such as high activity levels at night.<sup>10</sup> These patients frequently have structural brain damage, a part of which is caused by cerebral small vessel disease. Our finding that cerebral small vessel disease is related to disturbed 24-hour activity rhythms suggests that the disturbances seen in the sleep-wake rhythm in Alzheimer's patients arise early in the disease process before clinical signs are present (Chapter 3.1). Possibly, therapy aimed at improving the circadian rhythm, such as exercise or light therapy, can delay the progression of this disease.

### **Suggestions for future research**

Future research is needed to study whether the associations between sleep/circadian rhythm parameters and brain structure are indeed bidirectional or whether problems in one of the two primarily causes problems in the other. A limitation of our studies of sleep and the brain was the cross-sectional design. Therefore, we were not able to study the temporality of the associations. One possibility is that a better brain health at older age can lead to a better sleep quality. Better brain health can be attained by prevention of vascular damage (a main cause of white matter lesions) and atrophy. Another possibility is that by improving sleep quality, the general health, and thereby brain health of persons is improved. Xie et al. (2013) found that during sleep potentially neurotoxic waste products are removed from the brain interstitial space.<sup>55</sup> Problems in this sleep-process can lead to brain damage. To find out which preventive strategy will be more successful, more has to be known about the temporality of the associations (cause and effect). Nevertheless, healthcare professionals have to realize that persons with brain disorders often have sleep disturbances, and the other way around, persons with sleep problems might have (subclinical) brain damage. In the Rotterdam Study, the second time point measurement of actigraphy is now available. Together with the multiple MRI measurements that are already available in the Rotterdam Study, this will be one of the few cohorts that will be able to study the longitudinal relation of objective sleep and circadian rhythm parameters with brain structure, and thereby be able to assess reversed causality, and the directionality of the association between brain structure and sleep.

Information on the direction of effect is also important for treatment. For example, is treatment focussing on brain health, e.g. by treating cardiovascular risk factors, also beneficial for sleep disorders and age-related sleep complaints? Is treatment focussing on improving sleep and circadian rhythms, e.g. by light therapy or increasing exercise during the

day, beneficial for a healthy brain and survival? An interesting start for this kind of research might be in insomnia patients in whom we found that structural brain variations seem to underlie insomnia. Furthermore, sleep and the brain could be investigated by comparing persons with a sleep disorder (e.g. insomnia) and structural brain damage with sleep disorder patients without brain damage. Or vice versa: study persons with structural brain damage, such as stroke or Alzheimer's disease patients, and compare patients with and without sleep problems. This will indicate which risk and protective factors are important for sleep and a healthy brain.

In our studies, the associations between sleep and the brain were not straightforward. For example, in Chapter 3.3 structural brain variation was not related to objective total sleep time, sleep efficiency and subjective sleep quality. On the other hand, in Chapter 3.4 we found that the size of brain structures corresponded to the severity of insomnia. More research is needed to get better insight in the relation between sleep and the brain. First, structural brain variation is possibly only in specific brain regions related to sleep parameters. We measured global gray and white matter volumes, but we did not investigate specific lobes or smaller brain regions. Also, with our PSG analyses we only studied EEG power estimated from lead C3/A2. The combination of using more electrodes during PSG and studying brain structures in more specific regions may provide more information about the association of sleep and the brain. Second, brain function might have a more important role than brain structure in the association with sleep. Studies measuring brain function, e.g. using functional MRI, could perhaps shed more light on this. An important caveat, however, is that brain activity differs during day and night-time, and that performing functional MRI during sleep is practically very challenging.

Research on the associations between PSG and circadian rhythm measures is scarce. The Rotterdam Study is one of the few population-based studies that implemented both ambulant PSG and actigraphy. Although sleep and the circadian rhythm are two different processes, they are closely related. As described above, the mechanism behind sleep is thought to consist of two interacting processes<sup>14</sup>: Process S, representing sleep pressure, and Process C, representing the circadian rhythm. Following this theory, disturbed circadian rhythms will impair sleep, but also sleep problems can disturb the circadian rhythm. To illustrate, sleep apnea is characterized by cessation of breathing, but also by light sleep and waking up during sleep, causing fragmented sleep. This sleep fragmentation will automatically lead to more fragmentation over a 24-hour day. Increased fragmentation is an important circadian activity rhythm measure related to depressive symptoms and mortality. More research is needed to study the associations between PSG and circadian rhythm measures, to get more insight in the interrelation of sleep and circadian rhythms. This will aid the about 80% of elderly persons with sleep complaints.



Furthermore, much is unknown about the influence of genetics on sleep. Genetic studies to sleep have been only limitedly successful. Probably, this is partly due to the use of subjective sleep measures. Sleep questionnaires are usually not very specific. For example, women report poorer sleep than men.<sup>56</sup> Therefore, very large numbers are needed to find associations between genetics and sleep. Currently, the amount of polysomnographic data is increasing, opening ways for large collaborative efforts to address these issues. Future genetic research can benefit from this increase in objective sleep parameters.

### **Concluding remarks**

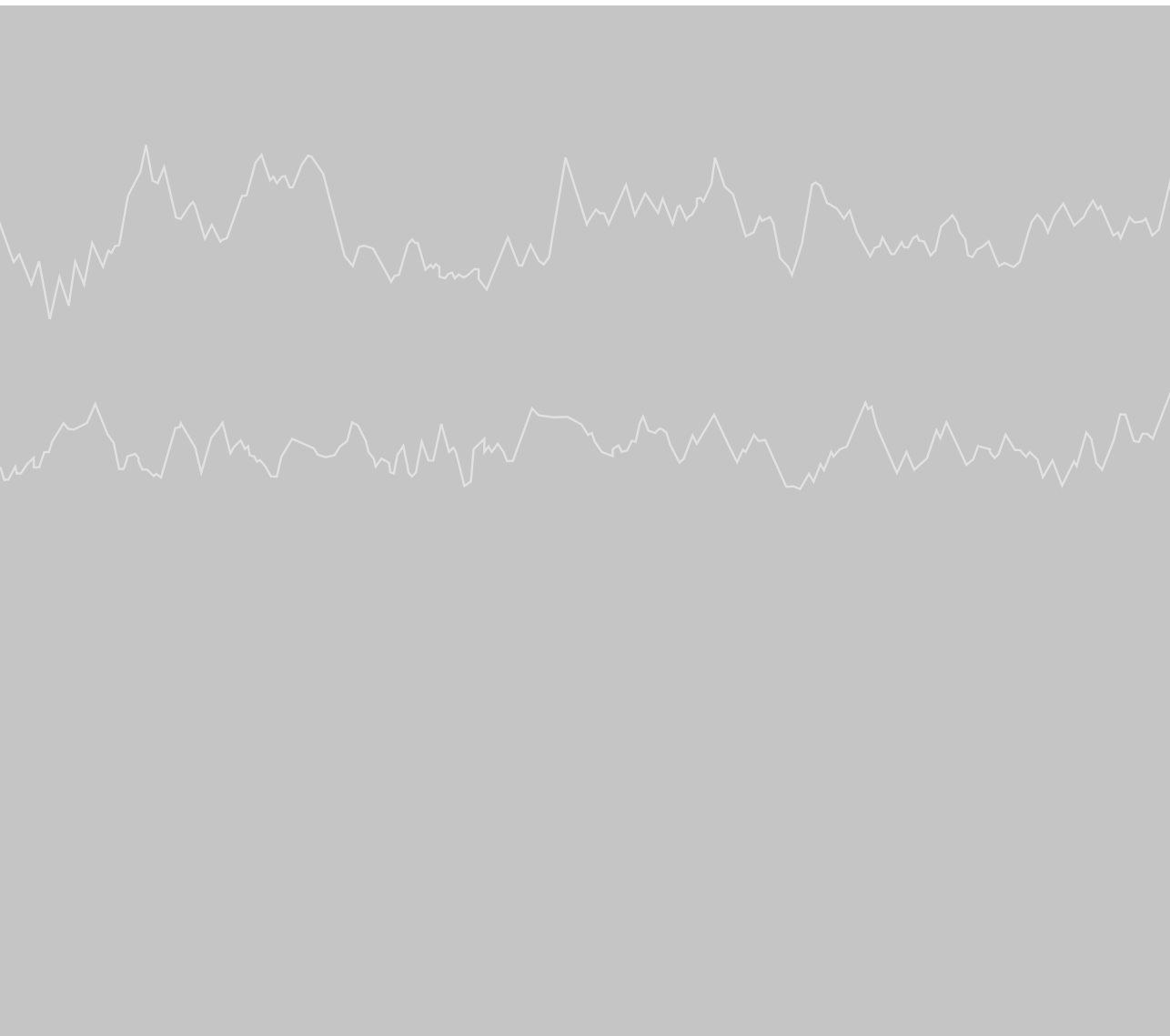
Sleep, 24-hour activity rhythms and the brain are three very important factors for a healthy life. With my thesis I brought these factors together in a middle-aged and elderly population. This unique project combined polysomnography, actigraphy and MRI and pointed out the complex nature of the relations between sleep, 24-hour activity rhythms and the brain. I believe that the combination of the interesting fields of sleep and the brain will reveal much more fascinating insights in the future.

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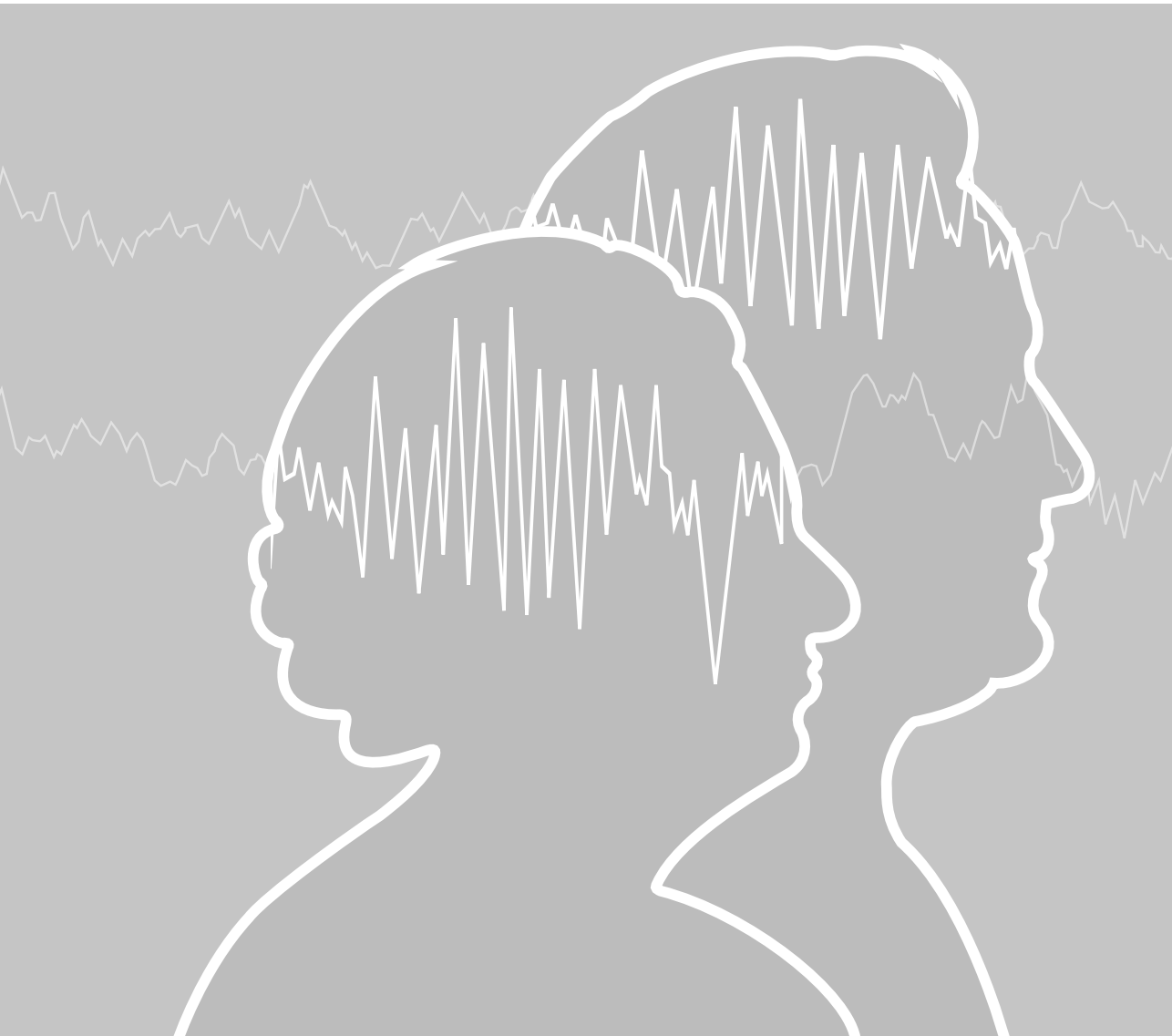
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# Chapter 5

**Summary**  
**Samenvatting**





## Summary

A good night of sleep is very important for a healthy life (**Chapter 1**). However, sleep problems and circadian rhythm problems are very common, especially in the older population. About 80% of the elderly report sleep complaints. For example, elderly persons report trouble falling asleep, waking up too early, a low sleep quality, involuntary napping during the day, and obtaining not enough sleep during the night. The sleep and circadian rhythm changes with ageing are paralleled by structural brain changes that are also more prevalent in elderly populations. Typical brain changes observed in elderly populations are loss of brain tissue (i.e. cerebral atrophy), cerebral vascular lesions, and reduced connectivity between brain areas. These brain changes often occur unnoticed. However, even small brain changes can affect health and behavior. To date, there is only scarce knowledge whether brain changes typically observed in an ageing population relate to altered sleep patterns. The aim of this thesis was to investigate the associations between sleep, 24-hour activity rhythms and health parameters in a middle-aged and elderly population, particularly focusing on the relation between sleep and age-related structural brain changes. The analyses performed in this thesis were based on data from the Rotterdam Study, a large population-based cohort among adults aged 45 and over living in Rotterdam, the Netherlands.

In **Chapter 2** I describe the associations between sleep, 24-hour activity rhythms and several health aspects. **Chapter 2.1** describes the influence of demographics, lifestyle and sleep on 24-hour activity rhythms. 24-Hour activity rhythms were quantified by the stability and the fragmentation of the rhythm. Older age was associated with a more stable and a more fragmented 24-hour activity rhythm. Furthermore, a high body mass index, smoking and having more depressive symptoms were related to unstable and more fragmented rhythms. This suggests that stability and fragmentation of the 24-hour activity rhythm are important for major health issues in elderly. In **Chapter 2.2** we studied the mortality risk of sleep and 24-hour activity rhythms. Whereas sleep measures were not related to mortality after adjustment for health parameters, unstable and fragmented activity rhythms were related to a higher all-cause mortality risk. This strengthens the idea that disturbed circadian activity rhythms reflect age-related alterations in the biological clock and could be an indicator of disease. Chapter 2.2 was extended with **Chapter 2.2a**. In this chapter I discuss the influence of circadian misalignment to the circadian activity rhythm and mortality association. Circadian misalignment refers to the difference between the timing of sleep on workdays compared to weekend days. I describe that although employment status did not influence the associations between the 24-hour activity rhythm and mortality, more circadian misalignment was observed in employed participants compared to unemployed participants. Furthermore, this circadian misalignment was associated with the stability of the activity rhythm. However, circadian misalignment indicators did not predict mortality.



In **Chapter 2.3** I present a study on the differential effects of clinical heart failure and echocardiographic indicators of cardiac dysfunction on sleep quality. Clinical heart failure predicted a reduction of sleep quality in longitudinal assessment. This association was driven by the sleep onset latency and sleep quality components of the Pittsburgh Sleep Quality Index. Cardiac dysfunction, measured by echocardiography, was not related to sleep quality in cross-sectional or longitudinal analyses. These findings suggest that clinical manifestations of heart failure negatively affect sleep.

In **Chapter 3** associations between sleep, 24-hour activity rhythms and structural brain parameters are reported. **Chapter 3.1** discusses the relation of cerebral small vessel disease with disturbed 24-hour activity rhythms. Cerebral small vessel disease may manifest as lacunar infarcts, white matter lesions or cerebral microbleeds. I found that white matter lesion volume and presence of cerebral microbleeds are related to disturbed activity rhythms. This suggests that subclinical brain damage affects the 24-hour activity rhythm. In **Chapter 3.2** I investigate different aspects of sleep apnea, the apnea-hypopnea index (AHI), nocturnal oxygen desaturation and arousals, in relation with brain structure. Only nocturnal oxygen desaturation was related to white matter brain atrophy. The amount of apneas, hypopneas or sleep apnea related arousals did not explain this association. **Chapter 3.3** describes the associations between brain structure and sleep parameters, measured both objectively with polysomnography and subjectively. The results demonstrated that brain structure was not associated with delta power (slow wave sleep), but it related to beta power during NREM sleep. Beta power during wake is associated with vigilance and cognition. Gray matter atrophy was associated with more beta power, white matter atrophy and less white matter integrity were related to less beta power. Also, more beta power was associated with a shorter total sleep time, a lower sleep efficiency and poor subjective sleep quality, all indicating poor sleep. However, these associations were independent of brain structural variations. Beta power during sleep is considered unfavorable, causing hyperarousal, but during wake beta power is a meaningful signal. Probably, the important role of white matter in connectivity of brain regions during the day, is also reflected in more beta power at night. In **Chapter 3.4** differences in brain morphology between insomnia cases and good sleepers are explored. I found that persons with insomnia complaints had smaller white matter volumes compared to the good sleepers, suggesting that structural brain variations underlie insomnia, or that insomnia causes structural brain variations. Moreover, the size of brain structures corresponded to the severity of insomnia: a smaller white matter volume was related to a lower sleep efficiency, a smaller gray matter volume was related to a poorer sleep quality, and a smaller hippocampal volume was related to a shorter sleep duration and to a poorer sleep quality.

In **Chapter 4** I review the main findings of this thesis. Furthermore, in this chapter I discuss methodological considerations, clinical implications and suggestions for future research.

## Samenvatting

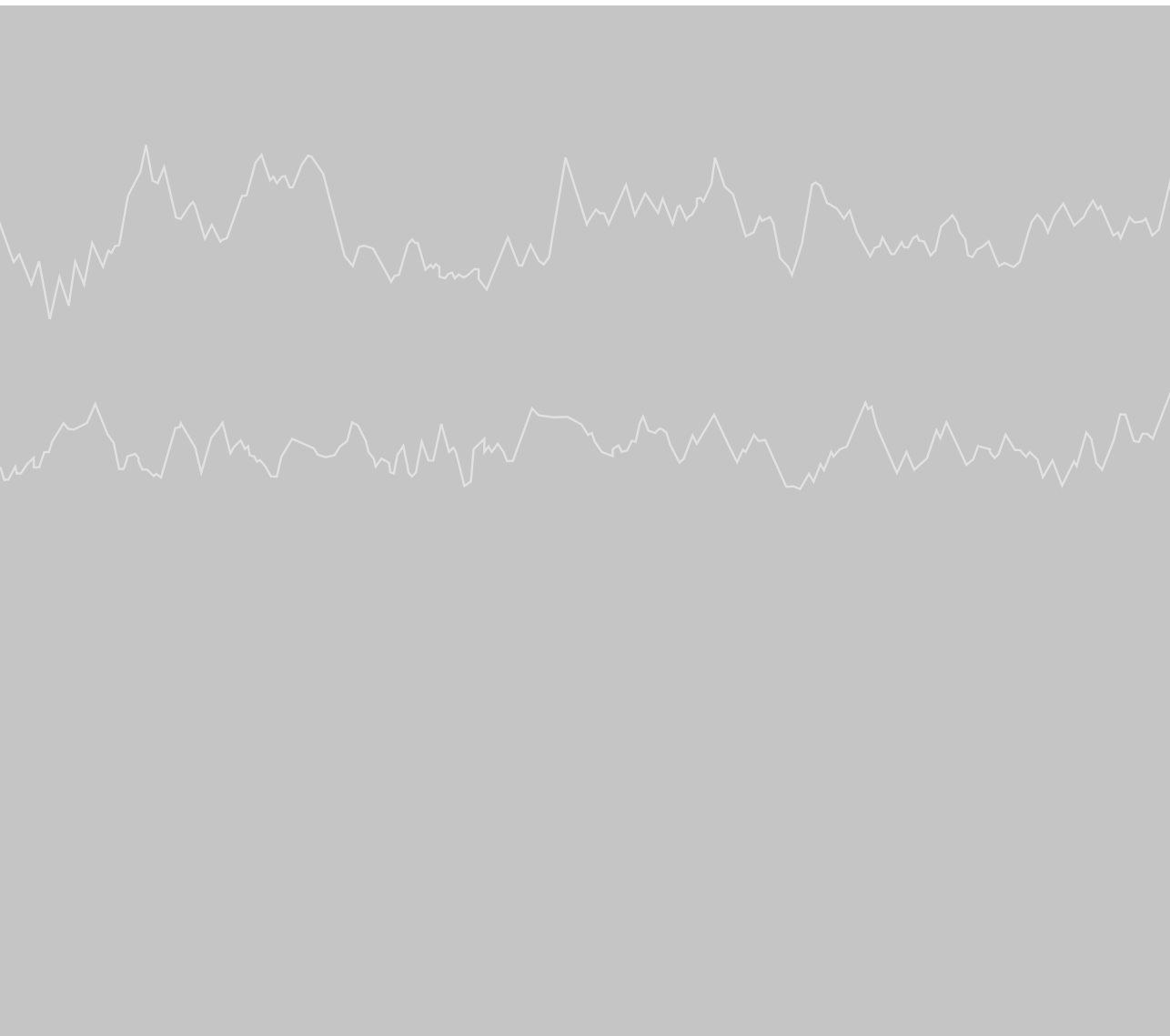
Een goede nachtrust is belangrijk voor een gezond leven (**Hoofdstuk 1**). Slaapproblemen en problemen met het circadiaans ritme komen veel voor, vooral in de oudere populatie. Ongeveer 80% van de ouderen heeft slaapkachten. Ouderen hebben moeite met in slaap vallen, worden te vroeg wakker, rapporteren een slechte slaap kwaliteit, doen (al dan niet ongewild) dutjes overdag en krijgen niet genoeg slaap tijdens de nacht. Naast veranderingen in slaap en circadiaans ritme, komen ook structurele hersenveranderingen bij ouderen meer voor. Kenmerkende hersenveranderingen bij ouderen zijn een verlies aan hersenweefsel (cerebrale atrofie), cerebrale vasculaire laesies en een verminderde connectiviteit tussen hersengebieden. Deze hersenveranderingen worden door ouderen zelf niet altijd opgemerkt. Desondanks kunnen deze kleine hersenveranderingen gezondheid en gedrag beïnvloeden. Er is nog weinig bekend over de relatie tussen leeftijdsgerelateerde hersenveranderingen en veranderende slaappatronen. Inzicht in de relatie tussen deze veelvoorkomende problemen is belangrijk voor klinische oplossingen. In dit proefschrift onderzoek ik de relaties tussen slaap, het 24-uurs bewegingsritme en gezondheidsparameters, met name structurele hersenveranderingen gerelateerd aan ouderdom, in mensen van 45 jaar en ouder. Al deze mensen namen deel aan het Erasmus Rotterdam Gezondheid Onderzoek (ERGO), een groot bevolkingsonderzoek onder mensen van 45 jaar en ouder die in de wijk Ommoord in Rotterdam wonen.

In **Hoofdstuk 2** beschrijf ik de relatie tussen slaap, 24-uurs bewegingsritmes en verschillende gezondheidsfactoren. **Hoofdstuk 2.1** laat zien hoe demografische kenmerken, lifestyle en slaapsamenhangen met het 24-uurs bewegingsritme. Hiervoor bestudeerden wij de stabiliteit en fragmentatie van het 24-uurs bewegingsritme, gemeten met actigrafie. Een oudere leeftijd was geassocieerd met zowel een meer stabiel als een meer gefragmenteerd 24-uurs ritme. Daarnaast waren een hoge BMI, roken en meer depressieve symptomen gerelateerd aan onstabiele en gefragmenteerde 24-uurs ritmes. Dit suggereert dat de stabiliteit en fragmentatie van het 24-uur bewegingsritme belangrijk zijn bij gezondheidsproblemen in ouderen. In **Hoofdstuk 2.2** zijn slaap en het 24-uurs bewegingsritme gebruikt om het overlijdensrisico te voorspellen. Hoewel slaap niet gerelateerd was aan het overlijdensrisico als er rekening werd gehouden met verschillende gezondheidsparameters, waren onstabiele en gefragmenteerde 24-uurs ritmes wel gerelateerd aan een hoger overlijdensrisico. Deze bevindingen versterken de hypothese dat verstoorde circadiaanse ritmes leeftijdsgerelateerde veranderingen in de biologische klok weergeven, waarmee zij een indicatie voor een slechtere gezondheid zijn. In aanvulling op deze bevindingen bespreek ik in **Hoofdstuk 2.2a** hoe 'sociale jetlag' een rol kan spelen in de relatie tussen het 24-uurs ritme en de kans op overlijden. De 'sociale jetlag' refereert naar het verschil in het 'biologische' ritme en het 'sociale' ritme, het ritme dat door de omgeving opgelegd wordt,

bijvoorbeeld door werk en kinderen. Ondanks dat de relatie tussen een verstoord 24-uurs ritme en de kans op overlijden niet verdwijnt als er gecontroleerd wordt voor werkstatus, hebben werkende mensen wel meer last van een 'sociale jetlag' dan mensen die niet (meer) werken. De 'sociale jetlag' was geassocieerd met stabiliteit van het 24-uurs ritme, maar voorspelde zelf niet het overlijdensrisico. **Hoofdstuk 2.3** gaat over de effecten van klinisch hartfalen en verminderde hartfunctie (gemeten met echocardiografie) op de subjectieve slaapkwaliteit. Klinisch hartfalen voorspelt het rapporteren van een slechte slaapkwaliteit (Pittsburgh Sleep Quality Index) en het langer duren van het inslaap vallen. Een verminderde hartfunctie was niet gerelateerd aan gerapporteerde slaapproblemen. Dit suggereert dat de ernst, of het bewustzijn, van hartfalen de slaap negatief beïnvloeden.

In **Hoofdstuk 3** bespreek ik de associaties tussen slaap, 24-uurs bewegingsritmes en de hersenstructuur. **Hoofdstuk 3.1** gaat over de relatie tussen schade aan kleine bloedvaten in de hersenen, zoals schade aan de witte stof en microbloedingen, en verstoorde 24-uurs ritmes. Ik heb gevonden dat een groter volume van de beschadigingen aan de witte stof en meer cerebrale microbloedingen gerelateerd zijn aan meer verstoorde 24-uurs ritmes. Dit impliceert dat subklinische hersenschade het 24-uurs ritme beïnvloed. In **Hoofdstuk 3.2** heb ik verschillende factoren van slaap apneu onderzocht, de apneu-hypopneu index (AHI), nachtelijk zuurstoftekort en arousals ('word wakker' reflex) in relatie met hersenstructuur. Alleen het nachtelijke zuurstoftekort was gerelateerd aan witte stof atrofie. **Hoofdstuk 3.3** beschrijft de associaties tussen hersenstructuur met objectieve slaapparameters (gemeten met polysomnografie) en subjectieve slaapparameters. Hersenstructuur was niet gerelateerd aan delta golven, die voornamelijk zichtbaar zijn tijdens de diepe slaap, maar wel aan de hoeveelheid bèta golven tijdens de non-REM-slaap. Bèta golven zijn gerelateerd aan alertheid. Een groter grijze stof volume was geassocieerd met minder bèta golven, terwijl een groter witte stof volume en betere witte stof integriteit geassocieerd waren aan meer bèta golven. Meer bèta golven tijdens de NREM slaap relateerden aan een kortere totale slaaptijd, minder slaap efficiency en een slechte subjectieve slaapkwaliteit, dus aan een slechtere slaap. Deze associaties tussen bèta golven en slaap waren onafhankelijk van hersenstructuur. Mogelijk verklaart de belangrijke relatie tussen witte stof verbindingen en bèta golven tijdens de dag, de verhoogde hoeveelheid bèta golven tijdens de nacht. In **Hoofdstuk 3.4** heb ik verschillen in hersenstructuur tussen mensen met subjectieve insomnie klachten en goede slapers onderzocht. Ik heb gevonden dat mensen met insomnie klachten een kleiner witte stof volume hebben. Dit suggereert dat variaties in hersenstructuur ten grondslag liggen aan insomnie, of dat door insomnie klachten hersenveranderingen ontstaan. Bovendien hing binnen mensen met insomnie, de grootte van hersenstructuren samen met de ernst van insomnie: een kleiner witte stof volume was gerelateerd aan een lagere slaap efficiëntie, een kleiner grijze stof volume was gerelateerd aan een slechtere slaap kwaliteit en een kleinere hippocampus was gerelateerd aan een kortere slaapduur en een slechtere slaapkwaliteit.

In **Hoofdstuk 4** bespreek ik de hoofdbevindingen van dit proefschrift. Daarnaast bespreek ik in dit hoofdstuk methodologische overwegingen, klinische implicaties en suggesties voor toekomstig onderzoek.



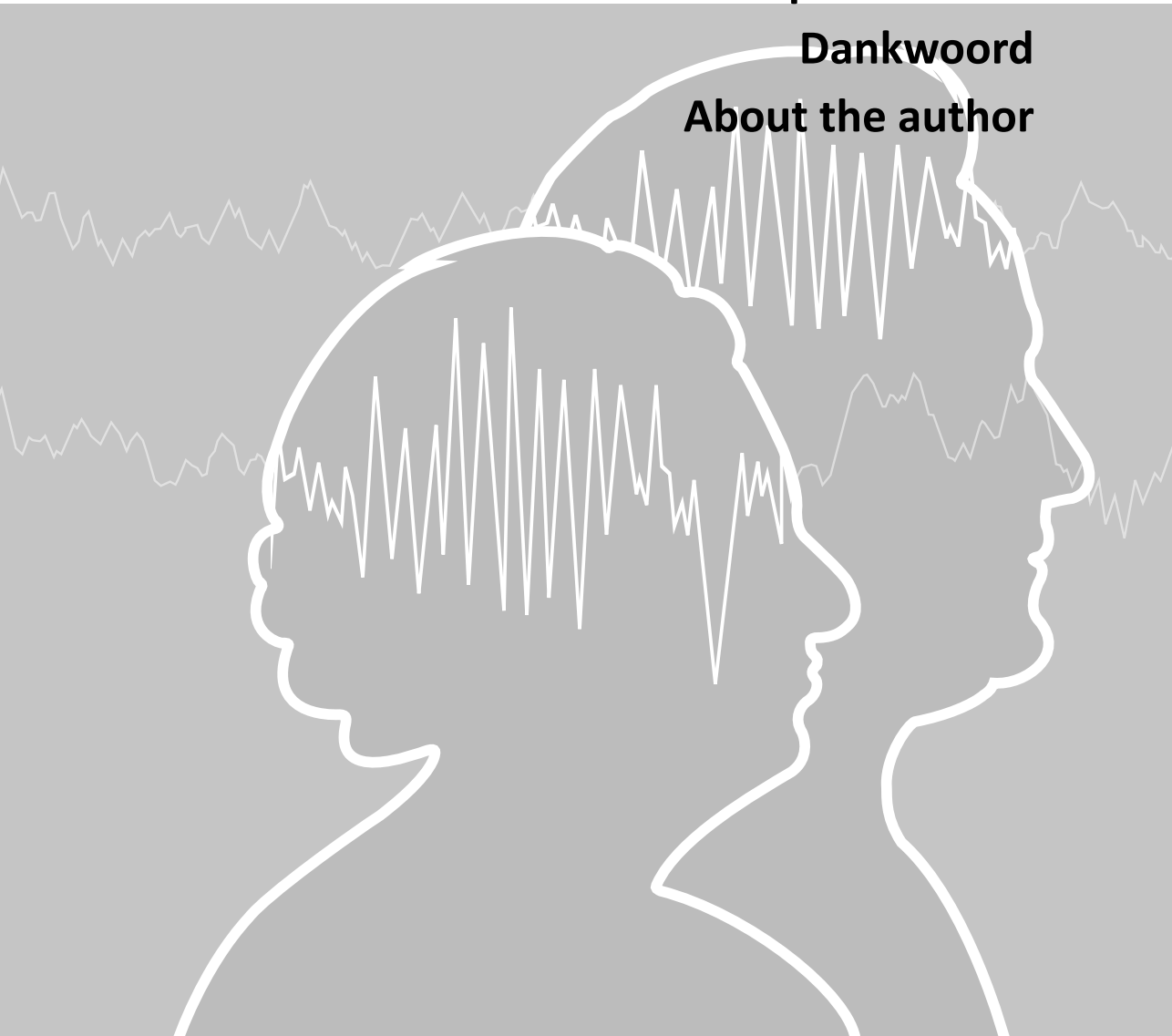
# Chapter 6

**PhD Portfolio**

**List of publications**

**Dankwoord**

**About the author**





## PhD Portfolio

Name: Lisette A. Zuurbier  
 Erasmus MC Department: Epidemiology  
 Research School: Netherlands Institute for Health Sciences (NIHES)  
 PhD period: 2011 – 2015  
 Promotors: Prof. dr. H. Tiemeier & Dr. M.W. Vernooij

	Year	ECTS
<b>MSc Clinical Epidemiology, NIHES</b>	2011-2013	
<i>Courses</i>		
○ Study Design		4.3
○ Classical Methods for Data-analysis		5.7
○ Clinical Epidemiology		5.7
○ Methodologic Topics in Epidemiologic Research		1.4
○ Biostatistical Methods II: Classical Regression Models		4.3
○ Principles of Research in Medicine		0.7
○ Clinical Decision Analysis		0.7
○ Methods of Public Health Research		0.7
○ Pharmaco-epidemiology		0.7
○ Genome Wide Association Analysis		1.4
○ Case-control Studies		0.7
○ History of Epidemiologic Ideas		0.7
○ Markers and Prognostic Research		0.7
○ The Practice of Epidemiologic Analysis		0.7
○ Logistic Regression		1.4
○ Repeated Measurements in Clinical Studies		1.4
○ Psychiatric Epidemiology		1.1
○ Missing Values in Clinical Research		0.7
○ Courses for the Quantitative Researcher		1.4
<b>Other courses</b>		
○ Biomedical English Writing and Communication	2013	4.0
○ Internship scoring of sleep phases, University of Chicago	2013	2.0
<b>Seminars</b>		
○ Seminars Department of Epidemiology	2011-2015	1.0



**(Inter)national conferences and presentations**

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|--|------|-----|
| ○ Najaarssymposium NSW0, Sint-Michielsgestel   | 2012 | 0.3 |
| ○ World Congress on Sleep Medicine, Valencia, Spain.<br>Oral presentation: "Circadian activity rhythm disturbances predict mortality"                                  | 2013 | 1.0 |
| ○ Oral presentation: "The Rotterdam Study", Section of Endocrinology in the Department of Medicine, University of Chicago, USA   | 2013 | 1.0 |
| ○ CTR meeting, Amsterdam   | 2013 | 0.3 |
| ○ SLEEP 2014, Minneapolis, USA. Oral presentations: "Cerebral small vessel disease and 24-hour activity rhythms and sleep: A population-based study"                   | 2014 | 1.0 |
| ○ SLEEP 2014, Minneapolis, USA. Poster presentation: "Cerebral small vessel disease and Actigraphically measured circadian rhythm and sleep: A population-based study" | 2014 | 1.0 |
| ○ Oral presentation: "Sleep and circadian activity rhythms: The Rotterdam Study", Department of Psychiatry, Erasmus MC   | 2014 | 1.0 |
| ○ Najaarssymposium NSW0, Eindhoven   | 2014 | 0.3 |

**Teaching**

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|--|-----------|-----|
| ○ Lectures Wetenschapsknooppunt (primary schools)  | 2012-2014 | 1.0 |
| ○ Teaching assistant 'Principles of Research in Medicine' Erasmus Summer Programme   | 2014      | 1.4 |
| ○ Supervising Master's thesis, Madhu Soekhai, "The relation of gray and white matter volumes with sleep EEG frequencies in the ageing brain" | 2014-2015 | 3.0 |
| ○ Supervising Master's thesis, Lindsay de Ligt, "The associations of slow wave sleep with cognition and memory"                              | 2014-2015 | 3.0 |

**Reviewing**

- |  |           |     |
|--|-----------|-----|
| ○ Reviewing articles for 'European Journal of Epidemiology' and 'BMC Psychiatry' | 2013-2015 | 1.0 |
|--|-----------|-----|

## List of publications

### *Manuscripts described in this thesis*

Luik AI, [Zuurbier LA](#), Hofman A, Van Someren EJ, Tiemeier H. Stability and fragmentation of the activity rhythm across the sleep-wake cycle: the importance of age, lifestyle, and mental health. *Chronobiol Int* 2013;30:1223-30.

[Zuurbier LA](#), Luik AI, Hofman A, Franco OH, Van Someren EJ, Tiemeier H. Fragmentation and stability of circadian activity rhythms predict mortality: the Rotterdam study. *Am J Epidemiol* 2015;181:54-63.

[Zuurbier LA](#), Kocavska D, Tiemeier H. Three Authors Reply. *Am J Epidemiol* 2015;182:470-1.

[Zuurbier LA](#), Luik AI, Leening MJ, Hofman A, Freak-Poli R, Franco OH, Stricker BH, Tiemeier H. Associations of heart failure with sleep quality: the Rotterdam Study. *J Clin Sleep Med* 2015;11:117-21.

[Zuurbier LA](#), Ikram MA, Luik AI, Hofman A, Van Someren EJ, Vernooij MW, Tiemeier H. Cerebral small vessel disease is related to disturbed 24-h activity rhythms: a population-based study. *Eur J Neurol* 2015;22:1482-7.

[Zuurbier LA](#), Vernooij MW, Luik AI, Kocavska D, Hofman A, Whitmore H, Ikram MA, Tiemeier H. Apnea-hypopnea index, nocturnal arousals, oxygen desaturation and structural brain changes: A population-based study. Submitted.

[Zuurbier LA](#), Luik AI, Tiemeier H, Niessen WJ, Whitmore H, Ikram MA, Vernooij MW. Brain structure, EEG activity during sleep and sleep quality: A population-based study of middle-aged and elderly persons. Submitted.

[Zuurbier LA](#), Vernooij MW, Spiegelhalder K, Hofman A, Niessen WJ, Van Someren EJ, Ikram MA, Tiemeier H. Gray matter, white matter and hippocampal volume in middle-aged and older adults with insomnia: A population-based study. In preparation.

### *Other manuscripts*

Aarts N, [Zuurbier LA](#), Noordam R, Hofman A, Tiemeier H, Stricker BH, Visser LE. Use of Selective Serotonin Reuptake Inhibitors and sleep quality: A population-based study. *Journal of Clinical Sleep Medicine*, accepted for publication.

Dashti HS, [Zuurbier LA](#), De Jonge E, Voortman T, Jacques PF, Lamon-Fava S, Scheer FAJL, Kieft-de Jong JC, Hofman A, Ordovás JM, Franco OH, Tiemeier H. Actigraphic sleep fragmentation, efficiency, and duration associate with dietary intake in the Rotterdam Study. *Journal of Sleep Research*, accepted for publication.

Luik AI, Direk N, [Zuurbier LA](#), Hofman A, Van Someren EJ, Tiemeier H. Sleep and 24-h activity rhythms in relation to cortisol change after a very low-dose of dexamethasone. *Psychoneuroendocrinology* 2015;53:207-16.

- Luik AI, Noteboom J, Zuurbier LA, Whitmore H, Hofman A, Tiemeier H. Sleep apnea severity and depressive symptoms in a population-based study. *Sleep Health* 2015;1:128-32.
- Luik AI, Zuurbier LA, Direk N, Hofman A, Van Someren EJ, Tiemeier H. 24-Hour Activity Rhythm and Sleep Disturbances in Depression and Anxiety: A Population-Based Study of Middle-Aged and Older Persons. *Depress Anxiety* 2015;32:684-92.
- Luik AI, Zuurbier LA, Hofman A, Van Someren EJ, Ikram MA, Tiemeier H. Associations of the 24-h activity rhythm and sleep with cognition: a population-based study of middle-aged and elderly persons. *Sleep Med* 2015;16:850-5.
- Luik AI, Zuurbier LA, Whitmore H, Hofman A, Tiemeier H. REM sleep and depressive symptoms in a population-based study of middle-aged and elderly persons. *J Sleep Res* 2015;24:305-8.
- Milic J, Saavedra Perez H, Zuurbier LA, Boelen PA, Rietjens JA, Hofman A, Tiemeier H. The longitudinal and cross-sectional associations of grief and complicated grief with sleep quality in older adults. Submitted.
- Springelkamp H, Zuurbier LA, Luik AI, Wolfs RCW, Hofman A, Klaver CCW, Tiemeier H, Jansonius NM. Relationship between sleep apnea and open-angle glaucoma: a population-based cohort study. In preparation.
- Stoffers D, Zuurbier LA, van Tol M, Penninx B, Veltman D, Van der Wee N, Tiemeier H, Van Someren EJ. A psychiatric phenotype-specific brain structural correlate of insomnia severity. Submitted.
- Zuurbier LA, Nikolova YS, Ahs F, Hariri AR. Uncinate fasciculus fractional anisotropy correlates with typical use of reappraisal in women but not men. *Emotion* 2013;13:385-90.

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## About the author

Lisette Zuurbier was born on March 22, 1988 in Langedijk, the Netherlands. She graduated from pre-university education at Han Fortmann in Heerhugowaard in 2006. Subsequently, Lisette started to study Psychobiology at the University of Amsterdam. After her graduation (cum laude) in 2009, Lisette started the Master's Programme Neuroscience and Cognition at Utrecht University. As part of this Master, she did her research internships at Duke University, North Carolina, USA and at the Helmholtz Institute, Utrecht. Lisette got her Master of Science degree in 2011. In August 2011, Lisette started the work presented in this thesis at the department of Epidemiology of the Erasmus University Medical Center in Rotterdam under the supervision of Prof. Dr. Henning Tiemeier and Dr. Meike Vernooij. She obtained a Master's degree in Clinical Epidemiology at the Netherlands Institute for Health Sciences (NIHES) in 2013. In 2014 Lisette got awarded with an American Academy of sleep medicine (AASM) 2014 Circadian Rhythms Section Investigator Award. Since May 2015, she is employed as a data scientist at DASC in Utrecht.



