Neuropsychiatric studies of sleep and 24-hour activity rhythms:

A population-based approach

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Neuropsychiatrisch onderzoek naar slaap
en het 24-uurs bewegingsritme in de algemene bevolking

Proefschrift

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'Enkel als ik slaap, ben ik even weg van al mijn dromen'
(The Opposites, Slapeloze nachten, 2012)

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Chapter 1

Introduction

It is the behavior we all do every day and we tend to like it, at least if everything goes well: sleeping. If we make it up to 90 age of years, we spend over 30 years doing it; we can simply not resist this crucial behavior, not being able to sleep can even result in death.[1] However, it is still not clear why sleeping is so crucial to us. It has been suggested that sleep is needed for energy conservation, but for energy conservation the loss of consciousness would not be necessary. Also, sleeping during the night versus not sleeping during the night only saves us 134 kilocalories,[2] which makes it unlikely that energy conservation is the function of sleep. Another suggestion is that sleep is needed for restoration purposes, it gives the body the time to repair and rejuvenate itself. This idea is supported by the detrimental effects of sleep deprivation on the immune system.[3] More recently, it has been suggested that sleep is crucial for brain function, this theory is supported by research on the effects of sleep on increased brain plasticity and decreased memory performance,[4] and the effects of sleep deprivation on several cognitive functions in laboratory studies.[5]

But what is sleep? One of the most common behavioral definitions describes sleep as 'a reversible behavioral state of perceptual disengagement from and unresponsiveness to the environment'.[6] The most influential model explaining the mechanisms behind sleep is the two-process model, first described in the 1980s. According to this model sleep consists of two processes.[7] Process 'S' entails the sleep pressure or sleep propensity. The sleep propensity rises during waking and declines during sleep. Process 'S' interacts with process 'C' which is the circadian component. The circadian component reflects a clocklike mechanism that is basically independent of prior sleep and waking and determines the approximately 24-hour rhythm of the sleep-wake pattern. These 24-hour, or circadian, rhythms are found in behavior and in physiological processes throughout the body[8] and are regulated by the suprachiasmatic nucleus (SCN). This brain structure accommodates the central timekeeping mechanism,[9] it integrates internal rhythms with external time cues, such as light and exercise.[10]

Sleep and rhythmicity can be measured in multiple ways. Polysomnography (PSG) is the 'gold standard' of sleep research. PSG consists of different electrophysiologic measures, most important are the electroencephalography (EEG) channels which allow the assessment of brain activity during sleep. But not only the brain is active during sleep, PSG also includes bilateral electrooculography (EOG) to measure eye movements, electromyography (EMG) to measure muscle tone, and electrocardiography (ECG) to assess heart function. Further, respiratory belts on the chest and abdomen, oximetry, a nasal pressure transducer and an oronasal thermocouple measure breathing patterns. The Rotterdam Study[11] is one of the few cohorts in the world to have implemented in home, or ambulant, PSG. However, while PSG is very well suited to study sleep, a single night of PSG is not informative about the 24-hour organization of the sleep-wake rhythm. Wearing PSG equipment for multiple days during normal live is simply not feasible. To study 24-hour rhythms we used an accelerometer,

this watch-like-device measures the movement of the wrist. As movement is strongly related to periods of rest and wake, this device allows us to examine the sleep-wake rhythm. Wearing a watch is not considered invasive by most people, therefore actigraphy can be used to study 24-hour rhythms over longer periods of time.

But why would we study sleep and rhythms? As we all know from personal experience, a night of poor sleep can seriously mess up our day. And nights of poor sleep are not uncommon, about 33% of the general population report difficulties in initiating and maintaining sleep.[12] Next to sleep complaints, around 6% of the populations fulfills the criteria for a clinical diagnosis of insomnia.[12] The difference between the large number of persons with sleep complaints versus a relatively small number with clinical insomnia may be explained by the non-chronic nature or severity of the sleep complaints but insomnia might also be underdiagnosed. Sleep apnea is the other colloquial sleep disorder, in men the prevalence is estimated as high as 31% and in women as high as 21%.[13]

Sleep problems can occur as a single problem, but can also co-occur with other disorders. Sleep disorders, specifically insomnia, are frequently comorbid with psychiatric disorders. Persons complaining of sleep difficulties are 3-4 times more likely to be depressed.[14] Vice versa, up to 90% of depressed patients report difficulty falling asleep, staying asleep or early morning awakenings.[15] Sleep disturbances are also seen in persons with panic disorder, generalized anxiety disorder and post-traumatic stress disorder.[16,17] Not only complaints of insomnia are related to depression, an association between sleep apnea and depression has also been reported .[18] Cognitive functions are also strongly related to sleep and rhythms. This is most dramatically reflected by disasters such as the Challenger and Chernobyl accidents in which cognitive errors due to sleep loss are thought to play a role.[19] From sleep deprivation studies we learned that not sleeping severely disrupts cognitive performance.[20] Recent population-based studies have shown an association of disturbed sleep and rhythms with worse cognitive functioning in the general population also.

The goal of this thesis is to assess the variation of sleep and the 24-hour activity rhythm in middle-aged and elderly persons of the general population and to study how this variation is related to psychological and psychiatric problems. The thesis starts with a description of correlates of the 24-hour activity rhythm. In the first chapter the influence of demographics, lifestyle and sleep on the 24-hour activity rhythm are studied (Chapter 2.1). Disturbed rhythms can have detrimental effects independent of sleep, and can even shorten your life (Chapter 2.2). However, it is not just physical health that relates to sleep and 24-hour rhythms; psychiatric and psychological symptoms and diseases are even more intertwined with sleep and rhythms. In the next chapter, it is assessed how 24-hour activity rhythms and sleep are related with cognitive performance (Chapter 3.1), how 24-hour activity rhythms and sleep are associated with depression and anxiety (Chapter 3.2), and

whether 24-hour activity rhythms and sleep relate to how we respond to stress. This stress response was assessed with the biomarker cortisol after the intake of a very low-dose of dexamethasone (Chapter 3.3). As discussed above sleep is best assessed with PSG and implementing ambulant PSG in the Rotterdam Study was the core of this PhD-project. The fourth chapter discusses the first results of this PSG sleep research in the Rotterdam Study, we assessed how the microstructure of REM-sleep is related with depressive symptomatology (Chapter 4.1). Additionally, we tried to entangle the relation between sleep apnea, depressive symptoms and fatigue (Chapter 4.2). Chapter 5 comprises a general discussion of the work summarized in this thesis. In this chapter, I discuss methodological considerations and future directions for clinical practice and scientific research. Last, chapter 6 gives a short summary of this thesis.

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Chapter 2

24-Hour activity rhythms and habitual sleep in middle-aged and elderly persons

Chapter 2.1

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

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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 ± 14 hours, mean ± SD) were collected from 1734 people (age 62 ± 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 (β=.07, p=.02), but also with a more fragmented distribution of periods of activity and inactivity (β=.20, p<.001). Having more depressive symptoms was related to less stable (β=−.07, p=.003) and more fragmented rhythms (β=.10, p<.001). A high Body Mass Index and smoking were also associated with less stable rhythms (BMI: β=−.11, p<.001, smoking: β=−.12, p<.001) and more fragmented rhythms (BMI: β=.09, p<.001, smoking: β=.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.[1] 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,[2] a higher fragmentation of the rest and activity pattern,[3] a tendency to fall asleep earlier,[4] and a tendency to wake up earlier.[5] 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,[6,7] 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.[8] 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.[9] In a cross-sectional, population-based study, regular daily caffeine intake was also, albeit less clearly associated with disturbed sleep and daytime sleepiness.[10] 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.[11] 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[12] and as well in demented elderly people.[13]

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[14,15] 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. 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.[16] 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.[17]

From December 2004 until April 2007, in total 2632 consecutive participants were invited to enter the actigraphy study; 2063 (78%) agreed. The actigraphy study comprised participants of the second cohort (second examination, December 2004 - December 2005), and the new cohort (baseline examination, January 2006 - April 2007). No exclusion criteria were used; participants only 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 (84%) were available for further analyses.

Assessment of the activity rhythm

All participants were asked to wear an actigraph around the non-dominant 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.[18] 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 was 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. To distinguish sleep from waking, a previously described Actiwatch algorithm[19,20] with a low threshold of 20 was used.[21] Total sleep time was calculated as the total time of the epochs classified as sleep between sleep end and sleep start, sleep onset latency is the time between lights out and sleep start and wake after sleep onset is the total time of the epochs scored as wake between sleep start and sleep end.[22] Sleep end and sleep start were defined according to the Actiwatch manual.[19] Possible sleep apnea was assessed with two questions from the Pittsburgh Sleep Quality Index.[23] 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.[24]

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)[25] during one the visits to our center. Depressive mood was assessed using the Center for Epidemiologic Studies Depression (CES-D) scale[26] 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^2). 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,[27] 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. Box-Cox transformation[28,29] was applied to obtain normally distributed values for interdaily stability (λ=7.0), intradaily variability (λ=-3.9), actigraphic sleep onset latency (λ=-0.1) and actigraphic wake after sleep onset (λ=0.4). 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 ± 9.4 years. Participants were generally in good health as indicated by the low average ADL-score (0.29 ± 0.42) and the low prevalence of disease as reported in medical records; 2.6% of participants had had a cardiovascular accident, 2.0% had had a myocardial infarction and 12.9% was diabetic. 11.8% reported having had cancer in the past. The mean interdaily variability was .80 ± .10, the mean intradaily variability was .42 ± .14. Interdaily stability and intradaily variability were moderately negatively correlated (r=−.49, p<.01), see table 2 for correlations of interdaily stability, intradaily variability and sleep characteristics. 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 3 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 (β=.07, p=.020), i.e. more stable rhythms. Male gender (β=.11, p<.001) and being employed (β=−.11, p=.001) were related to a low interdaily stability. Persons with better cognitive functioning (β=.08, p=.001) and less depressive symptoms (β=−.07, p=.003) were more likely to have a high interdaily stability. A high Body Mass Index (β=−.11, p<.001) and smoking (β=−.12, p<.001) were associated with less interdaily stability. Individuals who had a high coffee intake (β=.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 (β=−.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 3). Older age (β=.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 (β=−.13, p<.001) and being employed (β=−.07, p=.010) were associated with a low intradaily variability. Persons with depressive symptoms (β=.10, p<.001) were more likely to have a high intradaily variability. A high Body Mass Index (β=.09 p<.001) and smoking (β=.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 (β=.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.

Several indicators of poor sleep were consistently associated with less interdaily stability and more intradaily variability (see table 4). More daytime napping (β=−.25 p<.001),

use of sleep medication (β=−.09, p<.001), and less subjective total sleep time (β=.07, p=.003) were all associated with less interdaily stability in the analyses fully adjusted for demographic,

Table 1. Population characteristics[*], N=1734

Demographics	
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
Health indicators	
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
Lifestyle	
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
Subjectively assessed sleep[†]	
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[†] (number)	1.51 ± 1.10
Napping (days per week)	1.66 ± 2.05
Objectively assessed sleep[‡]	
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
Sleep quality[†]	
Perceived sleep quality (average per week)	5.54 ± 1.61
Perceived impairment (days per week)	.75 ± 1.44
Activity rhythm[‡]	
Interdaily stability (score)	.80 ± .10
Intradaily variability (score)	.42 ± .13

[*]Mean ± SD, unless stated otherwise. [†]Assessed on daily basis within one week of actigraphy through self-report
[‡]Assessed by one week of actigraphy

Table 2. *Correlations[*] between the activity rhythm, sleep disorders, subjectively assessed sleep, objectively assessed sleep and sleep quality.*

		1	2	3	4	5	6	7	8	9	10	11	12
	Activity rhythm[†]												
1	Interdaily stability	-											
2	Intradaily variability	**−.50**	-										
	Sleep disorders[‡]												
3	Sleep medication	**−.10**	**.10**	-									
4	Possible apnea	**−.06**	**.11**	-.00	-								
	Subjectively assessed sleep												
5	Sleep onset latency	.02	.03	**.14**	-.02	-							
6	Total sleep time	**.09**	**−.10**	**−.12**	-.02	**−.29**	-						
7	Awakenings after sleep onset	.04	**.07**	**.11**	-.01	**.17**	**−.20**	-					
8	Napping	**−.26**	**.46**	**.06**	**.07**	.03	**−.10**	.02	-				
	Objectively assessed sleep												
9	Sleep onset latency	−.05	**.26**	**.09**	**.10**	**.12**	.02	**.06**	**.13**	-			
10	Total sleep time	**.30**	**−.24**	**.05**	−.04	**.11**	**.39**	**.08**	**−.14**	**−.12**	-		
11	Wake after sleep onset	**−.16**	**.22**	**.09**	.00	**.13**	**.09**	**.26**	**.05**	**.33**	**−.19**	-	
	Sleep quality[‡]												
12	Perceived sleep quality	**.11**	**−.09**	**−.27**	-.03	**−.27**	**.44**	**−.31**	**−.10**	.04	.00	**−.09**	-
13	Perceived impairment	**−.15**	**.16**	**.19**	.05	**.15**	**−.15**	**.16**	**.22**	.04	.01	**.08**	**−.53**

[*]Pearson correlation coefficients are presented for correlations between quantitative data; correlations between quantitative and binomial data are represented by point-biserial correlation coefficients. Significant correlations are printed in bold script.
[†]Assessed by one week of actigraphy
[‡]Assessed on daily basis within one week of actigraphy through self report

mental health and lifestyle parameters. Sleep parameters assessed by actigraphy had stronger associations with the stability of the rhythm; less objective total sleep time (β=−.15 p<.001), longer objective sleep onset latency (β=.25 p<.001) and more objective wake after sleep onset (β=−.15 p<.001) were all related to less stable rhythms. Persons who perceived their sleep as good (β=.08, p<.001) and who experienced less impairment due to tiredness (β=−.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 (β=−.02 p=.37) or intradaily variability (β=.03, p=.21) after full adjustment including BMI.

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.

Table 3. *Associations of demographics, mental health and lifestyle with interdaily stability and intra-daily variability**

	Interdaily Stability				Intradaily Variability			
	B	SE	β	p-value	B	SE	β	p-value
Demographic								
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
Mental Health								
Cognitive functioning	.05	.01	.08	.001	−.02	.01	−.04	.08
Depressive symptoms	−.01	.00	−.07	.005	.01	.00	.10	<.001
Lifestyle								
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.

Table 4. *Associations of sleep characteristics and sleep quality with interdaily stability and intradaily variability**

	Interdaily Stability				Intradaily Variability			
	B	SE	β	p-value	B	SE	β	p-value
Sleep disorders								
Sleep medication (ref=no)	−.26	.07	−.09	<.001	.16	.07	.06	.014
Possible apnea (ref=no)	−.04	.06	−.02	.51	.07	.05	.03	.21
Subjectively assessed sleep								
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
Objectively assessed sleep								
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
Sleep quality								
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.

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 can possibly explained by morbidity. For example, disrupted circadian organization of activity rhythms have been related to cerebral changes[30] cardiovascular disease,[6] and mortality.[31] The fragmentation of the rhythm may be 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 cannot easily be expected to 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[1] which have been related to more fragmented rhythms in a postmortem study on demented elderly people.[32] 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 18:00 h was related to less fragmentation of the rhythm. Earlier population-based studies found the opposite; high daily caffeine intake was related to poor sleep.[10] 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. 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.[12] 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[11,13] and even more extensively to sleep characteristics.[33]

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. Actigraphically assessed shorter sleep onset latency, more total sleep time and less wake after sleep onset were associated with more stable and less fragmented rhythms. This confirmed 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 asses a large number of variables and makes our results generalizable. 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. There are also 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, effects 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 typically 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 [34]. 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 change during aging. Little is known about the associations between circadian rhythms and mortality. We investigated whether 24-hour activity rhythms and sleep characteristics predict mortality independently. In 1,734 persons (aged 45-98 years) of the Rotterdam Study (2004 – 2013), actigraphy was used to obtain stability and fragmentation of the 24-hour activity rhythm. Sleep was assessed objectively with actigraphy and subjectively by sleep diary to estimate sleep duration, sleep onset latency and wake after sleep onset. The mean follow-up time was 7.3 years; in total 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 (hazard ratio=0.83 per standard deviation, 95% confidence interval: 0.71, 0.96) and a more fragmented rhythm with a higher mortality risk (hazard ratio=1.22 per standard deviation, 95% confidence interval: 1.04, 1.44). To conclude, low stability and high fragmentation of the 24-hour activity rhythm predicted all-cause mortality, while actigraphic and subjective sleep estimates 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 rhythms of approximately 24 hours, called circadian rhythms. Circadian rhythms and sleep change during aging.[1] Elderly people sleep less during the night, have more fragmented nights, have more difficulty in falling asleep, tend to fall asleep and wake up earlier, take more naps and report a lower sleep quality.[2-6] The longitudinal associations of these changes with adverse health consequences and mortality are not well understood. Previous studies found that short sleepers (≤6 hours) with poor sleep quality have a higher risk of diabetes and cardiovascular diseases.[7,8] Studies investigating sleep and mortality mainly focused on sleep duration. They suggest that the association between sleep duration and mortality is U-shaped; both subjective short and long sleep durations are predictors of all-cause mortality.[9,10]

Few studies have investigated the associations of circadian rhythms with mortality. In the elderly, the amplitude of several physiological circadian rhythms is reduced compared to younger people and the stability of the day-night rhythm declines.[1,4,11] 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.[12] The circadian rhythm in physical activity in elderly is better characterized by two 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.

Two previous studies which analyzed 24-hour activity rhythms parametrically, found that the least robust 24-hour activity rhythms had a 1.5 to 2 times increased all-cause mortality risk in older men and women.[13,14] In addition, abnormal sleep-wake cycles were associated with a three times increased mortality rate in elderly persons above 85 years of age.[15] These studies assessed 24-hour activity rhythms in very old persons with a relatively short follow-up period (on average 4.1, 3.5 and 2 years of follow-up, 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.[14] Another study assessed the sleep quality with the Pittsburgh Sleep Quality Index (PSQI) and daytime sleepiness with the Epworth Sleepiness Scale as determinants of mortality, but found that these parameters were not associated with mortality.[15] However, none of these studies took objectively assessed sleep characteristics such as sleep duration,

sleep onset latency and wake after sleep onset into account. In a large population-based study 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 with a longer follow-up period. We also ran analyses excluding mortality in the first two years as this minimizes the risk of reversed causality. Furthermore, actigraphic and subjective sleep diary estimates of sleep duration, sleep onset latency, wake after sleep onset and sleep quality are studied in relation to mortality, to investigate whether circadian rhythm and sleep characteristics predict mortality independently.

Materials and methods

Participants

This study was conducted within the Rotterdam Study, a prospective study of persons aged ≥45 years living in Rotterdam, The Netherlands that started in 1990. It examines the incidence and risk factors of neurological, cardiovascular, psychiatric, and other chronic diseases. More details about the Rotterdam Study can be found elsewhere.[16] 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, 2,632 successive participants were invited 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 due to technical problems or that contained less than 4 consecutive days and nights, 1,734 (84%) recordings (mean recording duration 138 (standard deviation (SD), 14) hours) were available for analyses.[17] No participants were lost to follow-up.

Actigraphy

We measured the 24-hour activity rhythm with an actigraph (Actiwatch model AW4, Cambridge Technology Ltd, Cambridge, United Kingdom) worn on the non-dominant wrist, as described previously.[18] In brief, participants were asked to wear the actigraph for seven consecutive days and nights, and to remove it only before bathing. Recordings were obtained in 30-second epochs. Because elderly can have less distinct circadian rhythms, the 24-hour activity rhythm was analyzed nonparametrically, thus no assumptions were made about the underlying shape of the circadian rhythm. The actigraph was used to calculate two 24-hour activity rhythm variables: interdaily stability and intradaily variability[19] and three sleep variables: sleep duration, sleep onset latency and wake after sleep onset[20] Interdaily stability indicates how stable the rhythm is over days, i.e. how similar the individual day-night patterns are over days. It is calculated as the ratio of the variance of the average activity

patterns around the mean and the overall variance.[21] Intradaily variability reflects the fragmentation of the rhythm, i.e. 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.[21] The variables have shown sensitivity in observational and experimental studies on aging.[22,23] Examples of activity rhythms characterized by a high or low stability and fragmentation are given in figure 1.

Sleep diary

During the week of actigraphy assessment, participants kept a sleep diary comprising questions about sleep characteristics, sleep quality, sleep medication, napping and alcohol use. To assess subjective sleep duration and sleep onset latency, participants were requested to answer the questions "In total, how many hours did you sleep last night?" and "How long did it take you to fall asleep?". In our analyses, daily values of these questions were averaged over the week. Sleep quality was measured by three 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?". The score of sleep quality was created by summing the three 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. Every day the participants specified sleep medications 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 one or more naps. The total number of days with a nap, taking into account the total number of days for which the participant contributed data, was used in analyses. Alcohol use was evaluated as the sum of units of alcohol after 18.00 hours in the week of actigraphy.

Pittsburgh Sleep Quality Index

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

Vital status

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 at 27 September 2013. The mean follow-up time was 7.3 years.
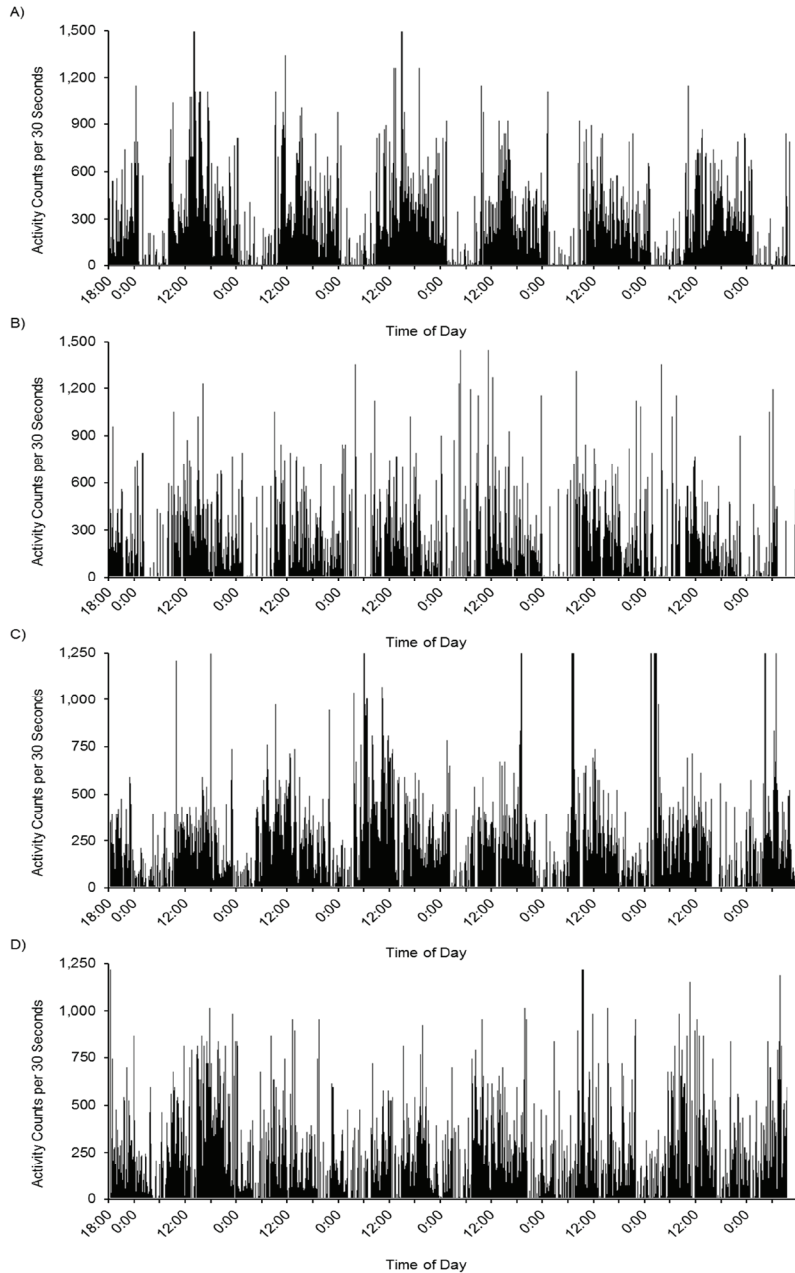
Figure 1. *Four examples of activity plots, Rotterdam Study, the Netherlands, 2004-2007. Plots show activity rhythms of participants from the Rotterdam Study. The x-axis represents time (0:00, midnight; 12:00 noon), the y-axis represents activity counts per 30 seconds. Participants started wearing the actigraph at 18:00 hours on day one. Note that these activity counts are scaled relative to the individual mean and cannot be compared easily across individuals. (A) A man aged 73 years, activity rhythm with high stability and low fragmentation, (B) a woman aged 84 years , activity rhythm with high fragmentation (C) a man aged 47 years, activity rhythm with low stability, and (D) a man aged 62 years, activity rhythm with low stability and high fragmentation.*

Covariates

We assessed the following variables as possible confounders based on previous literature:[14,17] gender, age, sleep medication, possible sleep apnea, napping, activities of daily living (ADL), education, cognitive functioning, depressive symptoms, body mass index, employment, current smoking, alcohol use, myocardial infarction, diabetes and stroke. Sleep medication, napping and alcohol use were estimated using the sleep diary. Information on possible sleep apnea, ADL, education, depressive symptoms, employment and current

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			
	Total (n=1734)	Alive (n = 1580)	Dead (n = 154)	
	Mean ± SD N (%)	Mean ± SD N (%)	Mean ± SD N (%)	Test Statistic[a]
Demographics				
Age (years)	62.2 ± 9.3	61.2 ± 8.6	72.6 ± 10.4	-13.1[d]
Gender (male)	808 (46.6)	723 (45.8)	85 (55.2)	5.0[b]
Employment (yes)	574 (33.1)	558 (35.3)	16 (10.4)	39.9[d]
Education				
low	264 (15.2)	227 (14.4)	37 (24.0)	
intermediate	1097 (63.3)	1000 (63.3)	97 (63.0)	14.5[c]
high	341 (19.7)	322 (20.4)	19 (12.3)	
Health status				
Activities of daily living (score)	0.29 ± 0.4	0.25 ± 0.4	0.66 ± 0.6	-8.3[d]
Cognitive functioning (score)	28.0 ± 1.8	28.0 ± 1.7	27.3 ± 1.9	5.0[d]
Depressive symptoms (score)	5.5 ± 7.)	5.4 ± 7.0	6.6 ± 7.9	-2.1[b]
Myocardial infarction (yes)	67 (3.9)	52 (3.3)	15 (9.7)	15.7[d]
Diabetes (yes)	205 (11.8)	167 (10.6)	38 (24.7)	26.8[d]
Stroke (yes)	45 (2.6)	30 (1.9)	15 (9.7)	34.1[d]
Body mass index (kg/m^2)	27.9 ± 4.2	27.9 ± 4.1	27.6 ± 4.1	0.82
Current smoking (yes)	358 (20.6)	328 (20.8)	30 (19.5)	0.29
Alcohol (cups per week)	5.7 ± 1.3	5.8 ± 7.3	5.3 ± 6.9	0.80
Circadian activity rhythm				
Interdaily stability (score)	0.80 ± 0.1	0.80 ± 0.1	0.77 ± 0.1	3.2[c]
Intradaily variability (score)	0.43 ± 0.1	0.42 ± 0.1	0.52 ± 0.2	-7.7[d]
Sleep				
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[d]
Wake after sleep onset (minutes)	69.5 ± 25.9	68.9 ± 25.2	74.5 ± 30.4	-2.6[b]

PSQI, Pittsburgh Sleep Quality Index; SD, standard deviation.[a] Statistical comparison of vital status at end of follow-up; t-test for continuous, X^2 for categorical variables, [b] *P* <0.05, [c] *P* <0.01, [d] *P* <0.001.

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

| | Status at end of follow-up | | | |
| | Total (n=1734) | Alive (n = 1580) | Dead (n = 154) | |
	Mean ± SD N (%)	Mean ± SD N (%)	Mean ± SD N (%)	Test Statistic[a]
Self-rated sleep				
Sleep medication (yes)	252 (14.5)	218 (13.8)	34 (22.1)	8.6[b]
Sleep apnea (yes)	507 (29.2)	448 (28.4)	59 (38.3)	6.8[b]
Napping (days per week)	1.7 ± 2.0)	1.6 ± 2.0)	2.7 ± 2.4)	-5.6[d]
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[§]	3.7 ± 3.5	3.6 ± 3.5	3.9 ± 3.7	-0.78

PSQI, Pittsburgh Sleep Quality Index; SD, standard deviation. [a] Statistical comparison of vital status at end of follow-up; t-test for continuous, X^2 for categorical variables, [b] P <0.05, [c] P <0.01, [d] P <0.001.

smoking (cigarettes, cigars or pipe) was obtained by a home interview. ADL was measured with the Stanford Health Assessment Questionnaire and was used to indicate general health.[26] Depressive symptoms were measured using the Center for Epidemiologic Studies Depression (CES-D) scale.[27] During a visit to our research center, cognitive functioning was measured by the Mini Mental State Exam;[28] length and weight were measured with light clothing and without shoes to calculate the body mass index (kg/m^2). Myocardial infarction, diabetes and stroke were determined by medical records.

Statistical analyses

The number of missing values of a variable never exceeded 5% (maximal amount of missing data 4.2% for ADL). Missing values of continuous variables were replaced by the median, and missing values of categorical variables were put into a separate missing category. Interdaily stability and intradaily 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 non-linear association by defining three categories (<6, 6-7.5 (reference group) and >7.5 hours).

Cox proportional hazards models were used to determine the hazard ratios (HR) and 95% confidence intervals (CI) of circadian rhythm and sleep parameters, and 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. Based on these criteria, education, employment and alcohol use were

not included in the full model. We tested two different models. Model 1 was adjusted for age and gender. Model 2 was adjusted for age, gender, ADL, current smoking, diabetes, myocardial infarction, stroke, cognitive functioning, depressive symptoms, body mass index, sleep medication, possible sleep apnea and napping. All statistical tests were two-sided and a P value <0.05 was considered statistically significant. We tested the proportional hazards assumption using Schoenfeld residuals.[29] The residuals did not significantly deviate from zero slope. Analyses were performed in SPSS version 20 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.).

Results

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

Interdaily stability and intradaily variability were moderately correlated (r=-0.49, P <0.001). The global PSQI score was moderately correlated to sleep quality obtained with 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 interdaily 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 the table 2.

Both circadian rhythm variables were significantly related to mortality (Table 3). After full adjustment, interdaily stability was associated with a lower mortality risk (HR=0.83 per SD, 95% CI: 0.71, 0.96) and intradaily variability (i.e. fragmentation) with a higher mortality risk (HR=1.22 per SD, 95% CI: 1.04, 1.44). To show the cumulative survival graphically, interdaily stability and intradaily variability were divided into 25% quartiles (Figure 2).

Table 2. *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**	-						
3 Objective sleep duration	**0.31**	**-0.26**	-					
4 Objective sleep onset latency	**-0.09**	**0.22**	**-0.11**	-				
5 Wake after sleep onset	**-0.19**	**0.28**	**-0.23**	**0.26**	-			
6 Subjective sleep duration	**0.09**	**-0.08**	**0.39**	0.01	**0.07**	-		
7 Subjective sleep onset latency	0.01	0.03	**0.11**	**0.14**	**0.13**	**-0.29**	-	
8 Sleep quality sleep diary	**0.11**	**-0.08**	0.01	-0.01	**-0.10**	**0.44**	**-0.27**	-
9 Global PSQI score	**-0.07**	**0.08**	0.01	**0.12**	**0.12**	**-0.41**	**0.37**	**-0.45**

Bold indicates p<0.05.

Because interdaily stability and intradaily variability are moderately correlated, we also analyzed an age and gender adjusted model including both 24-hour activity rhythm variables (intradaily variability, HR=1.25 per SD, 95% CI: 1.07, 1.47; interdaily stability, HR=0.84 per SD, 95% CI: 0.71, 1.00).

Actigraphically measured sleep onset latency and wake after sleep onset were marginally related to mortality in an age and gender adjusted model, but these associations were non-significant in the fully adjusted analysis (HR=1.01 per minute, 95% CI: 1.00, 1.02; HR=1.01 per minute, 95% CI: 100, 1.01, respectively). 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 gender adjusted model.

Table 3. *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[a]	
	HR	95% CI	HR	95% CI
Activity rhythm				
Interdaily stability (score)	0.75[c]	0.65, 0.86	0.83[b]	0.71, 0.96
Intradaily variability (score)	1.37[c]	1.20, 1.57	1.22[b]	1.04, 1.44
Objectively assessed sleep				
Continuous sleep duration				
Sleep duration (hours)	0.42	0.11, 1.57	0.69	0.19, 2.53
Sleep duration squared (hours^2)	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[b]	1.00, 1.02	1.01	1.00, 1.02
Wake after sleep onset (minutes)	1.01[b]	1.00, 1.01	1.01	1.00, 1.01
Subjectively assessed sleep				
Continuous sleep duration				
Sleep duration (hours)	0.23[b]	0.07, 0.74	0.40	0.11, 1.39
Sleep duration squared (hours^2)	1.12[b]	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

CI, confidence interval; HR, hazard ratio; PSQI, Pittsburgh Sleep Quality Index, [a] 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, [b] $P < 0.05$, [c] $P < 0.001$.

However, this association was non-significant after further adjustment (HR=1.07 per hour, 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 3).
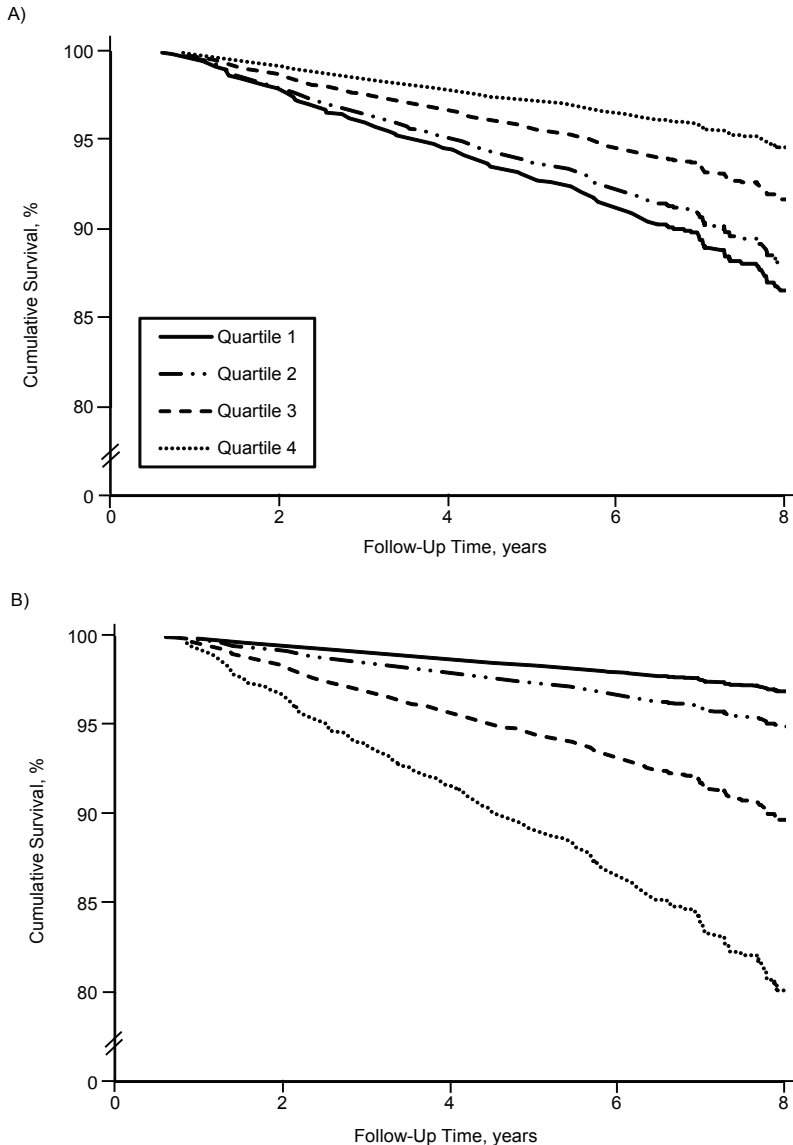

Figure 2. Crude cumulative survival plots per quartile of (A) interdaily stability and (B) intradaily variability, Rotterdam Study, the Netherlands, 2004-2013. Quartile 1 represents the lowest quartile and 4 the highest quartile. Survival was lower in participants with a low interdaily stability or a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

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

Discussion

In this prospective population-based cohort study, more fragmented and less stable 24-hour activity rhythms were associated to a 20% increase in all-cause mortality in a middle-aged and elderly population. These associations remained after adjustment for health parameters, possible sleep apnea and napping. When adjusted for age and gender only, subjective sleep duration showed a U-shaped marginal association with mortality. However, after fully adjustment no sleep parameter, whether estimated objectively using an actigraph 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.

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 may differ per level of circadian organization.[12] For example, changes may occur in the suprachiasmatic nucleus, the central clock of the brain, in peripheral oscillators, or in the ability to drive peripheral oscillators by the suprachiasmatic nucleus. In humans, post-mortem studies demonstrated a reduction in the number of vasopressin-expressing neurons in the suprachiasmatic nucleus at old age.[30] 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.[12,30-35] 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.[36] As a result people suffer from a higher disease susceptibility. Second, napping increases the fragmentation of the rhythm,[17] and can also be an indicator of bad health.[37] However, previous literature on the association of napping and mortality is inconsistent. It was found that people who take naps regularly might have a higher mortality

rate,[38,39] especially those who sleep >9 hours per night.[40] On the other hand, another study found no significant benefit or harm of napping,[41] while yet another study found a protective association of napping on mortality for short sleepers.[40] 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.[21,42] 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.[17,23,43-45]

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 excluding mortality in the first two years to test for reversed causality. The observed HR was very similar to the analyses including 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 change during aging. For example, elderly people sleep less during the night and tend to fall asleep and wake up earlier.[2-6] Nevertheless, in our study the correlations between sleep and 24-hour activity rhythm parameters were weak. We found that more fragmented and less stable circadian activity rhythms predicted mortality, but that 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 we had sufficient power to detect the moderate associations of 24-hour activity rhythm parameters on mortality. Arguably, we may not be powered to detect mild associations between sleep characteristics and mortality. Yet, the findings suggest that the circadian rhythm, as compared to sleep, is more strongly and independently associated with mortality.

There are few studies on activity rhythms and mortality.[13-15,46,47] Mortality was found to be higher in older men and women with less robust or abnormal 24-hour activity rhythms.[13-15] 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.[46,47] 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 non-significant when we adjusted for health parameters. A U-shaped relationship between sleep duration and mortality was found in several previous studies (for two meta-analyses see [9,10]).

In general, stronger associations between long sleep and all-cause mortality were observed in the more extreme categories (≥9 hours). In our study only 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 examining sleep duration stratified on health status are inconsistent.[48,49] One study observed an association between sleep duration and mortality in persons with pre-existing disease only,[48] while another study also found this association in healthy people.[49] In addition, part of the association of short sleep duration with mortality can be explained by sleep apnea.[50] We adjusted for possible sleep apnea, based on two questions of the PSQI.[24,25] Possible apnea was not a significant predictor in our fully adjusted model of sleep duration and mortality. However, the PSQI cannot be used to diagnose sleep apnea. We cannot rule out that sleep apnea, if assessed more in depth, explains part of the observed associations.

In this study, as in previous studies, perceived sleep quality was not related to all-cause mortality, whether measured with the sleep diary or measured with the PSQI.[51] Previously, sleep disturbances were associated with higher all-cause mortality only in men younger than 45 years, but not in women and men older than 45 years.[52]

One strength of our study is that it is embedded in the Rotterdam Study, a prospective population-based cohort study. This increases our generalizability and we could adjust for many covariates. A second strength was our use of both subjective and objective measurements to estimate the sleep duration and sleep onset latency. Because subjective and objective sleep variables are not strongly associated,[18] 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 rhythm was analyzed nonparametrically, so no assumptions were made about the underlying shape of its circadian rhythm. This study also has some limitations. First, data collection on sleep disorders, such as restless legs 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.

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Chapter 3

24-Hour activity rhythms and habitual sleep in relation to neuropsychiatric problems

Chapter 3.1

Associations of the 24-hour activity rhythm and sleep with cognition: A population-based study of middle-aged and elderly persons

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Abstract

Cognitive functioning changes with age, sleep and the circadian rhythm. We investigated whether these factors are independently associated with different cognitive domains assessed in middle-aged and elderly persons. In the Rotterdam Study, we collected actigraphy recordings of on average, 138 hours in 1723 middle-aged and elderly persons (age 62 ± 9.4 years, mean ± standard deviation). Actigraphy was used to quantify 24-hour rhythms by calculating the stability of the rhythm over days and the fragmentation of the rhythm. Sleep parameters including total sleep time, sleep onset latency and wake after sleep onset were also estimated from actigraphy. Cognitive functioning was assessed with the Word Learning Test (WLT), Word Fluency Test (WFT), Letter Digit Substitution Task (LDST) and Stroop Color Word Test (Stroop). Persons with less stable 24-hour rhythms performed worse on the LDST (B=0.42 per standard deviation (SD)-increase, p=0.004) and the Stroop interference trial (B=−1.04 per SD-increase, p=0.003) after full adjustment. Similarly, persons with more fragmented rhythms performed worse on the LDST (B =−0.47 per SD-increase, p=0.002) and the Stroop (B=1.47 per SD-increase, p<0.001). In contrast, longer observed sleep onset latencies were related to worse performance on the WLT delayed recall (B=−0.19 per SD-increase, p=0.027) and the WFT (B=−0.45 per SD-increase, p=0.007). Disturbances of sleep and the 24-hour activity rhythm were independently related to cognition; while persons with longer sleep onset latencies had worse memory, persons with 24-hour rhythm disturbances performed less on executive functioning and perceptual speed tasks.

Introduction

Disturbed sleep and disturbed circadian rhythms can exacerbate cognitive impairment. Recent population-based studies have shown compelling evidence for an association of sleep cognitive functioning in the elderly. Self-rated sleep characteristics have been associated with worse cognitive functioning in population-based samples.[1,2] Moreover, the association of sleep and cognition has also been assessed objectively by means of actigraphy in large population-based studies. Shorter total sleep time, longer sleep onset latencies, more wake after sleep onset, and lower sleep efficiency have been related to worse cognitive performance in elderly persons.[3,4]

Circadian rhythms have also been related to cognition. The fragmentation of the circadian rhythm is suggested to be particularly important for cognitive performance. For example, a fragmented 24-hour activity rhythm was significantly related to impaired mental speed and impaired executive functioning in 144 home-dwelling middle-aged and elderly persons.[5] In a larger sample of very old persons, fragmentation of the activity rhythm was associated with diminished performance on multiple cognitive tasks.[6] Moreover, a changes in the activity rhythm have been associated with an increased odds of developing mild cognitive impairment and dementia.[7] However, it is not clear if the associations of 24-hour activity rhythm disturbances with cognition can be explained by the association of sleep with cognition, as circadian rhythms are intrinsically related to sleep.

Previous research shows that the effects of circadian rhythm and sleep may differ per cognitive domain. Population-based studies on rhythm disturbances mostly demonstrate effects on non-memory tasks.[5,6] Disturbed sleep has been related to all cognitive domains,[3,4,7,8] but the relation between disturbed sleep and memory was mainly demonstrated in smaller populations. Experimental studies suggest that objectively measured sleep disturbance is also related to impaired memory, in particular to problems in memory consolidation.[8] The association between sleep and memory has hardly been tested in population-based studies.

Recent population-based studies of sleep, circadian rhythm and cognition have focused mostly on elderly populations with mean ages above 70 years. Although the effect of sleep on cognition has shown to be independent of age in most studies,[3,4,6] it is unclear whether these associations can already be observed at middle-age. Possibly, the aging process creates a vulnerability for the impact of sleep and circadian rhythm disturbances on cognitive performance. Detecting if these association are present in middle-aged and elderly persons could broaden the treatment choices for cognitive problems in this population. Activity rhythms and sleep are behaviors amenable to change and a possible target for intervention in patients with cognitive problems. Differential associations can indicate that manipulation of circadian rhythms and sleep both could be valuable for the treatment of selected cognitive problems.

In this population-based study of middle-aged and elderly persons we assessed the circadian rhythm and sleep with actigraphy and cognitive functioning with multiple cognitive tests. This allowed us to test whether (1) the 24-hour activity rhythm is associated with cognitive functioning independently of sleep, (2) whether the associations of sleep and the 24-hour activity rhythm are distinct per cognitive domain, and (3) whether the effects are specific to old age. We hypothesize that both the 24-hour activity rhythm and sleep have distinct and independent associations with cognition, as both the activity rhythm and sleep have been related to sleep extensively. More specifically, we posit that disturbances in the 24-hour activity rhythm are related to performance on non-memory tasks, while poor sleep explains performance in all cognitive tasks. We expect these associations to be stronger in old age for both the 24-hour activity rhythm and sleep, and for all cognitive domains.

Materials and Methods

Study Population

This study was conducted within the Rotterdam Study, a population-based cohort of middle-aged and elderly inhabitants of Rotterdam, the Netherlands. The study started in 1990 by inviting inhabitants of the district of Ommoord aged 55 years and over. In 2000, the study population was extended with a second cohort of inhabitants aged 55 and over. In 2006, a new cohort with inhabitants aged 45 and over was added. A more detailed description of the study can be found elsewhere.[9] The study is in accordance with the guideline proposed in the World Medical Association Declaration of Helsinki and approved by the Medical Ethics Committee of the Erasmus University Rotterdam. Written informed consent was obtained from all participants.

From December 2004 until April 2007, 2632 consecutive persons were invited to participate in the actigraphy study; 2063 (78%) agreed. The actigraphy study comprised participants of the second cohort (second examination, December 2004 - December 2005), and the new cohort (baseline examination, January 2006 - April 2007). The recordings of 340 participants (16%) were excluded; of 329 participants recordings either did not consist of 4 consecutive days and nights or had technical problems, of 11 participants there was no information on cognitive functioning. Recordings of 1723 participants were suitable for further analyses.

Assessment of the 24-hour activity rhythm and sleep

All participants were asked to wear an actigraph around the non-dominant wrist (Actiwatch model AW4, Cambridge Technology Ltd) continuously for 7 consecutive days and nights, and to remove it only while bathing. Actigraphs measured in 30-second epochs. 24-hour periods with more than three continuous hours missing were excluded from the

analyses to prevent a time-of-day effect.[10] Recordings had a mean duration of 138 hours (standard deviation, SD: 14 hours).

Activity rhythms were quantified using non-parametric indicators.[10,11] This allowed us to describe the rhythm without making strong assumptions about the shape of the rhythm.[12] We calculated the interdaily stability and the intradaily variability to assess the 24-hour activity rhythm. The interdaily stability indicates the stability of the rhythm, i.e. the extent to which the profiles of individual days resemble each other. Intradaily variability quantifies the fragmentation of the rhythm; more frequent alterations between an active and an inactive state lead to a higher intradaily variability.

We also used the actigraphy recordings to calculate total sleep time, sleep onset latency and wake after sleep onset with a validated algorithm.[13] Although sleep is best assessed with polysomnography, actigraphy is considered a reliable alternative to estimate sleep characteristics.[14] The procedure used to calculate these measures has been described in more detail elsewhere.[10]

Subjective sleep quality was assessed daily with a sleep diary which was kept during the week of actigraphy. Perceived sleep quality indicates the average of three questions about sleep quality (range 0-7).

Assessment of cognitive functions

Cognitive function was assessed with a neuropsychological test battery consisting of a 15-Word Learning Test (WLT, based on the Rey recall of words),[15] a categorical Word Fluency Test (WFT, animal categories),[16] the Letter Digit Substitution Task (LDST),[17] the Stroop Color Word Test (Stroop),[18] and the Mini Metal State Exam (MMSE, range 0-30).[19] The WLT consisted of three immediate recall trials (range 0-45 words) and a delayed recall trial (range 15 words). The examinations were performed by the same research team and in identical order for all participants.

We constructed a compound score for global cognition which has been described in more detail elsewhere.[20] The global cognition score is a summary measure of the Z-scores of all assessed cognitive tests, except the MMSE. All Stroop trials are included in the compound score, Z-scores of the Stroop are inverted in this compound score to ensure that a higher score reflects a better performance on all tasks.

Assessment of covariates

A-piori, we selected the following potential confounders based on previous literature.[6,10] Sex, age, employment status, education, smoking, body mass index (BMI), depressive symptoms, activities of daily living (ADL), myocardial infarction (MI), stroke, diabetes mellitus (DM), possible apnea and time of cognitive testing. All confounders were routinely collected in the Rotterdam Study. During a home interview all participants were asked about their employment status, education (low, intermediate or high), current

smoking, depressive symptoms and activities of daily living (ADL). Depressive symptoms were assessed using the Center for Epidemiologic Studies Depression (CES-D) scale[21] (range 0-60). ADL was assessed with the Stanford Health Assessment Questionnaire[22] (range 0-3). Height and weight were measured without shoes and heavy clothing during a center visit to calculate the BMI (kg/m^2). MI, stroke and DM were determined during the center visit and medical records. Possible apnea was assessed with two questions from the Pittsburgh Sleep Quality Index.[23] 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 per week.[24] Time of cognitive testing was assessed to prevent a time-of-day effect on cognitive functioning.

Statistical analyses

We assessed the associations of the 24-hour activity rhythm and sleep with cognitive functioning using linear regression analyses. We studied the associations of the 24-hour activity rhythm and sleep with global cognitive functioning (averaged z-scores), the WLT immediate recall (number of words over three trials), the WLT delayed recall (number of words), WFT (number of named animals), LDST (number of correct items) and the interference trial of the Stroop (time in seconds). Of the sleep variables, total sleep time was also tested in a quadratic model to test a possible u-shaped relation of total sleep time with cognitive function. Associations were tested in two successive models. The first model was adjusted for sex, age, employment status, education, BMI, smoking, depressive symptoms, ADL, MI, stroke, DM, possible apnea and time of testing. The second model was also mutually adjusted to test if the effects of indicators of the 24-hour activity rhythm and sleep were independent. Potential determinants were only tested in the second model if an association between the sleep or activity rhythm parameter and the respective cognitive task had been observed in the first step. Analyses were repeated excluding participants with a MMSE-score < 26,[19] to assess whether effects could be explained by severe cognitive impairment only. To assess whether the effects of the 24-hour activity rhythm and sleep differed by age, we assessed the interaction terms of age with activity rhythm and sleep parameters quantitatively. Because these interaction analyses were exploratory we used a more stringent cut-off for significance ($p<0.001$) to correct for multiple testing. To facilitate the interpretation of possible interactions, we will illustrate interactions observed between continuous variables by categorizing these variables. All analyses were performed using IBM SPSS Statistics, version 21 (IBM Corp., Somers, NY USA).

As the number of missing values per confounder never exceeded 5%, missing values in quantitative predictors were replaced by the median. A separate missing category was used for qualitative predictors. All 24-hour activity rhythm and sleep indicators and cognitive test scores were winsorized at 4 standard deviations from the mean. To obtain normally

distributed values, interdaily stability, intradaily variability, sleep onset latency, wake after sleep onset and subjective sleep quality were transformed using a Box-Cox transformation.[25,26] All activity rhythm and sleep parameters were standardized to facilitate interpretation.

Results

Mean age of participants was 62 years (standard deviation (SD): 9 years) and 53% was female. 15% had a low education and 33% were still employed (see table 1). Uncorrected correlations between activity rhythm indicators, sleep parameters and cognition measures can be found elsewhere.

First, we studied if 24-hour activity rhythms were associated with general cognitive functioning and domain specific tasks (see table 2). A lower intradaily variability, i.e. less fragmented rhythm, was associated with better global cognitive functioning (B=−0.05 per SD-increase, standard error (SE)=0.02, p=0.003), while the stability of the rhythm was not associated with global cognition (B=0.02 per SD-increase, SE=0.02, p=0.24) after adjustment for confounders. A more stable rhythm (B=0.42 per SD-increase, SE=0.15, p=0.004) and a less fragmented rhythm (B=−0.47 per SD-increase, SE=0.15, p=0.002) were both associated with a higher number of correct items on the LDST. Also, a more stable rhythm (B=−1.04 per SD-increase, SE=0.35, p=0.003) and lower fragmentation (B=1.47 per SD-increase, SE=0.36, p<0.001) were related with less time, thus better performance, on the Stroop interference trial. Stability and fragmentation of the rhythm were not associated with the immediate and delayed recall of the WLT, and the WFT.

Second, we studied the association of sleep with global cognitive functioning and domain-specific tests (table 2). Of the objectively measured sleep parameters, only a short sleep onset latency was associated with better global cognition (B=−0.05 per SD-increase, SE=0.02, p=0.015). Persons with a shorter sleep onset latency also knew more words on the delayed recall of the WLT (B=−0.19 per SD-increase, SE=0.09, p=0.027) and named more words on the WFT (B=−0.45 per SD-increase, SE=0.17, p=0.007). No other associations of sleep with cognitive tests were found.

Third, we assessed perceived sleep quality in relation to cognitive functioning. A lower reported sleep quality was not related to global cognitive functioning or specific cognitive tasks.

Results for the associations of the activity rhythm, sleep and sleep quality did not change largely when persons with MMSE<26 were excluded (n=138) from the analyses (results available upon request).

To test the independence of the effects of the 24-hour activity rhythm and sleep, the associations of the activity rhythm and sleep with global cognition, LDST-score and performance on the Stroop interference trial were assessed in a mutually adjusted model,

Table 1. *Population Characteristics, N=1723*

Demographic	
Female, %	53.5
Age (years)	62.23 ± 9.35
Employment, %	33.3
Education, %	
Low	15.2
Intermediate	63.4
High	19.6
Health indicators	
Stroke, %	2.5
Myocardial infarction, %	1.9
Diabetes Mellitus, %	8.9
Depressive symptoms, score (range 0-60)	5.46 ± 7.02
Activities of daily living, score (range 0-3)	.29 ± .42
Smoking, %	20.7
Body mass Index (BMI), kg/m^2	27.86 ± 4.16
Possible apnea, %	29.1
Use of medication, %	22.4
Circadian rhythm	
Duration actigraphy recording, hours	137.63 ± 14.19
Interdaily stability, score	.80 ± .10
Intradaily variability, score	.42 ± .13
Objectively assessed sleep	
Sleep onset latency, minutes	14.54 ± 12.35
Total sleep time , hours	6.38 ± 0.86
Wake after sleep onset, minutes	69.65 ± 26.26
Sleep quality	
Perceived sleep quality, score (range 0-7)	5.54 ± 1.61
Cognition	
Global cognitive functioning, averaged z-score	.01 ± .74
Word learning test (WLT) immediate recall, number of correct words (range 0-45)	22.55 ± 6.43
Word learning test (WLT) delayed recall, number of correct words (range 0-15)	7.47 ± 2.88
Word fluency test (WFT) , number of correct words	22.50 ± 5.87
Letter Digit Substitution Task (LDST),number of correct items	30.23 ± 6.75
Stroop color word test (Stroop) interference trial, seconds	47.40 ± 16.04

Values are stated as mean ± standard deviation or percentage.

as these outcomes were associated with multiple activity rhythm and sleep indicators. The association of intradaily variability (B=−0.05 per SD-increase, SE=0.02, p=0.011) with global cognition remained significant after mutual adjustment, as well as the association of intradaily variability with performance on the Stroop interference (B=1.32 per SD-increase, SE=0.43, p=0.002) Of the sleep variables, only the association of sleep onset latency) with

Table 2. Associations of the 24-hour Activity Rhythm and Sleep with Cognition.

	Global Cognition (N=1482)		Word learning test (WLT) Immediate recall (N=1554)		Word learning test (WLT) Delayed recall (N=1548)		Word fluency test (WFT) (N=1704)		Letter digit substitution task (LDST) (N=1682)		Stroop color word test (Stroop) Interference trial (N=1549)	
	B (SE)	P	B (SE)	P	B (SE)	P	B (SE)	P	B (SE)	P	B (SE)	P
Circadian rhythm												
Interdaily stability	.02 (.02)	0.24	-.11 (.15)	0.49	.00 (.07)	0.95	.15 (.14)	0.28	**.42 (.15)**	**0.004**	**-1.04 (.35)**	**0.003**
Intradaily variability	**-.05 (.02)**	**0.003**	-.17 (.16)	0.28	-.12 (.07)	0.096	-.19 (.14)	0.18	**-.47 (.15)**	**0.002**	**1.47 (.36)**	**<0.001**
Sleep												
Sleep onset latency	**-.05 (.02)**	**0.015**	-.31 (.19)	0.10	**-.19 (.09)**	**0.027**	**-.45 (.17)**	**0.007**	-.27 (.18)	0.13	.28 (.43)	0.51
Wake after sleep onset	-.02 (.02)	0.26	-.14 (.15)	0.35	-.09 (.07)	0.20	-.21 (.13)	0.11	-.07 (.14)	0.63	.55 (.34)	0.10
Total sleep time	.01 (.02)	0.75	-.11 (.16)	0.47	.00 (.07)	0.96	-.07 (.14)	0.62	.16 (.15)	0.28	.00 (.35)	0.99
Sleep quality												
Perceived sleep quality	-.03 (.02)	0.064	-.19 (.16)	0.24	-.14 (.07)	0.064	-.07 (.14)	0.63	-.23 (.16)	0.14	.36 (.37)	0.32

Linear regression models adjusted for sex, age, employment status, education, depressive symptoms, body mass index, activities of daily living, stroke, myocardial infarction, diabetes mellitus, possible apnea and time of testing. All variables were tested in a linear model. SE, standard error.
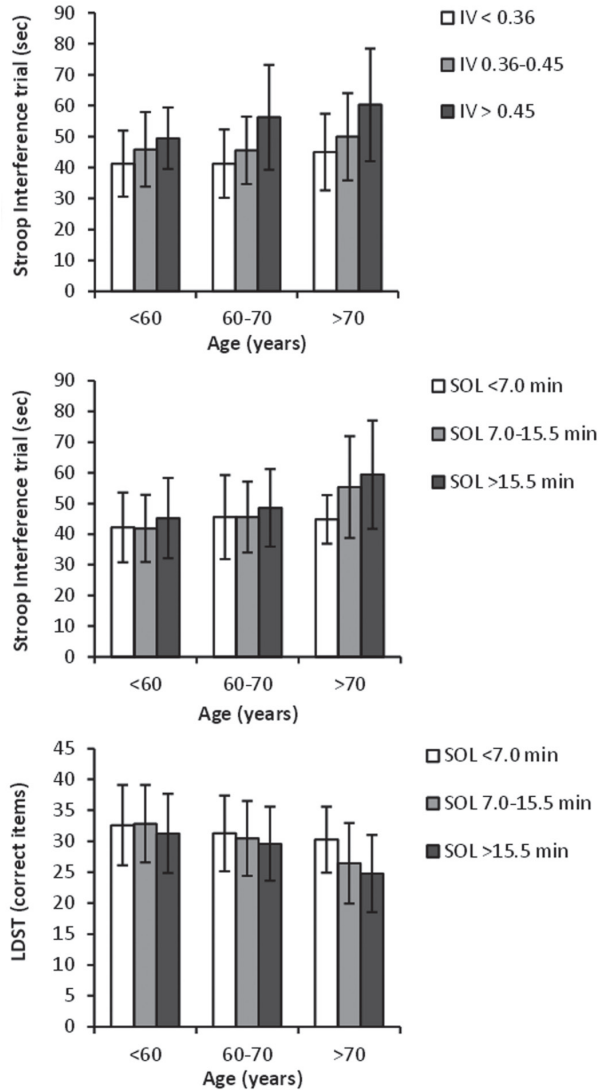
Figure 1. *Illustration of the continuous tested interaction of intradaily variability (IV) and age for the Stroop Interference trial, and the interaction of sleep onset latency (SOL) and age for the Stroop interference trial and the letter digit substitution task (LDST). Bars present means per group, error bars depict standard deviations of the group mean.*

global cognition remained significant (B=−0.06 per SD-increase, SE=0.02, p=0.013. None of the other associations of the 24-hour activity rhythm and sleep with cognitive tests remained significant

Lastly, we performed exploratory analysis to assess whether the effects of the 24-hour activity rhythm and sleep were modified by age; three interaction-terms met the stringent limit of p<0.001. Age modified the association of intradaily variability and

performance on the Stroop interference trial (p<0.001). In addition, age modified the association of sleep onset latency and LDST-score (p<0.001) and the association of sleep onset latency and time spent for the Stroop interference trial (p<0.001). Sleep onset latency and age were categorized to illustrate the quantitative interaction in figure 1. A more fragmented rhythm was more strongly associated with worse performance on the Stoop interference trial in older ages. Similarly, longer sleep onset latency was more strongly associated with worse performance on the LDST and the Stroop interference trial in older aged persons compared to middle-aged persons.

Discussion

In our population-based study, both aspects of the 24-hour activity rhythm and sleep were related to global cognitive functioning. More specifically, disturbances in the 24-hour activity rhythm were mostly related to tasks that draw on perceptual speed and executive functioning, while an increased sleep onset latency was related to tasks which are associated with memory performance.

Fragmentation of the activity rhythm and sleep onset latency both affected global cognition. Yet, these effects of the fragmentation of the rhythm and sleep onset latency on global cognition were independent from each other. Activity rhythm and sleep parameters were also only modestly correlated in our sample. This suggests that disturbances of circadian rhythms and disturbances of sleep affect cognitive functioning relatively independently; the small decreases in effect size after mutual adjustment suggested only limited shared variance. Circadian rhythm effects on cognition have previously been suggested to be independent of sleep in studies in which the human circadian rhythm was desynchronized.[27] Circadian control of pathways, synchronization of local clocks and neurogenesis have been named as possible mechanisms through which circadian disturbances might affect cognition.[28] On the other hand, sleep deprivation research has demonstrated that disturbed sleep can reduce neural activity,[29] and sleep has also been hypothesized to affect synaptic strength.[8] Further research is needed to disentangle the mechanisms which evoke the distinct effects of circadian rhythms and sleep on cognition.

Moreover the 24-hour activity rhythm and sleep parameters showed diverse association patterns with the different cognitive tasks. Fragmentation of the rhythm was particularly important for non-memory tasks. This extends on previous research where fragmentation has been associated with all cognitive subdomains except for episodic memory[6] or where associations with memory disappeared after correction for confounders.[5] In contrast, a long sleep onset latency was associated with worse memory performance. While this association has not been demonstrated population-based studies, it extends on clinical research of sleep and memory. Thus, in our study 24-hour activity rhythms were

related to non-memory executive tasks, whereas sleep characteristics, particularly sleep onset latency, were related to memory.

The association between disturbed rhythms and cognitive performance can be explained in multiple ways. First, the association could be a direct effect of disturbed rhythms on perceptual speed and executive functioning. Fragmentation, in our study, indicates fragmentation during the day as well as fragmentation during the night. It has been suggested that a high fragmentation not only indicates problems staying asleep during the night, but also problems in staying awake during the day.[30] We cannot assess the temporality of the effect in our cross-sectional study, so we can only carefully infer that persons with a high fragmentation may be less vigilant during the day which directly worsens the performance on perceptual speed and executive functioning tasks. Second, a shared underlying factor could explain the association between disturbed rhythms and worse cognitive functioning in the non-memory tasks. For example, an unhealthy lifestyle has been related to disturbed activity rhythms[10] and to worse cognitive performance.[31] Third, the direction of the effect can also be reversed, worse cognitive functioning might also lead to more disturbed rhythms. For example, dementia is accompanied by highly disturbed patterns of sleep. It has even been suggested that this is a major reason for hospitalization of demented patients.[11] Severe cognitive deficits can disturb activity rhythms in this situation. However, in our sample exclusion of participants who screened positive for dementia, did not change the results largely.

The association of lengthened sleep onset latency with poorer performance on the delayed recall of 15 words and generating animal names was not explained by disturbances in the activity rhythm. This is in line with the suggestion that memory performance is largely independent of the circadian rhythm disturbances.[32] It has been suggested that the association of sleep onset latency with memory can be explained by attention deficits; the inability to direct and control attention is not only detrimental for memory performance, but also for falling asleep as this requires the disregard of outside stimuli and thoughts.[33] However, in our sample sleep onset latency was not related with any other tasks which draw more on attention.

We found that age modified the association of sleep onset latency and non-memory tasks, as well as the association of fragmentation and performance on the Stroop interference trial. This demonstrates that age is an important factor in the relation of the 24-hour activity rhythm and sleep with cognition. Although our study is cross-sectional, it suggests that older people are more vulnerable for disturbed sleep and activity rhythms with respect to their cognitive performance, specifically in non-memory tasks. Most likely this is due to the aging of the brain, which diminishes the ability to compensate the effects of circadian and sleep disturbances on cognition.

The current study has several strengths. First, the embedding in an existing population-based study makes our findings more generalizable. Second, we assessed cognition with multiple, established cognitive tests. Third, activity rhythms and sleep have been assessed objectively over multiple nights. Fourth, we used non-parametric measures of the 24-hour activity rhythm. The main advantage of a non-parametric indicator, above a parametric indicator, is that no assumptions are made about the nature of the rhythm; such an assumption is particularly problematic in elderly populations with less pronounced circadian rhythms.[12] However, there are some limitations that should be considered. First, we cannot draw any conclusions on the temporality of the observed associations as our study is cross-sectional. Also, the design of our study was particularly suited to assess the 24-hour activity rhythm, as an indicator of the circadian rhythm. Actigraphy allows us to estimate sleep parameters, but it lacks the precision of polysomnography which is considered the gold standard in sleep research.[14] Next, we had self-rated but no objective information about sleep disordered breathing which may be a mediator between sleep and activity rhythm disturbances and cognition. Although a study in community-dwelling older men was not able to find an association between the apnea/hypopnea index and cognition,[34] it is not clear to what extent sleep disordered breathing affected our results. Last, persons with better cognitive functioning might be overrepresented in our study because they are less likely to refuse cognitive testing.

In conclusion, activity rhythm and sleep disturbances are independently related to cognition. Fragmentation of the activity rhythm is related to tasks that depend more strongly on executive functioning and perceptual speed, while sleep onset latency is associated with worse performance on memory-related tasks. Lastly, our research suggests that non-impaired circadian rhythms and good sleep are important for good cognitive functioning, in particular in the elderly who might be more vulnerable for the effects of circadian rhythm and sleep on cognition. Possibly, some cognitive problems can be ameliorated by treating circadian disturbances, e.g. with a lifestyle change or medication.

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Chapter 3.2

24-Hour activity rhythms and sleep disturbances in depression and anxiety: A population-based study of middle-aged and older persons

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Abstract

Disturbed circadian rhythms have been associated with depression and anxiety, but it is unclear if disturbances in the 24-hour activity rhythm and sleep are independently and specifically related to these disorders. In 1714 middle-aged and elderly participants of the Rotterdam Study, we collected actigraphy recordings of at least 96 hours (138 ± 14 hours, mean ± standard deviation). Activity rhythms were quantified calculating the fragmentation of the rhythm, the stability of the rhythm over days, and the timing of the rhythm. Total sleep time, sleep onset latency and wake after sleep onset were also estimated with actigraphy. Depressive symptoms were assessed with the Center for Epidemiologic Studies Depression scale, persons with clinically relevant depressive symptoms were interviewed to diagnose DSM-IV-depressive disorder. Anxiety disorders were determined with the Munich version of the Composite International Diagnostic Interview. More fragmented rhythms were associated with clinically relevant depressive symptoms (Odds Ratio (OR): 1.27, 95% confidence interval (CI): 1.04;1.54) and anxiety disorders (OR: 1.39, 95% CI: 1.14;1.70) after covariate adjustment. Less stable rhythms, longer sleep onset latency, and more wake after sleep onset were related to clinically relevant depressive symptoms or anxiety disorders only if not adjusted for covariates and other activity rhythm and sleep indicators. Our study in middle-aged and elderly persons suggests that fragmentation of the 24-hour activity rhythm is associated with depression and anxiety. Moreover, this association also largely accounts for the effect of disturbed sleep on these psychiatric disorders.

Introduction

Circadian rhythms are found in behavior and in physiological processes throughout the body.[1] The rhythms are regulated by the suprachiasmatic nucleus (SCN), which accommodates the central timekeeping mechanism.[2] The SCN integrates endogenous rhythms with external time cues, usually resulting in the typical 24-hour course of the diurnal activity rhythm.[3] Previously, we have related disturbances in these 24-hour rhythms to demographics, such as employment and having a partner, and lifestyle factors, such as coffee use and smoking.[4]

Disturbances in the 24-hour rhythm are known to affect mood in healthy persons.[5] It has been suggested that the circadian system is a vital regulator of multiple systems that have a key role in mood disorders.[6] A few studies related changes in the 24-hour activity rhythm to depressive symptoms in the general population.[7] Activity rhythms have rarely been studied in persons with anxiety disorders despite the overlap in symptoms, genetics and environmental risk factors of anxiety and depression. There is some evidence that patients with panic disorder have disturbed sleep-wake cycles.[8]

Psychiatric disorders are typically accompanied by changes in sleep. Persons complaining of sleep difficulties are 3-4 times more likely to be depressed.[9] Vice versa, up to 90% of depressed patients report difficulty falling asleep, staying asleep or early morning awakenings.[10] Disturbed sleep in depressed patients has also been studied using actigraphy.[11,12] Sleep disturbances are also seen in persons with panic disorder, generalized anxiety disorder and post-traumatic stress disorder.[13,14] Insomnia has also been linked to the onset of anxiety.[15]

According to one of the most influential models in sleep, the two process model, sleep and the circadian rhythm are intrinsically related.[16] This model explains sleep changes based on chronobiological assumptions, it describes sleep as a function of process 'C', a circadian component, and process 'S' which entails sleep pressure. The two-process model explains the relation between sleep and depression. Awakenings in depressed patients could be caused by a disturbed build-up of 'S' or an earlier timing of process 'C'.[17] However, circadian and sleep disturbances are not necessarily the cause of increased depressive symptoms, the overlapping symptoms between sleep as well as circadian rhythm disturbances and depression might also be accounted for by a common negative emotionality factor. Aging might make middle-aged and older persons more vulnerable to this negative emotionality. As a result, older adults may be more likely to misinterpret normal variations in activity rhythms, sleep, and depression.[18] Several other mechanisms have been proposed to explain the association of the circadian rhythms and sleep with depression, i.e. a phase shift in the circadian rhythm, abnormalities in the neuroendocrine system, and social rhythm disturbances.[19,20] Although we have demonstrated previously that depressive symptoms are

related to 24-hour activity rhythm disturbances,[4] it is not clear whether this association is independent of the association of sleep with depressive symptoms and also present for anxiety disorders. Anxiety has been hypothesized to co-occur with sleep problems through the maladaptive interpretation of stimuli and avoidance behaviors.[18] Yet, the mechanisms underlying the association of circadian rhythms and sleep with anxiety are much less explored than those explaining the occurrence of depression.

A better understanding of the unique effects of 24-hour activity rhythm and sleep disturbances on depression and anxiety may have important clinical implications, in particular for the treatment of depression and anxiety. Depressive and anxiety disorders can be treated by changing the rhythm or sleep,[21,22] i.e. pharmacological treatment[23] and bright light therapy.[24,25] Sleep deprivation is effective in patients with depression[26] but not in those with anxiety.[27] In elderly persons treatment choice is of particular importance as sleep and 24-hour activity rhythm disturbances occur frequently.[28]

In this study, we investigated (1) whether 24-hour activity rhythms and sleep are associated with depressive symptoms, (2) whether 24-hour activity rhythm and sleep are associated with anxiety disorder and (3) whether the 24-hour activity rhythm and sleep are each associated independently and specifically with depressive symptoms and anxiety disorders. We hypothesized that (H1) disturbed activity rhythms and sleep are each associated with more depressive symptoms. We also expected (H2) that persons with 24-hour activity rhythm or sleep disturbances have more anxiety disorders, although there is limited prior evidence from clinical and epidemiological studies. Last (H3), based on the two-process model, we expected the 24-hour activity rhythm to explain the association of sleep with depressive symptoms. We also tested whether any similarities in the associations of activity rhythms with depressive symptoms and anxiety disorders are explained by comorbidity.

Materials and Methods

Study Population

The current study was embedded in the Rotterdam Study, a population-based cohort study of middle-aged and elderly inhabitants of Rotterdam, the Netherlands.[29] The study was conducted in accordance with the guideline in the World Medical Association Declaration of Helsinki and approved by the medical ethics committee according to the Wet Bevolkingsonderzoek ERGO (Population Study Act Rotterdam Study), executed by the Ministry of Health of the Netherlands. Written informed consent was obtained from all participants.

From December 2004 until April 2007, 2632 consecutive persons were invited to participate in the actigraphy study; 2063 (78%) agreed. Women (60.5% versus 53.4%,

p=0.003) and older aged persons (mean age 67.5 years versus 62.3 years, p<0.001) were more likely to refuse participation in the actigraphy study. There was no significant difference in the prevalence of depressive symptoms or anxiety disorder between included persons and persons who refused participation in the study. We excluded 349 participants (17%); in 109 participants actigraphic recordings did not consist of 4 days and nights, 23 recordings were collected in a week of daylight saving time, in 197 persons the actiwatch malfunctioned, and in 20 persons there was no information on depressive symptoms. Depressive symptoms were scored incompletely in 1 person who refused to answer all questions, for the other 19 persons data are missing due to refusal or time constraints. In total, data of 1714 participants
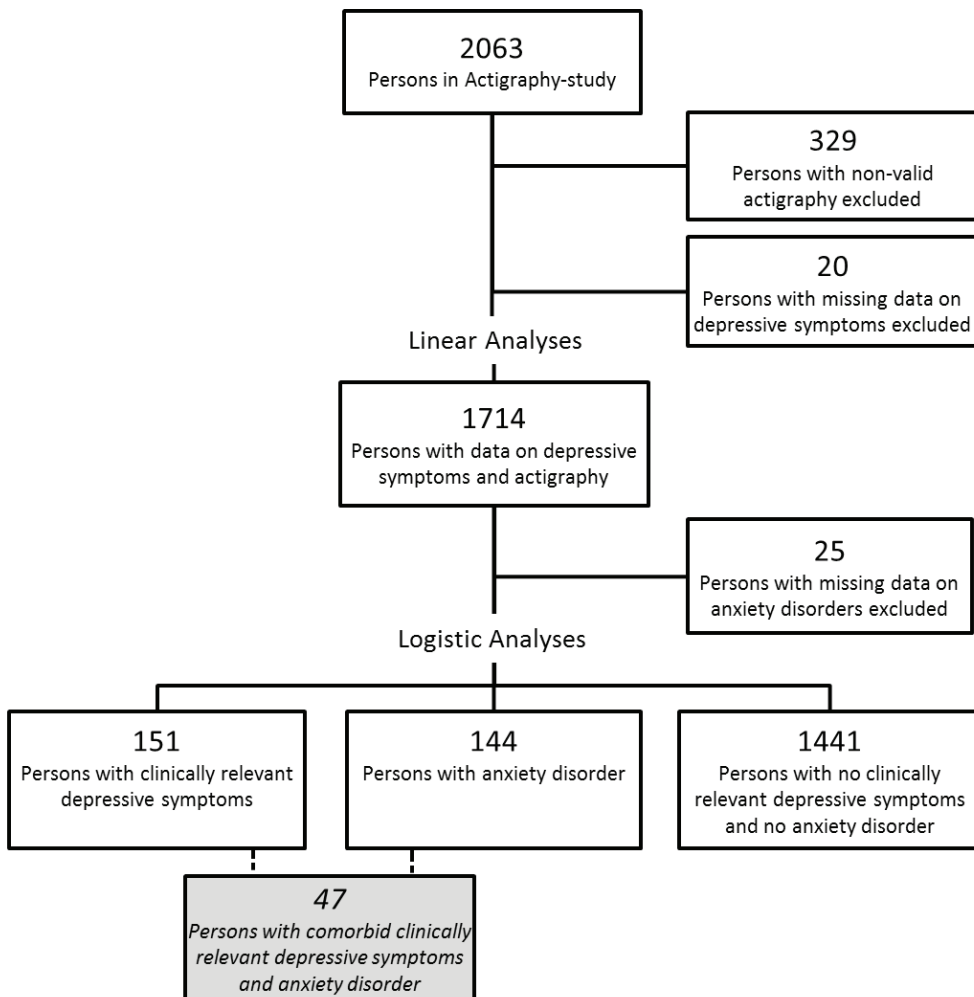
were eligible for further analyses. A flowchart of the study sample can be found in figure 1.

Figure 1 *Flowchart of study sample.*

Assessment of depression and anxiety

Depression was assessed with a two-step procedure. First, during the home-interview all participants were screened for depressive symptoms with the Center for Epidemiologic Studies-Depression (CES-D) scale.[30] A cut-off score of 16 defined participants with clinically relevant depressive symptoms.[31] Second, participants who screened positive for depressive symptoms underwent a semi-structured psychiatric interview with the Schedules for Clinical Assessment in Neuropsychiatry,[32] performed by trained and experienced clinicians. This clinical interview allowed us to diagnose major depressive disorder according to the Diagnostic and Statistical Manual for Mental Disorders IV (DSM-IV-TR).

During the initial home interview, a slightly adapted[33] Munich version of the Composite International Diagnostic Interview (M-CIDI) was administered to assess the following anxiety disorders according to the DSM-IV-TR: generalized anxiety disorder, specific phobia, social phobia, agoraphobia and panic disorder.[34] Anxiety disorders were grouped into one overlapping category of anxiety disorder according to the DSM-IV-TR. We had no information on anxiety in 25 participants. Data of 6 participants on anxiety were completely missing for unknown reasons; data of 19 persons were partly missing due to time constraints.

Assessment of the 24-hour activity rhythm and sleep

Actigraphy allowed us to assess the 24-hour activity rhythm. All participants were asked to wear an actigraph around the non-dominant wrist (Actiwatch model AW4, Cambridge Technology Ltd) continuously for 7 consecutive days and nights, and to remove it only while bathing. Actigraphs measured in 30-second epochs. All 24-hour periods with more than three continuous hours missing were excluded from the analyses to prevent a time-of-day effect. Recordings had to consist of at least 96 hours to be scored validly (109 excluded, 71 recordings did not consist of 96 hours before exclusion of missing periods and another 38 recordings did not consist of 96 hours after the exclusion of the 24-hour missing periods). In the 1734 recordings available for analysis, we excluded one 24-hour period in 140 persons (8.1%), two 24-hour periods in 41 persons (2.4%) and three 24-hour periods in 12 persons (0.7%).On average, the duration of the actigraphy recordings was 138 hours (standard deviation (SD) 14 hours).

Activity rhythms were quantified using non-parametric indicators to describe the rhythm without making strong assumptions about the shape of the rhythm.[4,35,36] We calculated three variables to assess the 24-hour activity rhythm: the interdaily stability, the intradaily variability, and the dominant rest phase onset. The interdaily stability indicates the stability of the rhythm, i.e. the extent to which the profiles of individual days resemble each other. Intradaily variability quantifies how fragmented the rhythm is relative to the overall variance. The intradaily variability is based on hourly values and reflects transitions

of relatively long periods of rest and activity; more frequent alterations between an active and an inactive state lead to a higher intradaily variability. Lastly, the dominant rest phase onset represents the clock time at which the 5-hour period of lowest activity in the average 24-hour pattern started.[37]

We also used the actigraphy recordings to estimate sleep characteristics. Although sleep is best assessed with polysomnography, actigraphy is a reliable alternative to estimate sleep characteristics.[38] We calculated total sleep time (TST), sleep onset latency (SOL) and wake after sleep onset (WASO) with a validated algorithm.[4,39,40] Subjective sleep quality was assessed with a sleep diary during the week of actigraphy. Participants answered three 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 the summed questions over 7 nights (range 0-7).[4]

Assessment of covariates

We selected possible covariates based on previous literature. First, we assessed demographics[4,7,11,14]: sex, age, partnership, employment status, and education. Second, we studied the medical status[4,7,11,14]: cognitive status, activities of daily living (ADL), body mass index (BMI), use of psycholeptics, use of psychoanaleptics, use of sleep medication during actigraphy, and use of medication prescribed for (1) blood and blood forming organs, (2) cardiovascular system, (3) genito-urinary system and sex-hormones, (4) systemic hormonal preparations, and the (5) respiratory system. Third, we explored lifestyle factors[4,41-43]: alcohol use, coffee use, and current smoking. Last, we assessed sleep characteristics[4,44]: duration of actigraphy and possible apnea. During a home-interview all participants were asked about their partnership, employment status, education, smoking, and medication use. Cognitive status was measured using the Mini Mental State Exam (MMSE).[45] Height and weight were measured without shoes and heavy clothing during a center visit to calculate the BMI (kg/m²). ADL was assessed with the Stanford Health Assessment Questionnaire.[46] Alcohol use was the self-reported number of units of alcohol which were consumed in the week of actigraphy. Coffee use indicated the average of the self-reported cups consumed after 18:00 during the week of actigraphy. Possible apnea was assessed with two questions from the Pittsburgh Sleep Quality Index.[47] 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 per week.[48] Use of medication was assessed at the home-interview and assigned to the different categories of the Anatomical Therapeutic Chemical (ATC)-classification. The use of sleep medication was self-reported during the week actigraphy on a daily basis.

Statistical analyses

We tested whether 24-hour activity rhythm and sleep were associated with depressive symptoms and anxiety disorders in three successive models. First, we studied the associations adjusted for sex and age. Second, we additionally adjusted for partnership, employment status, cognitive status, ADL, psycholeptics, psychoanaleptics, sleep medication, BMI and coffee use. We only included the a-priori selected covariate in the model if the covariate predicted depressive symptoms or anxiety ($p<0.10$). Third, to assess whether activity rhythms and sleep were independent predictors of depressive symptoms and anxiety, we studied the associations in a mutually adjusted model. Activity rhythm and sleep parameters were only included in this model when significantly associated with the outcome in the covariate adjusted model. The variance inflation factor (VIF) indicated that there was no multicollinearity (VIF<2).

We tested the effects of the 24-hour activity rhythm on depressive symptoms continuously using linear regression. In addition, we studied clinically relevant depressive symptoms (CES-D score ≥16) and anxiety disorders (yes/no) as categorical outcomes with logistic regression. Clinically relevant depressive symptoms were studied as the category of interest in these analyses to prevent for insufficient statistical power (compared to only including persons with major depressive disorder). To ensure results of clinically relevant depressive symptoms and anxiety disorders were comparable, we constructed one reference category for all these logistic analyses. This reference category comprised participants who screened negative for depressive symptoms (CES-D score <16) and had no anxiety disorder (n=1441). To study whether the comorbidity of clinically relevant depressive symptoms and anxiety explained similar associations, we have run the analyses excluding participants with both clinically relevant depressive symptoms and an anxiety disorder (N=47). Last, we assessed the relation of the 24-hour activity rhythm and sleep with major depressive disorder and individual anxiety disorders, i.e. generalized anxiety disorder, specific phobia, social phobia and panic disorder and/or agoraphobia. All analyses were performed using IBM SPSS Statistics version 21 (IBM Corp., Somers, NY USA).

As the number of missing values per parameter never exceeded 5%, missing values in quantitative predictors (missing values: cognitive status 2.2%, ADL 3.4%, BMI 1.1% and coffee use 3.0%) were replaced by the median.[49] A separate missing category was used for categorical predictors (missing values: partnership 0.1%). All 24-hour activity rhythm and sleep indicators were winsorized at 4 SD of the mean. We used a Box-Cox transformation[50,51] to obtain normally distributed values for interdaily stability (λ=7.0), intradaily variability (λ=-3.9), sleep onset latency (λ=-0.1), wake after sleep onset (λ=0.4) and subjective sleep quality (λ=5.3).

Results

Population characteristics of the total population (N=1714), persons with clinically relevant depressive symptoms (n=151), persons with an anxiety disorder (n=144) and the reference group (n=1441) can be found in table 1. Activity rhythm and sleep variables were low to moderately correlated (table 2). The interdaily stability, i.e. the stability of the rhythm, and intradaily variability, i.e. the fragmentation of the rhythm, correlated substantially (r=-0.50, p<0.001).

We studied whether 24-hour activity rhythm characteristics and sleep characteristics were related to clinically relevant depressive symptoms (table 3). A high intradaily variability (Odds Ratio (OR): 1.31 per 1-SD, 95% confidence interval (CI): 1.08;1.60, p=0.008) and worse perceived sleep quality (OR: 0.41 per 1-SD, 95%CI: 0.32;0.52, p<0.001) were associated with more clinically relevant depressive symptoms after covariate adjustment. The fragmented rhythm and worse sleep quality remained associated with clinically relevant depressive symptoms when mutually adjusted (respectively OR: 1.26 per 1-SD, 95%CI: 1.03;1.55, p=0.027 and OR: 0.41 per 1-SD, 95%CI: 0.32;0.53, p<0.001). After exclusion of persons with comorbid anxiety disorder, the association of intradaily variability with clinically relevant depressive symptoms was attenuated (OR: 1.18 per 1-SD, 95%CI: 0.94;1.48, p=0.163).

When we assessed depressive symptoms quantitatively, a low stability of the rhythm (B=-0.05, SE=0.02, p=0.047), high variability of the rhythm (B=0.10, SE=0.02, p<0.001), a later dominant rest phase onset (B=0.05, SE=0.02, p=0.015), more wake after sleep onset (B=0.05, SE=0.02, p=0.040) and worse sleep quality (B=-0.30, SE=0.02, p<0.001) were all associated with more depressive symptoms when adjusted for covariates (table 3). Persons with more fragmented rhythms (B=0.08, SE=0.03, p=0.003), and worse sleep quality (B=-0.29, SE=0.02, p<0.001) had significantly more depressive symptoms even if we mutually adjusted the model for rhythm and sleep characteristics.

Next, we assessed whether 24-hour activity rhythm and sleep characteristics were associated with anxiety disorders (table 4). Persons with a more fragmented rhythm and persons who perceived their sleep quality worse had more anxiety disorders independent of covariates (OR: 1.39 per 1-SD, 95%CI: 1.13;1.70, p=0.002 and OR: 0.68 per 1-SD, 95% CI: 0.55;0.84, p<0.001, respectively). Mutual adjustment for fragmentation and perceived sleep quality did not affect the associations with anxiety disorders (respectively OR: 1.37 per 1-SD, 95%CI: 1.11;1.68, p=0.003 and OR: 0.69 per 1-SD, 95%CI: 0.56;0.85, p<0.001). After exclusion of 47 participants with both clinically relevant depressive symptoms and an anxiety disorder, more fragmented rhythms (OR: 1.29 per 1-SD, 95%CI: 1.01;1.63, p=0.038) remained associated with anxiety disorder in the covariate adjusted model, while the association of subjective sleep quality with anxiety disorder was attenuated (OR: 0.84 per 1-SD, 95%CI: 0.66;1.06, p=0.14).

Table 1. Population characteristics.

	Total sample	No clinically relevant depressive symptoms - No anxiety disorder	Clinically relevant depressive symptoms	Any anxiety disorder
	N=1714	n=1441[1]	n=151[2]	n=144[2]
Socio-demographics				
Women (%) *	53.6	49.8	69.5	73.6
Age (years)	62.19 ± 9.36	62.22 ± 9.30	61.26 ± 9.18	61.16 ± 9.24
Education				
Low (%)	15.2	14.6	15.9	16.7
Intermediate (%)	63.4	63.4	63.6	64.6
High (%)	19.6	20.3	17.9	17.4
Health status'				
Employed (%)*	33.4	35.0	25.8	27.8
ADL (score)*	0.29 ± 0.42	0.26 ± 0.39	0.48 ± 0.50	0.43 ± 0.53
Cognitive status (score)*	28.00 ± 1.75	28.02 ± 1.69	27.58 ± 2.09	27.93 ± 1.97
Depressive symptoms (score)*	5.49 ± 7.13	3.55 ± 3.76	23.88 ± 7.28	12.04 ± 10.64
Alcohol use (units per week)	9.47 ± 9.35	9.77 ± 9.36	8.41 ± 9.50	7.88 ± 9.34
Use of medication for the respiratory system*	12.5	11.6	20.5	12.5
Use of medication for the cardiovascular system*	42.1	40.5	49.7	50.0
Use of medication for blood and blood forming organs*	18.6	17.8	27.2	19.4
Use of medication for the genito-urinary system and sex hormones	5.9	6.0	6.0	4.2
Systemic hormonal preparations*	4.4	3.5	11.9	8.3
Psycholeptics *	10.7	7.4	33.8	25.5
Psychoanaleptics*	6.2	4.0	17.9	20.1
Sleep medication in week of actigraphy*	14.6	11.8	31.8	28.5
Possible Apnea, %	13.9	14.1	14.6	11.8

Table 1. Population characteristics (continued).

	Total sample N=1714	No clinically relevant depressive symptoms - No anxiety disorder n=1441[1]	Clinically relevant depressive symptoms n=151[2]	Any anxiety disorder n=144[2]
Activity rhythm				
Duration actigraphy (hours)	137.52 ± 14.32	137.55 ± 14.21	136.53 ± 15.23	137.17 ± 14.10
Interdaily stability (score)*	0.80 ± 0.10	0.80 ± 0.10	0.78 ± 0.11	0.79 ± 0.11
Intradaily variability (score)*	0.42 ± 0.13	0.42 ± 0.13	0.46 ± 0.15	0.46 ± 0.14
Dominant rest phase onset (clocktime)	01:16 ± 1:07	01:14 ± 1:07	01:22 ± 1:09	01:28 ± 1:08
Sleep				
Total sleep time (hours)	6.38 ± 0.86	6.38 ± 0.86	6.33 ± 0.87	6.34 ± 0.94
Sleep Onset Latency (minutes)	14.51 ± 12.38	14.21 ± 11.82	15.69 ± 14.08	16.13 ± 15.78
Wake After Sleep Onset (minutes)*	69.49 ± 25.86	69.03 ± 25.80	73.72 ± 26.38	71.22 ± 25.31
Perceived Sleep Quality (score)*	5.53 ± 1.61	5.73 ± 1.46	4.09 ± 1.93	4.67 ± 1.98

Values given as mean ± standard deviation, unless stated otherwise, * indicates significant difference between persons with clinically relevant depressive symptoms and persons without clinically relevant depressive symptoms and no anxiety disorder at $p<0.05$ analyzed with a Chi-Square test for qualitative variables or a one-way analysis of variance for quantitative variables.
[1] Persons with clinically relevant depressive symptoms (n=151), persons with an anxiety disorder but no clinically relevant depressive symptoms (n=97) and persons with no information on anxiety and no clinically relevant depressive symptoms (n=25) were excluded from this group.
[2] These groups contain an overlap of 47 persons who have both clinically relevant depressive symptoms and an anxiety disorder.

Table 2. Correlations between circadian rhythm and sleep.

	1	2	3	4	5	6
1 Interdaily stability	-					
2 Intradaily variability	**-0.50**	-				
3 Dominant rest phase onset	**-0.14**	**0.14**	-			
4 Total sleep time	**0.29**	**-0.24**	-0.08	-		
5 Sleep Onset Latency	**-0.16**	**0.22**	0.10	**-0.19**	-	
6 Wake after Sleep Onset	-0.05	**0.26**	**0.18**	**-0.12**	**0.33**	-
7 Perceived Sleep Quality	**0.10**	**-0.07**	-0.03	-0.03	-0.08	0.05

Bold script indicates $p<0.01$.

Table 3. Associations of 24-hour activity rhythms, sleep and covariates with depressive symptoms.

	Clinically relevant depressive symptoms (n=151)[1]						Depressive symptoms (n=1714)[2]					
	Basic model			Multivariate model			Basic model			Multivariate model		
	OR	95% CI	p	OR	95% CI	p	B	SE	p	B	SE	p
Covariates												
Sex (male)	2.28	1.59;3.28	<0.001	1.50	1.00;2.24	0.049	0.35	0.05	<0.001	0.15	0.05	0.001
Age	0.99	0.97;1.01	0.30	0.95	0.92;0.97	<0.001	0.00	0.00	0.81	-0.02	0.00	<0.001
Partner		-		0.50	0.33;0.77	0.001		-		-0.30	0.06	<0.001
Employment		-		0.64	0.39;1.04	0.073		-		-0.01	0.06	0.84
Cognitive status		-		0.92	0.83;1.01	0.092		-		-0.02	0.01	0.12
Activities of daily living		-		2.43	1.60;3.70	<0.001		-		0.56	0.06	<0.001
Body mass index		-		0.97	0.93;1.02	0.20		-		-0.01	0.01	0.034
Coffee use (units/day)		-		1.33	1.07;1.64	0.009		-		-0.02	0.03	0.49
Psycholeptics		-		3.69	2.26;6.03	<0.001		-		0.36	0.08	<0.001
Psychoanaleptics		-		3.09	1.16;5.44	<0.001		-		0.36	0.09	<0.001
Sleep medication in week of actigraphy		-		1.60	0.98;2.60	0.059		-		0.30	0.07	<0.001
Determinants												
Interdaily Stability (per 1-SD)	0.78	0.65;0.93	0.005	0.91	0.75;1.10	0.31	-0.11	0.02	<0.001	-0.05	0.02	0.047
Intradaily Variability (per 1-SD)	1.54	1.28;1.85	<0.001	1.31	1.08;1.60	0.008	0.16	0.02	<0.001	0.10	0.02	<0.001
Dominant rest phase onset (per 1-hr)	1.14	0.98;1.33	0.085	1.02	0.87;1.20	0.81	0.07	0.02	<0.001	0.05	0.02	0.015
Total Sleep Time (per 1-hour)	0.84	0.69;1.04	0.10	0.86	0.70;1.07	0.17	-0.02	0.03	0.56	0.00	0.03	0.86
Sleep Onset Latency (per 1-SD)	1.26	1.02;1.56	0.032	1.06	0.84;1.33	0.62	0.02	0.03	0.41	-0.02	0.03	0.45
Wake After Sleep Onset (per 1-SD)	1.24	1.04;1.47	0.017	1.09	0.90;1.32	0.36	0.09	0.02	<0.001	0.05	0.02	0.040
Perceived Sleep Quality (per 1-SD)	0.37	0.30;0.47	<0.001	0.41	0.32;0.52	<0.001	-0.36	0.02	<0.001	-0.30	0.02	<0.001

CES-D, Center for Epidemiologic Studies-Depression; OR, Odds Ratios; CI, Confidence Interval; 1-SD, 1 standard deviation.
Basic model, adjusted for sex and age; Covariate adjusted model, adjusted for sex, age, partnership, employment, coffee use, body mass index, cognitive status, activities of daily living, use of medication prescribed for the nervous system; Mutually adjusted model: adjusted for sex, age, partnership, employment, coffee use, body mass index, cognitive status, activities of daily living, use of medication prescribed for the nervous system and mutually adjusted for interdaily stability, intradaily variability, phase onset, total sleep time, sleep onset latency, wake after sleep onset and perceived sleep quality.
[1] Logistic regression analyses, clinically relevant depressive symptoms: CES-D score ≥16, reference category: no clinically depressive symptoms and no anxiety (n=1441). Persons with anxiety disorder and no clinically relevant depressive symptoms (n=97) and no information on anxiety and no clinically relevant depressive symptoms (n=25) were excluded from these analyses.
[2] Linear regression analyses.

Table 4. Associations of 24-hour activity rhythms, sleep and covariates with anxiety disorders

	Anxiety disorders (n=144)[1]					
	Basic model			Multivariate model		
	OR	95% CI	p	OR	95% CI	p
Covariates						
Sex (male)	**2.79**	**1.90;4.11**	**<0.001**	**1.82**	**1.20;2.76**	**0.005**
Age	**0.99**	**0.97;1.01**	**0.245**	**0.95**	**0.93;0.98**	**<0.001**
Partner		-		**0.49**	**0.32;0.75**	**0.001**
Employment		-		0.65	0.40;1.06	0.084
Cognitive status		-		1.01	0.91;1.13	0.79
Activities of daily living		-		**2.06**	**1.34;3.14**	**<0.001**
Body mass index		-		1.00	0.95;1.04	0.86
Coffee use (per units/day)		-		1.18	0.94;1.47	0.15
Psycholeptics		-		**2.31**	**1.36;3.94**	**<0.001**
Psychoanaleptics		-		**4.35**	**2.57;7.39**	**<0.001**
Sleep medication in week of actigraphy		-		1.86	1.14;3.04	0.013
Determinants						
Interdaily Stability (per 1-SD)	**0.83**	**0.70;1.00**	**0.046**	0.97	0.80;1.18	0.77
Intradaily Variability (per 1-SD)	**1.63**	**1.34;1.98**	**<0.001**	**1.39**	**1.13;1.70**	**0.002**
Dominant rest phase onset (per 1-hr)	**1.24**	**1.06;1.44**	**0.008**	1.11	0.95;1.31	0.20
Total Sleep Time (per 1-hour)	0.85	0.69;1.05	0.13	0.87	0.70;1.07	0.19
Sleep Onset Latency (per 1-SD)	**1.30**	**1.04;1.61**	**0.021**	1.10	0.87;1.38	0.43
Wake After Sleep Onset (per 1-SD)	1.12	0.94;1.34	0.20	0.95	0.78;1.15	0.59
Perceived Sleep Quality (per 1-SD)	**0.60**	**0.50;0.73**	**<0.001**	**0.68**	**0.55;0.84**	**<0.001**

OR, Odds Ratios; CI, Confidence Interval; 1-SD; 1 standard deviation.
Sex-age adjusted model, adjusted for sex and age; Covariate adjusted model, adjusted for sex, age, partnership, employment, coffee use, body mass index, cognitive status, activities of daily living, use of medication prescribed for the nervous system; Mutually adjusted model: adjusted for sex, age, partnership, employment, coffee use, body mass index, cognitive status, activities of daily living, use of medication prescribed for the nervous system and mutually adjusted for interdaily stability, intradaily variability, phase onset, total sleep time, sleep onset latency, wake after sleep onset and perceived sleep quality if the predictor was significant in covariate adjusted model.
[1] Logistic regression analyses with reference category: no clinically depressive symptoms and no anxiety (n=1441). Persons with clinically relevant depressive symptoms and no anxiety disorder (n=104) and no information on anxiety (n=25) were excluded from these analyses.

Lastly, we performed exploratory analyses to assess the associations of the 24-hour activity rhythm and sleep with major depressive disorder and subtypes of anxiety. We observed associations of shorter total sleep time (OR: 0.47 per 1-hour, 95%CI: 0.29;0.76, p=0.002) and sleep quality (OR: 0.12 per 1-hour increase, 95%CI: 0.05;0.32, p<0.001) with major depressive disorder (n=22) in covariate adjusted analyses. Next, we studied specific anxiety disorders, diagnosed with the M-CIDI, as the outcome. More fragmented rhythms (OR: 1.75 per 1-SD, 95%CI: 1.20;2.55, p=0.004), a shorter total sleep time (OR: 0.66 per 1-hour, 95%CI: 0.45;0.97, p=0.033), and a worse sleep quality (OR: 0.64 per 1-SD, 95%CI: 0.43;0.94, p=0.022) were associated with generalized anxiety (n=39) in the covariate adjusted model. In addition, a worse sleep quality was associated with panic disorder and/

or agoraphobia (n=80, OR: 0.68 per 1-SD, 95%CI: 0.51;0.90 *p*=0.008) and with social phobia (n=26, OR: 0.33 per 1-SD, 95%CI: 0.18;0.61, *p*<0.001). Activity rhythms and sleep were not related to specific phobia (n=30).

Discussion

In this population-based actigraphy study of 1714 middle-aged and elderly persons, actigraphically assessed disturbances of the circadian rhythm and sleep were related to depressive symptoms and anxiety disorders. As expected, the stability, fragmentation and timing of the activity rhythm were all related to depressive symptoms. Of the sleep characteristics, only wake after sleep onset and self-rated sleep quality were related with more depressive symptoms. Although we expected 24-hour activity rhythms and sleep to be also associated with anxiety disorders, only fragmented rhythms and worse sleep quality were associated with anxiety disorder. Fragmented rhythms explained the associations of the other activity rhythm parameters and wake after sleep onset with depressive symptoms. However, fragmented rhythms could not explain the association of perceived sleep quality with depressive symptoms or anxiety.

Our research is cross-sectional, yet our results suggest that the circadian organization of the activity rhythm plays a key role in both depressive symptoms and anxiety in middle-aged and elderly persons. This is in line with previous evidence of disturbances in process 'C' of the two-process model[6] possibly due to the aging of the brain. Our study only partly supports the phase-shift hypothesis of depression which proposes that mood disturbances result from a phase advance or delay.[20] The dominant rest phase onset gives an indication of the start of the peak of the rest-activity nadir[37] this variable was only associated with depressive symptoms when modeled quantitatively and not when we dichotomized depressive symptoms using a cut-off for clinically relevant depressive symptoms or when major depressive disorder was studied as the outcome. This can be explained two ways, possibly, our study was underpowered to detect an association of phase onset with clinically relevant depressive symptoms and anxiety disorders. Alternatively, changes in the onset of the rest phase may impact the continuum of depressive symptoms only, and do not specially affect persons with clinically relevant symptoms most. Lastly, both for depression and anxiety, a tripartite approach to the associations of circadian rhythm, depression and anxiety has been suggested.[18] This hypothesis suggests that the overlapping symptoms between circadian rhythm and sleep disturbances with anxiety and depression can be accounted for by a common negative emotionality factor. Aging might make middle-aged and older persons more vulnerable to this negative emotionality. As a result, older adults may be more likely to misinterpret normal variations in activity rhythms, sleep, depression and anxiety. In addition, altered behaviors change the activity rhythm, sleep, depression and anxiety, e.g.

persons might avoid exercise because of a fear to trigger health difficulties and thereby impact sleep and depression.

The idea of a common negative emotionality influencing all aspects from rhythms, sleep, depression and anxiety is further supported by the robust associations of perceived sleep quality with depressive symptoms and anxiety. In addition, these associations were independent of the objectively measured sleep and circadian rhythm parameters. This suggests that perceived sleep quality reflects different aspects of sleep than the actigraphically assessed 24-hour rhythms. This might be due to sleep misperception, which is more common in patients with depression,[52] and is influenced by negative emotionality. After exclusion of persons with comorbid clinically relevant depressive symptoms and anxiety disorders, the association for sleep quality and anxiety disorders disappeared. This suggests that perceived sleep quality in persons with an anxiety disorder is influenced less by differences in the negative emotionality than that of persons with depressive symptoms.

Our findings suggest separate and shared mechanisms underlie the association of 24-hour activity rhythms and sleep with clinically relevant depressive symptoms and anxiety disorders. For example, total sleep time was related to the clinical diagnosis of major depressive disorder but not to depressive symptoms or any of the anxiety disorders. It is tempting to speculate that a shorter total sleep time is typical for clinical depression. Exploratory analyses of individual anxiety disorders demonstrated that the relation between fragmented rhythms and anxiety disorders was mainly accounted for by generalized anxiety disorder, which is frequently comorbid with heightened depressive symptoms. Also, generalized anxiety disorder is less dependent on specific stimuli, compared to other anxiety disorders.[13] Possibly, the differences in the associations of 24-hour rhythms and sleep with the individual anxiety disorders can be explained by the different etiological backgrounds of these anxiety disorders.

The current study had several strengths. First, we assessed depressive symptoms by self-report and depressive disorder and anxiety with a clinical interview in a large population-based sample. Second, we measured the circadian organization of the 24-hour activity rhythm objectively with actigraphy, which prevents the information bias which can easily occur when participants with heightened depressive symptoms or anxiety disorder judge their own sleep. Third, we used non-parametric measures of the 24-hour activity rhythm. The main advantage of a non-parametric indicator is that it does not make assumptions about the nature of the rhythm, which can be problematic in elderly populations.[36] Some limitations should also be considered. First, we cannot draw any conclusions on temporality since our study is cross-sectional. Second, although circadian rhythms are observable in the activity rhythm, this is an indirect assessment only. In addition, the design of our study was particularly suited to assess the 24-hour activity rhythm disturbances. Actigraphy allows us to estimate sleep parameters, but it lacks the precision of polysomnography.[38] Also, we had

only a small number of cases with major depressive disorder (n=22). This limited the statistical power of these analyses. Thus, we must extrapolate the results of persons with depressive symptoms to those with major depressive symptoms very carefully. Last, we combined the different anxiety disorders following the DSM-IV-TR, however our results suggest that the mechanisms underlying the individual anxiety disorders are not the same.

In conclusion, specifically the fragmentation of the 24-hour activity rhythm is related to clinically relevant depressive symptoms and anxiety disorders in this population-based study of middle-aged and elderly persons. The stability of the rhythm, the phase of the rhythm, the total sleep time, sleep onset latency and wake after sleep onset could not explain additional variance above fragmented rhythms for clinically relevant depressive symptoms and anxiety disorders. Together with robust associations of worse perceived sleep quality with depressive symptoms and anxiety, this suggests that fragmentation of the rhythm and negative emotionality are important in the relation between sleep and common psychiatric disorders in the middle-aged and elderly. This supports further integration of chronobiological and cognitive treatment of sleep problems in the management of depressive and anxiety disorders.

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Chapter 3.3

Sleep and 24-hour activity rhythm and sleep disturbances in relation to cortisol change after a very low-dose of dexamethasone

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Abstract

The hypothalamic-pituitary-adrenal (HPA) axis plays an important role in sleep. Nevertheless, the association of sleep and its 24-hour organization with negative feedback control of the HPA axis has received limited attention in population-based studies. We explored this association in 493 middle-aged persons of the Rotterdam Study, a large population-based study (mean age 56 years, standard deviation: 5.3 years; 57% female). The negative feedback of the HPA axis was measured as the change in morning saliva cortisol after the intake of 0.25 mg dexamethasone the night before. Actigraphy allowed us to measure the stability and fragmentation of the activity rhythm and to estimate total sleep time, sleep onset latency and wake after sleep onset. A sleep diary kept during the week of actigraphy was used to assess self-reported total sleep time, sleep onset latency, number of awakenings and perceived sleep quality. In our study, enhanced negative feedback of the HPA axis was found in association with unstable activity rhythms (B=0.106, 95% confidence interval (CI): 0.002;0.210), total sleep time (B=0.108, 95%CI: 0.001;0.215) and poor subjective sleep quality (B=0.107, 95%CI: 0.009;0.206) after multivariate adjustment. These results indicated that the 24-hour organization, duration and experience of sleep are all associated with the negative feedback control of the HPA axis.

Introduction

The hypothalamic-pituitary-adrenal (HPA) axis determines the stress reponse of humans as it regulates the release of cortisol by a negative feedback control.[1] Cortisol shows a typical diurnal pattern with peaks when stress is increased. The diurnal pattern is regulated by the suprachiasmatic nucleus (SCN), the body's central pacemaker, which is responsible for the overall co-ordination of the HPA axis and synchronizing the time of day and neuroendocrine output.[2]

The HPA axis plays an important role in the regulation of sleep.[3] However, research on the association of sleep parameters with cortisol secretion is not consistent.[4] In population-based studies, it has been found that saliva awakening cortisol was not associated with sleep quantity and quality in healthy middle-aged adults,[5] and that cortisol levels in urine were not associated with objective sleep duration.[6] In contrast, others have observed that self-reported sleep duration and disturbances were associated with the diurnal slope in cortisol secretion in the population.[7] None of these studies, however, assessed experimentally induced activation of the HPA-axis. Cortisol levels can be manipulated experimentally by performing a behavioral stress test. A recent publication found that sleep deprivation was associated with both elevated resting cortisol and an exaggerated cortisol response after the Trier Social Stress Test.[8] Cortisol levels can also be manipulated pharmacologically to assess the functioning of the HPA axis. Results of studies which assessed the HPA axis after pharmacological manipulation in relation to sleep are also mixed; poor sleep can lead to increased activity of the HPA axis, for example in chronic insomniacs.[9] However, self-rated sleep was not related to cortisol levels after dexamethasone intake in a combined dexamethasone/ corticotrophin-releasing-hormone (CRH) test,[10] nor were sleep disorders.[11] Research has been complicated by the use of objective versus subjective measures of sleep in different studies.[6] In addition, both the HPA axis and sleep behaviors are affected by stress. However, most studies on sleep and the function of the HPA axis have been done in the laboratory, and rarely in the home situation. This itself might affect hormone regulation, which could further complicate the interpretation and generalizability of the results. In addition, sleep and cortisol secretion both have strong circadian rhythms, which could affect the association between sleep and the HPA axis.[3] However, not much is known about the 24-hour organization of rest activity rhythms in reference to the negative feedback of the HPA axis in population-based samples.

In the current study we assessed the negative feedback of the HPA axis with a very low-dose dexamethasone suppression test (DST). The DST is specifically designed to measure the negative feedback of the HPA axis and has mostly been used in clinical populations. Initially, assessment of the negative feedback of the HPA axis was developed to diagnose Cushing's disease,[12] but it has also been proposed as a biomarker for psychiatric diseases.[13]

Diminished negative feedback of the HPA axis has been found in melancholic depression, eating disorders and alcoholism, while in contrast an enhanced negative feedback has been associated with posttraumatic stress disorder, stress-related bodily disorders and chronic fatigue syndrome.[14] Within the general population a dose of 1 mg dexamethasone, which is comparable to that applied in clinical populations, would suppress saliva cortisol almost completely in all persons.[15] Therefore we implemented a very low-dose DST to assess the effect of 0.25 mg of dexamethasone on cortisol in saliva. A dose of 0.25 mg dexamethasone has been suggested for a more informative assessment of the sensitivity of the HPA axis feedback in healthy adults.[15] We specifically tested the level of cortisol after a very low-dose of dexamethasone controlled for baseline cortisol.

We explored whether the 24-hour organization of the activity rhythm, objective and subjective sleep parameters, and perceived sleep quality were related with the negative feedback control of the HPA axis in the general population by conducting an experiment with a very low-dose DST. Enhanced negative feedback of the HPA was measured as the reduction in morning cortisol after a low dose of dexamethasone the prior evening. Both sleep and cortisol have a strong circadian organization, therefore we hypothesized that disturbed 24-hour activity rhythms were related with the negative feedback control of the HPA axis. Results for the association of sleep with the negative feedback control have been mixed; to our knowledge, objectively measured habitual sleep has only been studied in relation to the negative feedback control of the HPA axis in adolescents.[16] Lastly, we expected subjective sleep quality to be associated with the feedback of the HPA axis.

Methods

Study Population

The current study was embedded in the Rotterdam Study, a population-based cohort of middle-aged and elderly inhabitants of Rotterdam, the Netherlands.[17] In 2006, a new cohort with inhabitants aged 45 and over was added (RSIII-1). The study was conducted in accordance with the guideline proposed in the World Medical Association Declaration of Helsinki and 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. Written informed consent was obtained from all participants.

All participants in RSIII-1 were invited for the very low-dose DST at the baseline assessment. Of 1822 persons (response rate 63.9%) a valid very low-dose DST was available after the exclusion of persons with incomplete data (n=58), persons who used corticosteroids (n=3), or invalid timing of sampling (n=59). Of these 1822 persons, 627 persons were invited for the actigraphy study, 43 persons refused to participate (6.9%). In 17 persons actigraphic

recordings did not consist of 4 consecutive days and nights, in 16 persons recordings were collected within a week of daylight saving time, and in 58 persons the actiwatch malfunctioned. In total, 493 persons remained for analyses.

Assessment of the very low-dose dexamethasone suppression test

For the very low DST participants were asked to collect a saliva sample at 08:00 at day 1, take a very low dose of dexamethasone (0.25 mg, oral) at 23:00 at day 1, and collect saliva again at 08:00 at day 2. Sampling times were kept equal for all participants to prevent large individual differences in the time between dexamethasone intake and cortisol sampling. Saliva samples were collected using Salivette sampling devices (Sarstedt, Nümbrecht, Germany). Participants received oral and written instructions about the use of the sampling device. In addition, they were asked not to eat or brush their teeth 15 minutes before the collection of the samples and to report the exact date and time of the sampling. Samples were stored at -80 ºC until they were analyzed at the laboratory of Biopsychology, Technical University of Dresden, Germany. Salivary cortisol concentrations were measured using a commercial immunoassay with chemiluminescence detection (CLIA; IBL Hamburg, Hamburg, Germany), further details have been described elsewhere (N. Direk, M.J.H.J. Dekker, A.I. Luik, C. Kirschbaum, Y.B. De Rijke, A. Hofman, W.J. Hoogendijk, H. Tiemeier, unpublished observations).

Assessment of the 24-hour activity rhythm and sleep

Actigraphy allowed us to measure the 24-hour activity rhythm[18,19] and to estimate sleep parameters objectively.[20] All participants wore an actigraph around the non-dominant wrist (Actiwatch model AW4, Cambridge Technology Ltd) continuously for 7 consecutive days and nights, the actigraph was only to be removed while bathing. Actigraphs measured in 30-second epochs.[20] Recordings had to consist of at least 96 hours. All 24-hour periods with more than three continuous hours missing were excluded from the analyses to prevent a time-of-day effect. The average duration of the actigraphy recordings was 138 hours (standard deviation (SD): 14 hours).

Activity rhythms were quantified using non-parametric indicators[21,22] to describe the rhythm without making strong assumptions about the shape of the rhythm.[23] Two variables were calculated to assess the 24-hour activity rhythm: the interdaily stability and the intradaily variability. The interdaily stability indicates the stability of the rhythm, i.e. the extent to which the profiles of individual days resemble each other. Intradaily variability quantifies how fragmented the rhythm is relative to the overall variance. It is based on hourly values and reflects transitions of relatively long periods of rest and activity; more frequent alterations between an active and an inactive state lead to a higher intradaily variability.

We also used the actigraphy recordings to estimate sleep parameters. Actigraphy is

considered a reliable estimator of sleep parameters such as total sleep time.[24] We used a validated algorithm[20] to calculate total sleep time, sleep onset latency and wake after sleep onset using the actigraphy.[22,25]

Total sleep time, sleep onset latency and number of awakenings were also self-rated by the participant using a sleep diary which was kept during the week of actigraphy. Values were averaged over the 7 nights. Perceived sleep quality was evaluated with this same sleep diary; participants answered 3 dichotomous questions about their sleep quality. Perceived sleep quality indicates the average of the summed and weighted questions over 7 nights (range 0-7).

Assessment of covariates

We a-priori selected sex, age, partnership, education, employment status, exercise, body mass index (BMI), coffee use, alcohol use, current smoking, activities of daily living (ADL), cognitive status, depressive symptoms, diabetes mellitus, possible apnea and use of sleep medication, psycholeptics and/or psychoanaleptics, time of cortisol sampling, habitual wake up time, and the time difference between sleep measurements and cortisol sampling as covariates based on previous literature.[22,26] During a home interview all participants were asked about a partner, education, employment status, exercise, smoking, ADL, possible apnea and medication use. Exercise indicates whether the participants practiced any sports on the basis of a self-report questionnaire item. ADL was evaluated with the Stanford Health Assessment Questionnaire to indicate health status.[27] Cognitive status was measured with the Mini Mental State Exam (MMSE).[28] We used the Center for Epidemiologic Studies-Depression (CES-D) scale to measure depressive symptoms.[29,30] Diabetes mellitus was determined during the center visit on the basis of fasting or non-fasting glucose levels in combination with medical records. Possible apnea was assessed with two questions from the Pittsburgh Sleep Quality Index.[31] 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 per week.[32] Use of medication was based on self-report during the home interview and in the sleep diary. Height and weight were measured without shoes and heavy clothing during a center visit to calculate the BMI (kg/m^2). Coffee and alcohol use were the number of units consumed per day after 18:00h in the week of actigraphy, as reported in a daily question in the sleep diary. Time of cortisol sampling was self-reported in a form which was enclosed with the saliva sampling devices. Habitual actigraphic wake up time was averaged over the actigraphy period.

Statistical analyses

We assessed the associations of the 24-hour activity rhythm and sleep parameters with saliva cortisol levels after the intake of 0.25 mg dexamethasone with linear regression

in successive models. First, we studied the associations adjusting for baseline cortisol, sex and age. Second, we ran models which we adjusted for baseline cortisol, sex, age, partnership, education, employment status, BMI, alcohol use, current smoking, ADL, cognitive status, diabetes mellitus and use of sleep medication, psycholeptics and/or psychoanaleptics, habitual wake up time, and the time difference between sleep measurements and cortisol sampling. Lastly, we ran a model in which we adjusted the analyses additionally for depressive symptoms to test whether effects were confounded by mental health. We included the a-priori selected covariate in the model if the covariate predicted the 24-hour activity rhythm or sleep ($p<0.05$) or if it changed the effect estimate of the main determinants by more than 10%. Exercise, coffee use, possible apnea and time of cortisol sampling did not meet either of these criteria. Next, we assessed whether sex and medication modified the associations of 24-hour rhythms and sleep with cortisol. We additionally analyzed the association of the 24-hour activity rhythm and sleep parameters with the saliva cortisol level before and after dexamethasone intake without adjustment for baseline cortisol in all three models for the comparison of our results with results from the classical DST. In the classical DST, only cortisol after dexamethasone intake is assessed, instead of the change in cortisol levels before and after intake. All analyses were performed using SPSS Statistics (version 21, IBM Corp., Somers, NY USA).

Saliva cortisol levels were natural-log transformed due to the non-normal distribution. All 24-hour activity rhythm and sleep indicators were winsorized at 4 standard deviations to the mean. We used a Box-Cox transformation[33,34] to obtain normally distributed values for interdaily stability ($\lambda=7.0$), intradaily variability ($\lambda=-3.9$), actigrahic sleep onset latency ($\lambda=-0.1$), actigraphic wake after sleep onset ($\lambda=0.4$), self-rated sleep onset latency ($\lambda=0.3$), self-rated number of awakenings ($\lambda=0$, natural log transformation) and subjective sleep quality ($\lambda=5.3$). All 24-hour activity rhythm and sleep indicators were standardized to facilitate the interpretation. The number of missing values for the covariates was generally low (alcohol use 0.4%, ADL 6.3%, cognitive status 2.0%, depressive symptoms, 0.2%). Missing values in quantitative predictors were replaced by the median.[35]

Results

Population characteristics (N=493) are presented in table 1. The mean age of our sample was 55.6 years (standard deviation (SD): 5.34) and 57% was female. Average saliva cortisol levels were 14.75 mmol/L (SD: 8.65) in the morning before the intake of dexamethasone and 6.1 mmol/L (SD: 7.13) in the morning after the intake of 0.25 mg dexamethasone. Participants slept on average 6.3 hours (SD: 0.88) according to the actigraphy, self-rated total sleep time was 6.8 hours (SD: 0.95) on average. On average, the week of actigraphy, in which the sleep variables were also self-reported by means of a sleep

Table 1. *Population characteristics (n=493).*

Demographics	
Female, %	57.2
Age, years	55.58 ± 5.34
Partnership, %	81.3
Education, %	
Low	8.9
Intermediate	66.5
High	23.1
Employment, %	56.6
Health indicators	
Exercising, %	59.8
Body mass Index (BMI), kg/m^2	27.60 ± 4.28
Coffee use, cups per day	0.96 ± 0.84
Alcohol use, units per week	6.09 ± 8.00
Current smoking, %	25.8
Activities of daily living, score	0.15 ± 0.29
Cognitive status, score	28.11 ± 1.75
Depressive symptoms, score	5.87 ± 7.46
Diabetes Mellitus, %	8.3
Possible Apnea, %	26.4
Use of sleep medication, psycholeptics and/or psychoanaleptics, %	21.1
Cortisol	
Cortisol in saliva before dexamethasone intake, mmol/L	14.75 ± 8.65
Cortisol in saliva after dexamethasone intake, mmol/L	6.12 ± 7.13
Sampling time day 1, clocktime	7:57 ± 0:42
Sampling time day 2, clocktime	7:55 ± 0:33
Time between sleep measurements and cortisol sampling, days	34.83 ± 25.21
24-hour activity rhythm	
Interdaily stability, score	0.80 ± 0.10
Intradaily variability, score	0.39 ± 0.11
Duration of actigraphy, hours	137.72 ± 15.04
Actigraphic sleep	
Total sleep time, hours	6.31 ± 0.88
Sleep onset latency, minutes	6.37 ± 2.44
Wake after sleep onset, minutes	68.67 ± 23.93
Self-rated sleep	
Total sleep time, hours	6.77 ± 0.95
Sleep onset latency, minutes	17.90 ± 12.97
Number of awakenings, score	1.48 ±1.09
Actigraphic habitual wake up time, clocktime	07:34 ± 0:53
Sleep quality	
Perceived sleep quality, score	5.35 ± 1.68

Values are stated as mean ± standard deviation or percentage.

Table 2. Correlations of cortisol in saliva, 24-hour activity rhythms, and sleep (N=493).

	1	2	3	4	5	6	7	8	9	10	11
Cortisol											
1 Cortisol in saliva before dexamethasone intake	-										
2 Cortisol in saliva after dexamethasone intake	**0.40**	-									
Circadian rhythm											
3 Interdaily stability	**0.12**	**0.10**	-								
4 Intradaily variability	**−0.09**	**−0.09**	**−0.60**	-							
Actigraphic sleep											
5 Total sleep time	−0.04	−0.05	**0.33**	**−0.31**	-						
6 Sleep onset latency	−0.06	−0.02	**−0.27**	**0.34**	**−0.46**	-					
7 Wake after sleep onset	**−0.10**	**−0.11**	**−0.16**	**0.23**	**−0.17**	**0.74**	-				
Self-rated sleep											
8 Total sleep time	−0.03	−0.05	**0.09**	**−0.13**	**0.41**	−0.04	0.09	-			
9 Sleep onset latency	−0.01	−0.05	0.00	0.01	**0.10**	**0.14**	**0.21**	**−0.26**	-		
10 Number of awakenings	**−0.11**	**−0.10**	−0.01	0.01	**0.15**	0.05	**0.26**	**−0.17**	**0.25**	-	
Sleep quality											
11 Perceived sleep quality	**0.11**	**0.18**	0.07	−0.05	0.01	−0.04	−0.06	**0.33**	**−0.30**	**−0.35**	-

Bold indicates p<0.05. Pearson correlation coefficients. All cortisol levels were natural-log transformed, interdaily stability, intradaily variability, actigraphic sleep onset latency, actigraphic wake after sleep onset, self-rated sleep onset latency and self-rated number of awakenings and perceived sleep quality were box-cox transformed.

diary, took place one month (35 days, SD: 25.21) before the very low-dose DST.

Table 2 shows the correlations between saliva cortisol levels, the 24-hr activity rhythm and sleep parameters. Cortisol levels before and after dexamethasone intake correlated moderately with each other (r= 0.40, p<0.01) and minimally with depressive symptoms (r=-0.10, p=0.024 and r=-0.09, p=0.036 respectively). Parameters of the 24-hour activity rhythm correlated substantially with each other (r= 0.60, p<0.01). Similarly, actigraphic sleep onset latency and actigraphic wake after sleep onset (r=0.74, p<0.01) correlated highly.

Next, we explored the stability and fragmentation of the 24-hour activity rhythm with saliva cortisol after dexamethasone intake (adjusted for baseline cortisol), see table 3. A more stable rhythm was associated with higher levels of saliva cortisol after dexamethasone in the basic model (B=0.132, 95% Confidence Interval (CI): 0.032;0.233). This association remained after multivariate adjustment (B=0.106, 95%CI: 0.002;0.210) including adjustment for depressive symptoms (B=0.106, 95%CI: 0.002;0.210). A more fragmented rhythm was associated with lower saliva cortisol after dexamethasone in the basic model (B=−0.102, 95%CI: −0.203;0.000). This association was attenuated after multivariate adjustment

Table 3. *Associations of the 24-hour activity rhythm and sleep with saliva cortisol after dexamethasone intake when adjusted for baseline cortisol (N=493).*

	Cortisol in saliva after dexamethasone intake			
	Basic model		Multivariate model	
	B	95%CI	B	95%CI
24-hour activity rhythm				
Interdaily stability	**0.132**	**0.032; 0.233**	**0.106**	**0.002; 0.210**
Intradaily variability	**−0.102**	**−0.203; 0.000**	−0.073	−0.178; 0.032
Actigraphic sleep				
Total sleep time	0.034	−0.063; 0.130	**0.108**	**0.001; 0.215**
Sleep onset latency	−0.089	−0.253; 0.074	−0.087	−0.254; 0.079
Wake after sleep onset	−0.087	−0.189; 0.014	−0.074	−0.179; 0.031
Self-rated sleep				
Total sleep time	−0.038	−0.132; 0.055	0.012	−0.086; 0.110
Sleep onset latency	−0.011	−0.103; 0.081	0.004	−0.090; 0.098
Number of awakenings	−0.017	−0.110; 0.077	−0.028	−0.122; 0.066
Sleep quality				
Perceived sleep quality	**0.108**	**0.011; 0.205**	**0.107**	**0.009; 0.206**

CI: Confidence Interval. Bold indicates p<0.05. Linear regression analyses. Cortisol levels were natural-log transformed, interdaily stability, intradaily variability, actigraphic sleep onset latency, actigraphic wake after sleep onset, self-rated sleep onset latency, self-rated number of awakenings and perceived sleep quality were box-cox transformed to obtain a normal distribution. All activity rhythm and sleep variables were standardized. The basic model was adjusted for baseline cortisol, sex and age. The multivariate model was adjusted for baseline cortisol, sex, age, partnership, education, employment, body mass index, alcohol use, smoking, activities of daily living, cognitive status, diabetes mellitus, use of sleep medication or medication prescribed for the nervous system, actigraphic habitual wake up time and time difference between sleep measurements and cortisol sampling.

(B=−0.073, 95%CI: −0.178;0.032), most likely due to strong confounding of wake up time (B=−0.196, 95%CI: −0.306;−0.086).

In addition, we assessed actigraphically measured sleep parameters, as well as self-rated sleep parameters in relation to saliva cortisol levels after dexamethasone intake controlled for baseline cortisol (table 3). Total sleep time was related to saliva cortisol after dexamethasone, but only after multivariate adjustment (B=0.108, 95%CI: 0.001;0.215). Stepwise analyses showed that this association was affected most by the confounding of actigraphic wake-up time (results not presented). Further adjustment for depressive symptoms did not change the effect estimate (B=0.108, 95%CI: 0.001; 0.215). Other sleep parameters were not related to saliva cortisol after dexamethasone intake, independent of measurement method.

We also studied whether perceived sleep quality was associated with saliva cortisol after dexamethasone intake (table 3). In all models, a better perceived sleep quality was associated with higher levels of saliva cortisol after dexamethasone intake (basic model: B=0.108, 95%CI: 0.011; 0.205, and multivariate model: B=0.107, 95%CI: 0.009; 0.206). Further adjustment for depressive symptoms changed the effect estimate marginally (B=0.118, 95%CI: 0.014; 0.222).

Table 4. *Associations of the 24-hour activity rhythm and sleep with cortisol in saliva before dexamethasone intake (N=493).*

| | Cortisol in saliva before dexamethasone intake | | | |
| | Basic model | | Multivariate model | |
	B	95%CI	B	95%CI
24-hour activity rhythm				
Interdaily stability	**0.089**	**0.032; 0.146**	**0.064**	**0.005; 0.123**
Intradaily variability	**−0.066**	**−0.124; −0.008**	−0.030	−0.089; 0.028
Actigraphic sleep				
Total sleep time	−0.014	−0.069; 0.041	0.001	−0.060; 0.062
Sleep onset latency	−0.087	−0.180; 0.006	−0.064	−0.157; 0.030
Wake after sleep onset	**−0.064**	**−0.122; −0.006**	−0.041	−0.100; 0.018
Self-rated sleep				
Total sleep time	−0.020	−0.074; 0.034	−0.004	−0.059; 0.052
Sleep onset latency	0.000	−0.053; 0.053	0.035	−0.018; 0.088
Number of awakenings	**−0.056**	**−0.110; −0.003**	**−0.054**	**−0.106; −0.001**
Sleep quality				
Perceived sleep quality	**0.060**	**0.004; 0.116**	0.038	−0.018; 0.094

CI: Confidence Interval. Bold indicates p<0.05. Linear regression analyses. Cortisol levels were natural-log transformed, interdaily stability, intradaily variability, actigraphic sleep onset latency, actigraphic wake after sleep onset, self-rated sleep onset latency, self-rated number of awakenings and perceived sleep quality were box-cox transformed to obtain a normal distribution. All activity rhythm and sleep variables were standardized. The basic model was adjusted for sex and age. The multivariate model was adjusted for baseline cortisol, sex, age, partnership, education, employment, body mass index, alcohol use, smoking, activities of daily living, cognitive status, diabetes mellitus, use of sleep medication or medication prescribed for the nervous system, actigraphic habitual wake up time and time difference between sleep measurements and cortisol sampling.

Next, we studied whether sex and the intake of sleep medication, psycholeptics or psychoanaleptics modified the associations of the 24-hour rhythm and sleep with cortisol after dexamethasone intake. Significant interaction effects of sex were found on the associations of interdaily stability with cortisol after dexamethasone (p=0.049) and on the association of total sleep time with cortisol after dexamethasone (p=0.032). In men interdaily stability and cortisol after dexamethasone (n=211, B=0.192, 95%CI: 0.036; 0.348) were more strongly associated than in women (n=282, B=0.024, 95%CI: −0.119; 0.166). Likewise, total sleep time and cortisol after dexamethasone were more strongly associated in men (B=0.186, 95%CI: 0.032; 0.339) than in women (B=0.029, 95%CI: 0.123; −0.181). No significant interactions were found for an effect of medication on any of the associations.

Last, we present the associations of the 24-hour activity rhythm and sleep parameters with saliva cortisol before dexamethasone intake and after dexamethasone intake not adjusting for baseline cortisol (see table 4 and table 5). Persons with more stable rhythms had higher levels of cortisol both before and after dexamethasone intake (B=−0.064, 95%CI: −0.005; −0.123, B=0.149, 95%CI: 0.038; 0.260 respectively). Self-rated number of awakenings were associated with cortisol before dexamethasone intake after multivariate adjustment (B=−0.054, 95%CI: −0.106; −0.001), whereas they were not related to saliva cortisol after

Table 5. *Associations of the 24-hour activity rhythm and sleep with cortisol in saliva after dexamethasone intake (N=493).*

	Cortisol in saliva after dexamethasone intake			
	Basic model		Multivariate model	
	B	95%CI	B	95%CI
24-hour activity rhythm				
Interdaily stability	**0.196**	**0.088; 0.304**	**0.149**	**0.038; 0.260**
Intradaily variability	**−0.150**	**−0.259; −0.040**	−0.094	−0.206; 0.019
Actigraphic sleep				
Total sleep time	0.023	−0.081; 0.128	0.108	−0.006; 0.223
Sleep onset latency	−0.153	−0.330; 0.023	−0.131	−0.309; 0.047
Wake after sleep onset	**−0.134**	**−0.243; −0.024**	−0.102	−0.214; 0.010
Self-rated sleep				
Total sleep time	−0.053	−0.155; 0.048	0.009	−0.096; 0.114
Sleep onset latency	−0.011	−0.111; 0.089	0.028	−0.073; 0.129
Number of awakenings	−0.058	−0.159; 0.043	−0.065	−0.165; 0.035
Sleep quality				
Perceived sleep quality	**0.151**	**0.047; 0.256**	**0.133**	**0.028; 0.239**

CI: Confidence Interval. Bold indicates p<0.05. Linear regression analyses. Cortisol levels were natural-log transformed, interdaily stability, intradaily variability, actigraphic sleep onset latency, actigraphic wake after sleep onset, self-rated sleep onset latency, self-rated number of awakenings and perceived sleep quality were box-cox transformed to obtain a normal distribution. All activity rhythm and sleep variables were standardized. The basic model was adjusted for sex and age. The multivariate model was adjusted for baseline cortisol, sex, age, partnership, education, employment, body mass index, alcohol use, smoking, activities of daily living, cognitive status, diabetes mellitus, use of sleep medication or medication prescribed for the nervous system, actigraphic habitual wake up time and time difference between sleep measurements and cortisol sampling.

dexamethasone intake (B=−0.065, 95%CI: −0.166; 0.035). In addition, in persons with a worse perceived sleep quality no changes were observed in saliva cortisol before dexamethasone (B=0.038, 95%CI: −0.018; 0.094). However, in those with a worse sleep quality cortisol after dexamethasone was lowered (B=0.133, 95%CI: 0.028; 0.239) after multivariate adjustment. Associations of cortisol after dexamethasone had marginally increased effects sizes when they were not adjusted for baseline cortisol, but were largely similar to the associations that were adjusted for baseline cortisol.

Discussion

Our study demonstrated that a lower stability of the 24-hour activity rhythm is associated with enhanced negative feedback of cortisol in the HPA axis. Similarly, a poor perceived sleep quality was related to enhanced negative feedback. Total sleep time was associated with cortisol after dexamethasone only when controlled for wake-up time. Total sleep time and perceived sleep quality were associated uniquely with cortisol levels after the intake of dexamethasone, these sleep characteristics were not associated with cortisol before the intake of dexamethasone.

The stability of the 24-activity rhythm was associated with the change in saliva cortisol after intake of 0.25 mg dexamethasone. There are several explanations possible for the association of less stable rhythms with enhanced negative feedback of the HPA axis. First, a deterioration of the SCN may underlie both less stable activity rhythms as well as an enhanced negative feedback control of the HPA axis. A deteriorated SCN can result in problems in integrating internal and external time cues,[36] which can lead to unstable and fragmented rhythms and possibly disturbances in the cortisol rhythm.[37] However, research has suggested that CRH, which is released from the hypothalamus, is the key circadian alerting signal of the HPA axis instead of cortisol.[3] Second, the association of the stability of the rhythm with the negative feedback of the HPA axis could be due to depressive symptoms; cortisol after dexamethasone intake has been suggested as a biomarker for depression.[13] However, while depression has been related with more disturbed circadian rhythms,[38] it has mostly been related to a diminished negative feedback of the HPA instead of an enhanced negative feedback. This is not consistent with our results as we find that less stable rhythms are associated with enhanced negative feedback of the HPA axis. Also, adjustment for depressive symptoms did not change the association between the stability of the rhythm and the negative feedback control in the present study. Associations in the same direction as in the present study have been reported for other disorders such as post-traumatic stress disorder[14] and job-related exhaustion.[39] The association of job-related exhaustion with negative enhanced feedback has been explained by glucocorticoid receptor hypersensitivity accompanied by changes in GR induced gene expression. Possibly, the association of less

stable rhythms with enhanced negative feedback follows a similar mechanism, further studies are needed to test this hypothesis. Third, less stable activity rhythms may directly influence cortisol levels. Possibly, the association of a more stable rhythm and lower cortisol levels can be explained by better persistence of a rapidly rising cortisol awakening response, despite a low dose of dexamethasone, in those maintaining a more stable activity rhythm. Unstable rhythms may result in more awakenings or more fluctuations which are physical and psychological stressors that can increase cortisol levels.[40] These increased cortisol levels might need higher doses of dexamethasone to be suppressed. In our study we did not find an association of wake after sleep onset or fragmented rhythms suggesting that awakenings are not key in the association of unstable rhythms with enhanced negative feedback of the HPA axis. Fourth, previous research suggests that the association between the stability of the rhythm and the negative feedback control is bi-directional.[3] Thus, a less adaptive HPA axis could also lead to more unstable rhythms. Stability of the 24-hour rhythms can be highly dependent on amenable behaviors. Less regulation of the HPA axis might lead to less regulated behaviors, including less habituated sleep and wake times.

In our sample, total sleep time was related to saliva cortisol levels after the intake of a very low-dose of dexamethasone. This finding was dependent of the measurement method of sleep; it was only found when total sleep time was assessed objectively with actigraphy. Previous results have been mixed. Most associations of sleep with cortisol were found in clinical samples, i.e. in insomniacs,[9] or after experimental manipulation of sleep, i.e. after sleep deprivation.[8] Population-based studies have not found associations between objectively assessed sleep and cortisol,[5,6] however these cortisol levels were not experimentally manipulated. In line with this, we also found that indeed total sleep time was not associated with cortisol levels before dexamethasone intake. The discrepancy between cortisol levels before and after dexamethasone intake could be explained multiple ways. First, sleep may only affect the glucocorticoid receptor mediated negative feedback, which is the mechanism mainly influenced by the intake of dexamethasone, and no other mechanisms involved in cortisol production. Second, a rapid habituation of the production of cortisol to sleep disturbances has been reported,[41] possibly this habituation only affects certain aspects of the functioning of the HPA axis.

Poor perceived sleep quality was associated with enhanced negative feedback of the HPA axis. This association is unique for the negative feedback control of the HPA axis since the association was only observed with cortisol after dexamethasone intake and not with cortisol levels before dexamethasone intake. Altered HPA-axis activity has been reported in stress-related conditions, for example after acute induced stress,[40] after prenatal stress,[42] and in stress disorders.[43] Stress could also impact how persons experience their sleep quality. More generally, perceived sleep quality may be a marker for general social, psychological or physical issues, which might affect how persons react to pharmacologically

or psychologically induced stress. Yet, depressive symptoms could not explain the association between perceived sleep quality and the negative feedback control in our study. This is in line with the normalization of cortisol levels in depressed patients independent of their sleep quality.[44] In a previous population-based study, low waking salivary cortisol and a flatter slope in cortisol secretion were associated with fatigue.[45] Possibly, symptoms such as fatigue represent the mechanism which underlies the association of poor perceived sleep quality and enhanced negative feedback of the HPA axis in the present study. This would also be in line with previously described association of enhanced negative feedback with chronic fatigue syndrome[14] and job-related exhaustion.[39]

In our study, the associations of the stability of the rhythm and total sleep time with cortisol change differed by sex. It has been demonstrated that men exhibit greater saliva responses in studies with social stress situations, but studies which assessed the pharmacological manipulation of cortisol levels did either demonstrate no sex differences or a decreased feedback sensitivity in women, specifically at older age.[46] Our results were more pronounced in men, which is in line with previous evidence of a stronger negative feedback in men than women. The use of 0.25mg of dexamethasone might have been too low to trigger a response in the middle-aged and elderly women of our sample due to the decreased feedback sensitivity in this population.

Our study has several strengths. First, to our knowledge, we were the first to assess a very low-dose DST in relation to sleep in a population-based study in middle-aged and elderly persons which deals with the generalizability problem in clinical studies. Second, we were able to study the 24-hour organization of the activity rhythm as persons were studied with actigraphy over multiple days. And last, we studied sleep with both a sleep diary and actigraphy over the same period, which allowed us to differentiate between subjective and objective measurements of sleep. Nevertheless, there are also limitations to our study. First, we assessed the effects of a very low-dose dexamethasone in a sample of middle-aged and elderly persons. Dexamethasone metabolism has been suggested to alter with age.[47,48] Age did not have a significant confounding influence in our study, but we must be careful in generalizing these results to younger populations. Second, although actigraphy is a valid estimator of sleep, it lacks the precision of polysomnography.[24] Third, the very low-dose DST was performed at home by the participants which makes is susceptible for non-compliance. However, all of our participants participated voluntarily and could have easily withdrawn from participation. In other population-based studies non-compliance has also not been an issue.[26,49] Fourth, we evaluated the effects of dexamethasone on saliva cortisol levels. It has been suggested that low doses of dexamethasone only create a state of low brain cortisol which leads to a compensatory increase in central CRH.[3] However, we were not able to assess this with the current study. In addition, the impact of 0.25 mg of dexamethasone has been found to be less reproducible in saliva cortisol levels than in serum cortisol levels.[50] The

susceptibility for this low dose of dexamethasone has also been suggested to be dependent on a distinct GR polymorphism.[51] Next, the timing of cortisol sampling was set at 8 AM for everyone independent of their wake-up times at the days of saliva sampling. And, as we had only one baseline sample, we were not able to assess the cortisol awakening response. Last, the actigraphy and very low-dose DST were not administered in the same week, we thus assessed the effect of habitual rhythms and sleep on the negative feedback control of the HPA axis and not the effect of dexamethasone intake on sleep directly.

In conclusion, a less stable 24-hour organization, a shorter sleep duration, and a poor perceived sleep quality were associated with the negative feedback control of the HPA axis, as indicated by the difference in the cortisol concentrations in saliva before and after the intake of 0.25 mg of dexamethasone. Dexamethasone cortisol reactivity may help to understand the impact of circadian rhythm and sleep disturbances on the stress system.

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Polysomnographic sleep and depressive symptomatology in the general population

Chapter 4.1

REM-sleep and depressive symptoms in a population-based study of middle-aged and elderly persons

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Abstract

Alterations in rapid eye movement (REM)-sleep have been consistently related to depression in clinical studies. So far, there is limited evidence from population-based studies for this association of REM-sleep alterations with depressive symptoms. In 489 participants of the Rotterdam Study we assessed REM-sleep latency, REM-sleep duration and REM-density with ambulant polysomnography, and depressive symptoms with the Center of Epidemiologic Studies-Depression scale. A longer REM-sleep latency (B=.002, p=.025) and higher REM-density (B=.015, p=.046) were related to depressive symptoms after age-sex adjustment. When we excluded persons who used sleep medication or medication for the nervous system (n=124), only REM-density remained related to depressive symptoms (B=.018, p=.027). Our results suggest that REM-density is a marker of depressive symptoms in the general population and that associations of REM-sleep are modified by the use of medication.

Introduction

Clinical research suggests that a decreased REM-sleep latency, increased REM-sleep duration, and increased REM-density are prominent in persons with depressive disorders.[1] Changes in sleep are found during and before the onset of depressive disorders, suggesting that sleep alterations can be both a trait and state marker.[2]

Studies on the associations of REM-sleep and depression have been conducted in clinical populations with severe depressive episodes mostly. However, in the general population symptoms of depression are usually lingering and less severe. In two large population-based studies no associations of REM-sleep with depressive symptoms were found.[3,4] In a large sample of older men a lower percentage of time spent in REM-sleep was related to more depressive symptom.[5]

Clinical studies typically investigate medication naïve patients, as sleep medication and other medication can impact REM-sleep.[6] Population-based studies mostly adjust for medication use, such as anti-depressants, which are commonly prescribed. However, medication can both confound and modify the association between REM-sleep and depressive symptoms. In this study we examined whether REM-sleep latency, REM-sleep duration and REM-density are related to depressive symptoms in a population-based sample. We also assessed these associations excluding persons who used sleep medication or medication prescribed for the nervous system.

Methods

Study Population

The current study was embedded in the Rotterdam Study, a population-based cohort of middle-aged and elderly inhabitants of Rotterdam, the Netherlands.[7] The study was conducted in accordance with the guideline proposed in the World Medical Association Declaration of Helsinki and approved by the medical ethics committee according to the Population Study Act Rotterdam Study, executed by the Ministry of Health of the Netherlands. Written informed consent was obtained from all participants.

From January 2012 until June 2013, 876 persons were invited to participate in a polysomnography (PSG) study; 500 participants (57.1%) agreed. There was no significant difference in age or depressive symptoms between participants and those who declined to take part in the PSG study, although women were more likely to decline (53.8% versus 61.2%, p=0.028). 11 participants (2.2%) were excluded; for 9 participants the PSG recording was of insufficient quality and for 2 participants there was no information on depressive symptoms. Data of 489 participants was eligible for analyses.

Assessment of depressive symptoms

During a home-interview all participants were screened for depressive symptoms with the validated, Dutch version of the Center for Epidemiologic Studies-Depression (CES-D) scale.[8,9] The CES-D consists of 20 questions which measure negative affect, lack of positive affect, interpersonal and somatic problems scored on a 0-3 scale according to their severity, with a higher score indicating more depressive symptoms.

Assessment of sleep

Participants were scheduled for a home visit of a trained research assistant to place all sensors to record an ambulant PSG (Vitaport 4, Temec, Kerkrade, the Netherlands). The PSG included six electroencephalography channels, F3/A2, F4/A1, C3/A2, C4/A1, O1/A2, O2/A1, bilateral electrooculography, electromyography, electrocardiography, respiratory belts on the chest and abdomen, oximetry, and a nasal pressure transducer and oronasal thermocouple to measure airflow. All participants slept one night with the equipment and were instructed to spend the night as normal as possible. There were no further restrictions on bedtimes and the use of alcohol, coffee, and medication. All recordings were scored according to AASM (American Association of Sleep Medicine) guidelines[10] by a Registered Polysomnographic Technologist (RPSGT).

PSG recordings were manually scored in 30-second epochs for identification of sleep stages; each epoch was scored as Wake, N1, N2, N3 or REM sleep. For each of these sleep stages, the duration and latency was determined. In addition, we used PRANA (PhiTools, Strasbourg, France) software[11] to automatically measure REM-density (number of rapid eye movements per minute during REM-period). REMs were detected automatically when the electrooculography signal had an amplitude of at least 10μV and a slope of at least 10μV/s, the duration had to between 0.05 ms and 3.0 ms.

Assessment of covariates

We selected sex, age, education, employment status, alcohol, and coffee use at night of PSG, body mass index, apnea-hypopnea index, use of sleep medication at night of PSG, and use of medication prescribed for (1) blood and blood forming organs, (2) cardiovascular system, (3) genito-urinary system and sex-hormones, (4) systemic hormonal preparations, (5) nervous system and (6) respiratory system (according to the anatomical therapeutic chemical (ATC) classification system) as possible confounders.

Statistical analyses

Associations of REM-sleep latency, REM-sleep duration and REM-density with depressive symptoms were assessed with linear regression. We conducted age-sex adjusted and multivariate adjusted analyses (including all covariates). In addition, we ran the analyses separately for participants free of sleep medication and free of medication prescribed for

the nervous system. Depressive symptoms were log transformed and standardized in all analyses. All analyses were performed using IBM SPSS Statistics, version 21 (IBM Corp., Somers, NY USA).

As the number of missing values per parameter never exceeded 1%, missing values in quantitative predictors were replaced by the median. A separate missing category was used for qualitative predictors.

Table 1. *Population Characteristics, N=489*

Demographic	
Female, %	52.4
Age, years	61.85 ± 5.37
Employment, %	50.2
Education, %	
Low	8.6
Intermediate	58.2
High	32.9
Health indicators	
Body mass Index (BMI), kg/m^2	27.84 ± 4.71
Alcohol use at night of polysomnography, , %	29.4
Coffee use at night of polysomnography, %	61.6
Use of medication for the nervous system (total)[1], %	23.3
Psychoanaleptics, %	6.7
Psycholeptics, %	7.6
Anti-Parkinson drugs, %	0.4
Anti-epileptics, %	2.9
Analgesics, %	11.0
Other, %	1.6
Use of medication for the respiratory system, %	8.6
Use of medication for the cardiovascular system, %	42.3
Use of medication for blood and blood forming organs, %	15.7
Use of medication for the genito-urinary system and sex hormones, %	3.1
Systemic hormonal preparations, %	5.1
Use of sleep medication at night of polysomnography, %	6.9
Depression	
Depressive symptoms, score	5.43 ± 6.47
Sleep	
Apnea Hypopnea Index (AHI), events per hour	15.71 ± 14.65
Total sleep time (TST), minutes	382.60 ± 63.13
Sleep onset latency (SOL), minutes	21.37 ± 26.08
Rapid Eye Movement (REM)-sleep latency, minutes	88.48 ± 53.09
Rapid Eye Movement (REM)-sleep duration, % total sleep time	18.2
Rapid Eye Movement (REM)-density, n per minute	5.76 ± 6.09

Values are stated as mean ± standard deviation or percentage.
[1] The total amount includes all persons who use one or more medications from this category.

Results

A description of the study population can be found in table 1.

Table 2. *The associations of REM-sleep with depressive symptoms.*

	Depressive Symptoms					
	Total sample n=489			Persons free of medication[1] n=365		
	B	SE	P	B	SE	P
Rapid Eye Movement (REM)-sleep latency (minute)[2]						
Sex-age adjusted	**.002**	**.001**	**.025**	.002	.001	.21
Multivariate adjusted	.001	.001	.16	.002	.001	.19
Rapid Eye Movement (REM)-sleep duration (%)						
Sex-age adjusted	−.004	.008	.64	.000	.010	.99
Multivariate adjusted	−.002	.008	.76	.002	.010	.81
Rapid Eye Movement (REM) density (n/minute)[2]						
Sex-age adjusted	**.015**	**.007**	**.046**	**.018**	**.008**	**.027**
Multivariate adjusted	.012	.007	.089	**.017**	**.008**	**.032**

Bold indicates p<0.05. Linear regression analyses. Depressive symptoms were log-transformed and standardized. Multivariate adjusted models are adjusted for sex, age, education, employment status, alcohol use, coffee use, body mass index, sleep medication use (only in total sample), general use of medication prescribed for the nervous system (only in total sample), respiratory system, cardiovascular system, blood and blood forming organs, genito-urinary system and sex hormones, and systemic hormonal preparations.
[1] Persons who used sleep medication at the night of PSG and/or used medication for the nervous system (n=124) are excluded.
[2] 1 participant did not have any REM-sleep, this person is excluded.

A longer REM-sleep latency and a higher REM-density were associated with more depressive symptoms when adjusted for age and sex (respectively B=.002, SE=.001, p=.025 and B=.015, SE=.007, p=.046; table 2). However, these associations disappeared after multivariate adjustment.

Exclusion of persons who used medication for the nervous system and sleep medication at the night of PSG (n=124), substantially increased the strength of the association of REM-density with depressive symptoms in the multivariate adjusted model (B=.017, SE=.008, p=.032, figure 1). This association was not found in persons who did use medication (B=−.002, SE=.017, p=.892). The association of a longer REM-sleep latency with depressive symptoms, adjusted for age and sex, was unchanged but no longer significant (B=.002, SE=.001, p=.21) in persons who did not use medication.

Discussion

REM-density was consistently related to depressive symptoms in this general population-based study. This association was particularly prominent in participants who did

not use any medication prescribed for the nervous system or sleep medication at the night of the PSG. No associations of REM-sleep latency and REM-sleep duration with depressive symptoms were found in multivariate adjusted models.
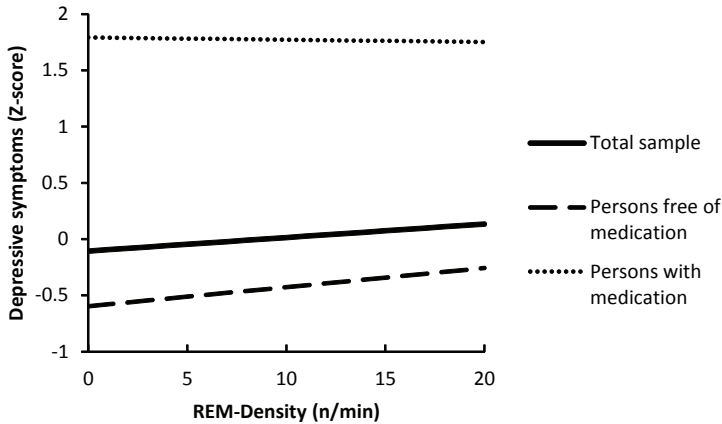

Figure 1. *The association of REM-density with depressive symptoms (z-score). Regression lines are plotted separately for the total sample (continuous line), persons free of any sleep medication or medication for the nervous system (dashed line) and persons with these medications (dotted line). Estimates were extracted from the multivariate adjusted model.*

To our knowledge the association of REM-density with depressive symptoms has not been assessed before in a population-based setting. Our results suggest that, of the REM-sleep characteristics, only REM-density is a marker of subclinical depressive symptoms in middle-aged and elderly persons in the general population. Possibly our findings can be explained by the relatively old age of our sample, as REM-sleep latency tends to become progressively shorter with middle age, while REM-density does not vary with age.[12] In addition, it has been suggested that REM-density is altered in both remittent and depressive states,[13] which might explain this association in the present subclinical population where depressive symptoms are less severe but continuously lingering. Research on high risk probands demonstrated that REM density is also elevated in healthy relatives of patients with depression, and that elevated REM density is predictive for the onset of psychiatric disorders.[14] Our results support the conclusion that increased REM density is a possible endophenotype of depression.

The focus on prevalent depressive symptoms but not on severe depression is therefore a strength and a limitation of our study. Another limitation is that we cannot draw any conclusions on temporality as our data is cross-sectional. In addition, our study consisted of one night of PSG and is thus susceptible for first-night effects. However, all persons were studied at their own home to minimize the first-night effect. Our study sample has a high average apnea-hypopnea index which might affect our results, to prevent for confounding

we included apnea-hypopnea index in our model.

In our study, the use of medication influenced the associations of REM-sleep latency and REM-density. After excluding participants who used medication that can affect sleep and depression, only the association of REM-density and depressive symptoms became more pronounced. Several medications, including antidepressants, are known to inhibit REM-sleep.[6] In our study, medication use masked the association of REM-density with depressive symptoms.

Our findings emphasize that REM-density may be considered as an important marker of depression in subclinical populations, next to clinical populations, and that medication use may obviate this marker.

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Chapter 4.2

The interrelation of sleep apnea, depressive symptoms and fatigue in a population-based study

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Chapter 5

Discussion

In this thesis I have investigated the variation of sleep and the 24-hour activity rhythm in middle-aged and elderly persons of the general population. In particular, I studied how this variation was related to neuropsychiatric problems. First, I focused on the associations of the 24-hour activity rhythm with demographics and lifestyle in middle-aged and elderly persons, and the effects of disturbances in the 24-hour activity rhythm on mortality. In the second part of my thesis, I examined the relation of actigraphically measured sleep and rhythms with cognition, depression, anxiety, and the negative feedback of the hypothalamic-pituitary-adrenal (HPA) axis. In the last part, I focused on polysomnographically (PSG) assessed sleep. The association between rapid eye movement (REM) sleep and depressive symptoms as well as the interrelation between sleep apnea, depressive symptoms and fatigue was explored. In this last chapter I have reviewed the main findings of this thesis, addressed methodological considerations and discussed implications and recommendations for the future.

Main findings

Chapter 2: 24-Hour activity rhythms and habitual sleep in middle-aged and elderly persons

Disturbances in the 24-hour rhythm are common in middle-aged and elderly persons.[1] We demonstrated that activity rhythms are not only disturbed in older age, but that rhythm disturbances are also associated with other demographics and lifestyle (chapter 2.1). Moreover, rhythm disturbances predicted earlier mortality (chapter 2.2). We tested rhythm disturbances by assessing the stability and fragmentation of the rhythm. The stability and fragmentation are unique aspects of the activity rhythm, which is demonstrated by the association of age with these two aspects. Stability and fragmentation are moderately and negatively correlated with each other, but older age increases the stability of the rhythm as well as the fragmentation of the rhythm. In chapter 2.2 we observed that more stable rhythms and less fragmented rhythms both decreased the mortality risk even if accounting for age. If we combine these results an unexpected pattern emerges. We observed that more fragmented rhythms are related to older age and, in accordance with this observation, that more fragmented rhythms were related to an increase in mortality risk. Possibly, a fragmented rhythm is one of the factors underlying this association. However, a contrasting pattern is observed for the triangle of stability of the rhythm, old age and mortality; while a more stable rhythm is related to older age, a more stable rhythm is also related to a lower mortality. We discussed in chapter 2.1 that stable rhythms might reflect a health-related behavior instead of a physiologic phenomenon. Stable activity rhythms are not likely to be a physiologic result of non-optimal health, which usually accompanies old age. Stabilizing the 24-hour rhythm might be an adaptive response to entrain the circadian clock, when health is not optimal.[2] Becoming more rigid in behavior, which is commonly observed in elderly

persons, might, intentionally or unintentionally, optimize health and decrease the mortality risk.

Until now the additional value of assessing the 24-hour organization of the activity rhythm above actigraphic estimates of sleep has been unclear. Therefore, the associations of rhythm disturbances with poor health could reflect the associations of sleep with poor health. We studied the 24-hour activity rhythm and sleep simultaneously in one study and demonstrated that 24-hour activity rhythms are related to sleep parameters but that they are not proxies of each other. In other words, the measures tap different behavioral phenomena. The results of our study on mortality are in line with this interpretation; we only observed an effect of 24-hour activity rhythms on mortality, and no effect of sleep on mortality. The associations of activity rhythms and sleep with other health constructs in chapters 3.1, 3.2 and 3.3 also suggest that activity rhythm parameters are unique constructs and cannot be viewed as sleep parameters.

Determinants of sleep changes have been studied extensively. For example, lifestyle and dietary habits, such as alcohol consumption, coffee use and smoking, are known to affect sleep.[3-5] We demonstrated that lifestyle factors are not only related to sleep, but also to changes in the 24-hour activity rhythm. An unhealthy lifestyle, reflected by a higher body mass index and smoking, was associated with more fragmented and less stable rhythms. These factors are also associated with mortality, but the effect of fragmented rhythms on mortality could not be explained by these unhealthy behaviors in our study. Thus, fragmented rhythms are not only an indicator or mediator of an unhealthy lifestyle, but pose an additional risk for a shorter life. Coffee use increased the stability of the activity rhythm and has been suggested to decrease mortality risk in other studies.[6] Possibly, coffee has a stabilizing effect on the 24-hour rhythm. Recent animal research suggests that caffeine increases the responsiveness to light of the master clock.[7] This stabilizing effect of coffee on the rhythm might be a mechanism which underlies the association of coffee use and mortality. However, coffee use could also be an indicator of good health, coffee intake is one of the behaviors that persons quickly alter when their health or sleep becomes poor. Indeed, the associations of the activity rhythm with healthy behavior are most likely bidirectional. Therefore, we cannot conclude whether rhythm disturbances are truly one of the many mechanisms through which lifestyle influences mortality risk.

Chapter 3: 24-Hour activity rhythms and habitual sleep in relation to neuropsychiatric problems in middle-aged and elderly persons

In chapter 3 we assessed how 24-hour activity rhythms and sleep were related with cognitive functions and psychiatric problems. First, we demonstrated that disturbances of sleep and the 24-hour activity rhythm were related to cognition. While persons with longer sleep onset latencies had worse memory function, persons with 24-hour rhythm disturbances

performed less on executive functioning and perceptual speed tasks (chapter 3.1). Associations of rhythm disturbances and perceived sleep quality with depressive symptoms and anxiety were demonstrated in chapter 3.2. Disturbances in the 24-hour activity rhythm and a worse perceived sleep quality were also associated with the negative feedback of the HPA axis, reflected by cortisol after the intake of a very low-dose of dexamethasone.

The results of this chapter reflect the importance of the 24-hour rhythm for cognitive functions and psychiatric problems, while limited evidence was found for any associations of actigraphic sleep parameters with these functions. Actigraphic sleep was only related to memory function, while the fragmentation of the rhythm was related to executive functioning, depression and anxiety. Possibly, depression and anxiety disturb executive functioning,8 or, vice versa, worse executive functions might create a vulnerability for depression and anxiety.9 Arguably, disturbances in the domain of the executive functions can result in depression or anxiety by making a person interpret stimuli more negatively,9 e.g. via ruminative responses to negative mood states or the inability to use positive and rewarding stimuli to regulate negative mood. However, in our study, the associations of disturbed rhythms with cognition were not explained by depressive symptoms, or reversely, cognitive status could also not explain the association of disturbed rhythms with depressive symptoms. Possibly, a common degenerative process in the brain underlies rhythm disturbances, executive function problems, depression and anxiety. In other words, they might be different manifestations of a single pathology. A possibly pathology underlying all these problems could be a poor function of the HPA axis. However in our study, the stability, and not the fragmentation, was related to the negative feedback of the HPA axis. Yet, we can conclude that overall the 24-hour organization of the activity rhythm is associated with different psychological processes, from cognitive problems to psychiatric symptoms.

Perceived sleep quality was the one sleep characteristic that was associated with all neuropsychiatric processes (cognition, depressive symptoms anxiety and the function of the HPA axis) reported in chapter 3. Multiple explanations are possible for the associations of perceived sleep quality with cognition, depressive symptoms anxiety and the function of the HPA axis. First, processes underlying the association of cognition and depression, e.g. ruminative responses to negative mood states or the inability to use positive and rewarding stimuli to regulate negative mood, might also affect how persons perceive or evaluate their sleep. It is not unlikely that these processes are also related to the negative feedback loop of the HPA axis, as the HPA axis regulates the release of stress hormones. For example, rumination, which has been suggested as a mechanism between cognition and depression, is also related to cortisol levels.10 Second, perceived sleep quality could be an overall health indicator. Perceived sleep quality is related to quality of life, which is dependent on health. The option of sleep as an indicator of poor health is further discussed in the section 'Methodological considerations' of this chapter.

Chapter 4: Polysomnographic sleep and depressive symptomatology in the general population

In chapter 4 we described that only REM-density is a marker of depressive symptoms in the general population. This association was particularly prominent in participants who did not use any medication prescribed for the nervous system or sleep medication at the night of the PSG. We also demonstrated that sleep apnea is not directly related with depression in the general population, although both were related with fatigue. Furthermore, fatigue moderated the association of sleep apnea and depressive symptoms.

The results of chapter 4 were affected by two important features of the study design. First, associations depend on the population studied. Generally, clinical studies tend to find stronger relations as they consider extremes of the population, for example persons with a clinical disease (e.g. persons with an apnea index above 15) are compared with healthy -often extremely healthy- persons (e.g. persons with an apnea index below 5). In contrast, a population-based study includes persons who have no complaints, those who meet the criteria for a clinical diagnosis, and also those who have some complaints albeit not enough to be diagnosed. We thus considered the continuum of the disease. In our study, only REM-density was a marker of subclinical depressive symptoms. The macrostructure of REM-sleep, for example REM-sleep latency and REM-sleep duration, have been suggested to change in persons with clinical depression. However, these characteristics did not differ for disease symptoms in the general population. Sleep apnea was not related to depressive symptoms in our population-based study, while this association has been found in clinical studies. Both these results demonstrate that associations can depend on the population studied. Second, the results of every study depend on the precision of the measurement used. For sleep research a new eras has started in which we can assess sleep validly with ambulant PSG in the participants home.[11] Population-based studies, like the Rotterdam Study, are particularly qualified to take this opportunity. Ambulant PSG allowed us to study the apnea-hypopnea index as a marker of sleep apnea and microstructural variables such as REM-density. In our study, measures of the macrostructure of sleep, such as the apnea hypopnea index or REM-latency, were not able to explain depressive symptoms, while the microstructural variable REM-density was related to depressive symptoms. This extends chapter 3.2 where we demonstrated that sleep, when assessed with actigraphy which only estimates sleep globally, was not related with depressive symptoms. Associations thus rely on the level of precision of the variable, and not necessarily on the instrument itself.

In chapter 4, we also demonstrated the importance of assessing effect modification. Sleep characteristics can be modified easily by external factors, such as the use of medication, and by levels of fatigue. The use of medication influenced the associations of REM-density with depressive symptoms. After excluding participants who used medication that can affect sleep and depression, the association of REM-density and depressive symptoms became

more pronounced. Several medications, e.g. antidepressants, are known to inhibit REM-sleep. These results demonstrate the importance of not only assessing confounding effects of medication, but to also to assess effect modification by medication. Interestingly, we also found that the association of sleep apnea with depressive symptoms can be obscured by fatigue. Sleep should thus be assessed in close relation with other health variables, as these might explain or modify the effects. This is particularly important for comorbid disorders as health problems often co-occur with sleep disorders.[12] Symptoms and consequences of sleep disorders are often non-specific, and could be mistaken for symptoms of consequence of other diseases. Careful assessment of the etiology is thus essential to treat sleep and other health problems effectively.

Methodological considerations

Assessing circadian rhythms in middle-aged and elderly persons

Circadian rhythms are found in behavior and in physiological processes.[13] These rhythms are regulated by the suprachiasmatic nucleus (SCN), which integrates endogenous rhythms with external time cues, usually resulting in the typical 24-hour course of the diurnal activity rhythm.[14] To measure 24-hour rhythms continuous sampling is needed, or at least multiple samples over a longer period time. This large amount of measurements complicates the assessment of 24-hour rhythms in large population-based samples. Assessment of hormone levels in the blood as a measure of the circadian rhythm is for this reason simply not feasible in large study samples. Rhythmicity of hormone levels can also be assessed in saliva, however as this requires participants to chew on cotton multiple times a day for a week, it would be considered too burdensome by many potential participants. Self-report of the 24-hour rhythm is a low impact measurement, but it is not precise and prone to social bias and recall bias. An objective, and less burdensome method to measure 24-hour rhythms is actigraphy. Participants only have to wear a watch-like-device, which measures movement. If this actigraph is worn for a longer period of time, it allows the estimation of the 24-hour organization of the rhythm. Actigraphy is considered to be a reliable measurement[15] with relatively little burden for the participant, and has been used commonly in large population-based studies.[16-18]

Fitting an adapted cosinor curve to the rhythm is a common method to assess the 24-hour organization of the activity rhythm.[19] However, unlike the curvature of certain hormone levels, the activity rhythm does not reflect a sinusoidal waveform. The shape of the activity rhythm would resemble a more square-shaped rhythm in which the periods of rest and activity have clear and steep boundaries. Using a cosinor function to assess the activity rhythm can be misleading; specifically when the rhythms have many transitions from rest to wake, as this leads to a poor fit of the cosinor curve. These rest-wake transitions

are particularly common in elderly persons; they tend to rest more often and do short activities. Thus, it has been suggested that activity rhythms should be assessed non-parametrically.[20] Non-parametric methods do not impose a model on the data, which prevents for errors in the analysis of highly fluctuating rhythms.[21,22] Non-parametric methods can describe the stability and fragmentation of the rhythm. These parameters give an indication of the level of disturbance in the 24-hour organization of the activity rhythm. Hence, we used a non-parametric approach for the assessment of the 24-hour activity rhythm in our study of middle-aged and elderly persons.

Polysomnography in large populations

PSG is considered the 'gold standard' of sleep research for decades now.[23] With PSG we are able to assess the brain activity. Brain activity differs not only between sleep and wake, but also within sleep. In deep sleep, brainwaves have a large amplitude and a low frequency, but in REM-sleep, the sleep in which we dream the most, brainwaves have a smaller amplitude and higher frequency, similar to brain waves during wake. However, we can clearly distinct REM-sleep from wake; in REM-sleep we see rapid eye movements and the muscle tone becomes very low. PSG therefore comprises of more than only electroencephalography (EEG), physiologic signals such as electromyography (EMG) and electrooculography (EOG) also register important information about sleep.

For decades, a PSG study was only possible in a hospital or research laboratory. PSG equipment was too large to facilitate research outside these facilities. In the very beginning, PSGs were recorded with a simple pen that traced the brain activity on long sheets of paper. With the introduction of the computer, PSG became digital and much more user-friendly. By now, ambulant equipment for PSG is available. Ambulant equipment permits performing a complete PSG at any place, without a restriction on the amount of information collected. The complete equipment (in our study we used the Vitaport 4, Temec, Kerkrade) can be worn on a shoulder band, a laptop is only needed for starting and checking the equipment. All tracings are stored on a small memory card. The ambulatory PSG equipment has been used in clinical practice and research for over a few years now and is gaining popularity. There are some limitations to ambulant PSG; it does not allow for real-time overnight monitoring, electrodes could get lost as there is no possibility for replacement and the environment can be less well monitored. However, depending on the research question, these limitations do not outweigh the advantages of ambulant PSG. The major strength of ambulant PSG is being able to assess sleep within a persons' own environment, in his own bed in his own room. For clinical purposes this is of interest because the environment can be part of the sleep problem, for research this is of particular interest as it makes sleep studies more generalizable. Ambulant PSG is generally also experienced less burdensome than an overnight PSG in a hospital or laboratory. In addition, ambulant PSG is cost effective,

which is important in a world where both hospitals and research institutions have to cut their spending.

The development of ambulant PSG improved the feasibility of recording a full PSG in large samples. Although there are studies in which large samples have been invited to a sleep lab,[24,25] these studies suffer from high costs and can have issues with generalizability. The Sleep Heart Health Study (SHHS) was the first study to implement ambulatory PSG in a home setting,[26] albeit with a limited number of EEG-channels. This has sparked other studies to add full or partial ambulant PSG sleep research to their cohorts,[27-29] the Rotterdam Study is now one of the few studies which is in possession of a large set of full ambulant PSG recordings in middle-aged and elderly persons. A repeat of this assessment would make the Rotterdam Study truly unique in sleep research, as this allows for a longitudinal, detailed assessment of sleep, see also the section 'Recommendations' in this chapter.

Sleep and rhythms as an indicator of general health

Sleep is a common behavior and large part of our life, which is bound to be influenced by sickness and health, and arguably also influences health.[30] Sleep and rhythms have been related to many different health factors, which could lead to the proposition that disturbed rhythms are merely an indicator of poor health and not reflecting a specific effect, mechanism or cause. Other health indicators are constructs such as quality of life (QOL) or activities of daily living (ADL), these constructs typically give a general indication of a person's health status. Generally these constructs are assessed with questionnaires. Persons can also self-rate sleep characteristics such as sleep duration, sleep quality, and chronotype in a questionnaire. Self-rated sleep characteristics did not have any effects on health in our thesis, but we did find associations of self-reported sleep quality and health. Sleep quality was related to almost all of the health problems we have addressed in this thesis, which increases the possibility that the experience of sleep could be an indicator of the status of our health, similarly to constructs such as the quality of life index.

We also assessed sleep and rhythms objectively with actigraphy. Actigraphically measured sleep characteristics were only related to specific cognitive problems and the negative feedback of the HPA axis, and not with depression, anxiety and mortality (chapter 2 and chapter 3). This would suggest that sleep is not an indicator of overall health, but is only associated with certain diseases. The notion of sleep as a marker of overall health is further corroborated by studies which have used PSG to assess sleep (chapter 4). However, a fragmented 24-hour organization of the activity rhythm was related to a variety of health problems studied in this thesis. This suggests that while sleep may not be an indicator of poor health, objectively measured fragmented rhythms possibly are a general indicator of poor physical and mental health. Disturbed rhythmicity cannot only be found in the activity rhythm, it can be found throughout the body, from cells up to complicated behaviors such

as cognition and mood.[31-33] Fragmented endogenous rhythms might disturb processes all over the body, thereby generating a wide array of health problems and diseases. Further research in rhythms on all levels is needed to provide evidence for this speculation.

I suggest that, fragmented activity rhythms are a stronger marker of poor health than objective sleep per se. Perhaps the most global indicator of general health is perceived sleep quality. Of course, perceived sleep quality is also related to the fragmentation of the activity rhythm. Possibly, fragmented 24-hour rhythms might worsen perceived sleep quality more than a short sleep duration would.

Categorization of variables

Most studies use categorical variables, in addition to continuous variables. Advantages and disadvantages of categorization, such as the loss of statistical power, multiple hypothesis testing, and the difficulty comparing results using different cut-offs across studies have been discussed extensively before.[34-36] In this paragraph I will expand these topics by discussing the motivation for the use of categorizations, despite the well-known disadvantages.

Certain variables are defined in "natural" categories, the most prominent example being biological sex, and the use of these categorizations, of course, is straightforward. For these variables, other categorizations are mostly irrelevant and the data could never be assessed continuously. For other variables, all categorizations are debatable, e.g. for education. In the Rotterdam Study alone this variable has been categorized in multiple ways. In this thesis, we analyzed education by dividing it in three levels: high, moderate and low. In other studies within the Rotterdam Study, it has been analyzed with four levels: low, low-intermediate, intermediate-high and high.[37] In other studies using Rotterdam Study data education has also been studied quantitatively as number of years of education.[38] But even within a single paper the categorization of a variable may differ. For example in chapter 2.2 of this thesis the association of 24-hour activity rhythms with depression is reported in three ways. First, the outcome 'depressive symptoms' reflects the quantitative value of the weighted total score of the CES-D assessment of depressive symptoms. Second, the variable 'clinically relevant depressive symptoms' reflects the same values of the CES-D, however the variable is now dichotomized with a validated cut-off. Third, the variable 'major depressive disorder' reflects persons who were diagnosed with a clinical major depressive disorder with a semi-structured clinical interview (SCAN). Our group has published papers with again other definitions, or additional definitions of depressive symptoms or depressive disorder.[39,40]

This raises the important question why researchers, and also epidemiologists, commonly evaluate data in categories. Multiple explanations are possible. First, categorizations of exposure make it possible to think in terms of low, medium and high-risk groups. Moreover, the association between exposure and outcome can be described in terms of a relative risk between these groups. Medical research often makes easy clinical

interpretation subordinate to best research practice. Clinicians prefer to have clear cut-offs on which they can base the decision whether the patient should be treated. However, the use of cut-offs are likely to increase the number of false positive findings. Adherence to unequivocal outcomes, common standards and stringent methods is likely to increase the proportion of true findings,[41] however common standards are not yet always available. The use of tertiles, quartiles and quintiles is also a common way of analyzing the data in epidemiologic studies, however this method of dealing with the data has similar issues as categorizations based on other cut-offs. It involves multiple hypothesis testing, it assumes homogeneity of risk within groups leading to both a loss of power and inaccurate estimation, and it leads to difficulty comparing results across studies due to the data-driven cut points used to define categories.[42]

Second, categorizations are often used to deal with non-linear associations. Authors commonly claim that these categorizations facilitate the interpretation of their data or that other models are prone to overfit.[43] However, if categorization is used to deal with non-linearity, the potential of the data is not used to its fullest, which again may cause false results. Several statistics have been developed to assess non-linear associations without the need for categorization. Possibly the most simple version is by introducing a squared term if the association is thought to be parabolic. However, more refined statistical methods to deal with other non-linear associations have also become available, for example the use of splines.[42]

Third, categorization allows for manipulation of the data. With an increasing pressure to publish in research, the number of cases of fraud seems to have risen in scientific research in the last years. In the Netherlands, several cases were exposed in the last years, ranging from reporting non-existing data to not having sufficient informed consent of participants. Results can also be manipulated by testing associations using different categorizations to explore which categorization has the most interesting effects sizes or which categorization gives "significant" results. Categorization can also be used to appear more clinically meaningful as results often seem to be more dramatic when the extremes of a variable are compared. This may increase the likelihood to get published, but can also increase the number of false findings in scientific journals due to multiple hypothesis testing. Therefore, the scientific field has to be extremely careful in the use of categorizations. I would propose that we need standard guidelines for the use of categorizations which should be applied whenever publishing in a scientific journal. These guidelines should include new statistic tests that have been developed to deal with non-linear associations for example, but also stimulate reporting associations of uncategorized data and present results using alternative categorizations in supplemental material.

Recommendations

Clinical implications

This thesis confirms the importance of sleep and rhythmicity in various neuropsychiatric problems. In general, sleep has received increasing attention in clinical practice,[44] general practitioners and the public become increasingly aware of sleep disorders. Although awareness is increasing, knowledge about sleep problems is still not optimal. The prevalence of sleep disordered breathing has risen largely in the last decades,[45] but sleep apnea is still not the first diagnoses which comes to the mind of the general practitioner when a middle-aged, overweight man with complaints of tiredness and concentration problems comes to his practice. A lack of awareness is more prominent for other sleep disorders. Persons with insomnia or insomnia complaints often do not get any other treatment than sleep medication, while alternative treatments such cognitive behavioral therapy can have long lasting effects on insomnia.[46] Restless legs syndrome (RLS), is often unrecognized by the general practitioner and can take a long time to be diagnosed, while asking four simple questions can establish the problem quite accurately.[47] The treatment of rhythm disorders such as jet lag disorder and shift work disorder often remains limited to providing advice about sleep hygiene.[44] It is the responsibility of both the clinician and the researcher to cover the gap between the knowledge gathered in sleep research and the clinical practice.

In this thesis we mainly assessed the associations of disturbances of sleep and rhythms with symptoms of disease in the general population, and not with specific clinical disorders. However, our research does give some important suggestions for the clinical practice. Chapter 2 stresses the importance of rhythms. Rhythm disturbances can shorten our life, and should thus be given attention in the clinical practice, next to sleep. We demonstrated in chapter 3 that disturbed rhythms are associated with more depressive symptoms and anxiety, and less cognitive functions. We do not have any longitudinal studies yet, but we would carefully infer that for numerous diseases, a disturbed rhythm might speed up the disease process or decrease the effectiveness of treatment. This is confirmed by research on the effects of biological rhythms on pharmacology; the timing of pharmacological treatment can improve or decrease effectiveness of medication.[48] Similarly, chronobiological treatment approaches have proven to be successful for depression and anxiety, e.g. by bright light therapy.[49,50] Possibly, chronotherapy might be able to target psychiatric complaints and sleep disturbances simultaneously, as it might underlie sleep disturbances and complaints about depression and anxiety. Last, disturbed rhythms were also found to affect cognition, which extends on the research that has been done previously on disturbed rhythms in demented and institutionalized elderly.[22,51] These results are a strong argument for keeping strict daily rhythms, specifically in institutions. No more pajama

days, sleeping in to eleven and going to bed at seven, behaviors not uncommonly seen in care facilities. Bright light treatment might help stabilizing and defragmenting rhythms[52] and be beneficial to minimize cognitive deterioration.

Future directions for research

This thesis is completely based on cross-sectional data, with the exception of chapter 2.2 which presents the results mortality. Longitudinal data are needed to test whether the associations studied in this thesis hold over time and to assess the temporality of these associations. The first longitudinal studies in which sleep has been assessed at baseline are published, but studies with objective measurements of sleep in large populations remain rather scarce. To our knowledge, no longitudinal studies with repeated objective assessments of sleep are available, and only extending the follow up of the health consequences will not deal with problems such as reversed causality. In the Rotterdam Study, the second round of actigraphy, about 5-7 years after the first round, has been finished. With these data, the Rotterdam Study comprises one of the few repeated actigraphic measurements in a longitudinal cohort. These data will make it possible to assess reversed causality in disturbed rhythms and depression for example, but also for other diseases. However, there is a possibility for the Rotterdam Study to become truly one of a kind in sleep research. If we can repeat the PSG measurement in a few years in our original cohort, a wealth of data with tremendous possibilities for studying the changes in specific sleep parameters in relation to disease would be created.

With these large studies becoming more and more available in sleep medicine, a gap emerged between small sample clinical and laboratory studies and large sample population-based studies. While the results of animal research haven been increasingly integrated with human lab studies in the field of sleep, integration with large population-based studies has been scarce. Laboratory studies in smaller samples of participants of large cohorts, would demonstrate how these findings from the laboratory compare to population-based studies, and translate into the general population. By keeping participants and methodologies equal, inter-disciplinary collaboration can help to detect what is prevalent in the general population and clinically meaningful. This would be particularly interesting in studies of the relation between sleep and cognitive deficits. For example, disastrous effects of sleep deprivation on driving abilities have been found.[53] However, most people would not drive a car completely sleep deprived, they would probably do drive a car partially or chronically sleep deprived. Car accidents have been associated with sleep loss and insomnia retrospectively,[54] but prospectively this is much harder to study with sufficient power, specifically in a naturalistic setting. Sleep studies in large prospective cohorts might open up the possibility of prospective assessment, as it would be able to answer the question whether persons with chronic short sleep or chronic fatigue were involved in more car accidents. A large prospective cohort

would create the possibility to answer these questions with ecological validity and avoid recall bias. If we additionally could bring those persons who had short sleep and car crashes to a sleep laboratory, we would be able to test these persons with driving simulators after partial or complete sleep restriction. This would demonstrate whether participants who had traffic accidents after chronic short sleep were indeed more vulnerable for sleep restriction than persons who had no traffic accidents and chronic short sleep.

Sleep problems, or sleep disorders, suffer from the problem that they are commonly comorbid with other disorders. The distinction between primary insomnia and secondary insomnia of the DSM-IV-TR demonstrated the idea that sleep was often a 'secondary' problem. However, with the publication of the DSM-5, the diagnoses of primary and secondary insomnia have disappeared. These changes underline the importance of treating insomnia, or any sleep disorder, not just as a consequence of any other disorder. In research, this leads to the problem of exclusion, do we exclude persons with comorbid disorders or not? While it was not uncommon in sleep research to study only those persons with the diagnoses of primary insomnia, and exclude those with secondary insomnia, the DSM-5 changes call for a new evaluation of this strategy. Population-based studies especially tend to suffer from a high prevalence of comorbidity, as having multiple diseases or problems, and not just one specific disease, is common in the population. However because of this heterogeneity, population-based studies might also hold the key to how comorbidity in sleep disorders can be addressed best. Longitudinal research in these studies should be able to address the problem of reverse causality for disorders that are comorbid frequently. Is there a bidirectional association, or is A mainly causing B or vice versa. In addition, disorders such as insomnia and depression can manifest themselves differently among persons. Large samples offer the possibility to not only assess these diseases based on the theoretical phenotype, but also on an empirical phenotype designed from the data. Within a large dataset, analyses such as latent class analyzes can be used to discern different manifestations of the disease based on the symptoms of the disease.[55,56] Latent class analysis posits that a heterogeneous group can be reduces to several homogeneous subgroups through evaluating and then minimizing associations among responses across multiple variables. These empirical distinctions raise new possibilities to find underlying biological mechanisms, as these may differ and depend on the empirical phenotype. Empirical based phenotypes might also be beneficial for genetic studies as these tend to suffer from imprecise phenotypes. Genetic studies are important in finding the biological mechanisms which underlie sleep and the 24-hour rhythm, for example by the use of genome wide association studies (GWAS). However, so far results have been limited, which seems to be at least partly due to questionnaire based sleep phenotypes, which tend to be less specific and therefore needs extremely large number to find associations. As first GWAS on sleep duration had just been published, the sample size included over 45,000 persons.[57] In addition, determining empirical

phenotypes for sleep characteristics, genetic sleep studies might also benefit from the increase in polysomnographic data that is available. As an increasing number of cohorts have both polysomnographic data and genotype data, it is a matter of time before the first GWAS with this data becomes available. With this, a new era in genetic sleep research is on the way.

Concluding remarks

I started this thesis in the introduction with the words 'It is the behavior we all do every day and we tend to like it, at least if everything goes well: sleeping. If we make it up to 90 years of age, we spend around 30 years doing it'. This is where my fascination for sleep research once started and this thesis proves that we should keep on sleeping, our health will benefit from it. But our health will benefit probably even more, or at least just as much, from having undisturbed 24-hour rhythms. We might notice it less, it might take in a less prominent place in our lives, but rhythms are key to our healthy live.

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Chapter 6

Summary

Samenvatting

Summary

We spend roughly a third of our life sleeping, but much is still unknown about this behavior (**Chapter 1**). Sleep is thought to be the consequence of two processes which interact, the sleep pressure or sleep propensity and the circadian component. The circadian component reflects a clocklike mechanism that is basically independent of prior sleep and waking and determines the approximately 24-hour rhythm of the sleep-wake pattern. Sleep is measured most accurately with polysomnography. PSG consists of different electrophysiologic measures, most important are the electroencephalography (EEG) channels which allow the assessment of brain activity during sleep. However, polysomnography is not suited to measure the 24-hour organization of rhythms, as it is not feasible to wear the equipment for multiple days. I therefore studied 24-hour rhythms by means of actigraphy, for which persons had to wear an actigraphy for 7 consecutive days and nights. Disturbances in sleep and the 24-hour rhythm can occur as a single problem, but are also often comorbid with other disorders. Specifically neuropsychiatric problems and diseases are often related to sleep and rhythm disturbances. The goal of this thesis is to assess the variation of sleep and the 24-hour activity rhythm in middle-aged and elderly persons of the general population and to study how this variation is related to neuropsychiatric problems.

In **chapter 2** I start with describing correlates of the 24-hour activity rhythm in middle-aged and elderly persons of the Rotterdam Study. **Chapter 2.1** reports on the associations of demographics, lifestyle and sleep parameters on the 24-hour activity rhythm. The results indicate that older age is associated with more stable and more fragmented rhythms. I 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. In addition, less healthy behavior e.g., a higher body mass index and smoking, are also associated with more rhythm disturbances. Lastly, while actigraphic estimated sleep is associated with the 24-hour rhythm, they cannot be used as proxies. Disturbed rhythms can also have detrimental effects on health. In **chapter 2.2**, the effect of disturbed rhythms on mortality is reported. Both a more fragmented rhythm and a less stable rhythm increase the mortality risk, independent of age and other health behaviors. Disturbed 24-hour activity rhythms thus reflect alterations in the biological clock and could be an indicator of disease.

The associations of disturbed rhythms and actigraphic estimates of sleep with neuropsychiatric problems are described in **chapter 3**. First, the associations of 24-hour rhythms and sleep with five cognitive tests are reported in **chapter 3.1**. Cognitive functioning changes not only with age, but also due to alterations in sleep and rhythms. Our results demonstrate that disturbances in sleep are mainly associated with memory-related tasks,

while disturbances in the rhythm relate to worse performance on tasks that tap highly on executive functioning and perceptual speed. **Chapter 3.2** reports on the relation of disturbed rhythms and sleep with two common psychiatric disorders, depression and anxiety. Depression, and in a lesser extent anxiety, is closely related to sleep and bidirectional associations have been suggested. The relation with 24-hour rhythms are much less explored. Our results show that fragmented rhythms are related with both depression and anxiety, while actigraphic sleep estimates are not related to depression and anxiety in our sample. Perceived sleep quality is also associated with anxiety and depression. It thus seems that, instead of sleep per se, disturbed rhythms and perceived sleep quality are related to depression and anxiety. In **Chapter 3.3** a similar conclusion is reported for the negative feedback of the hypothalamic-pituitary-adrenal (HPA) axis. The results demonstrate that, the stability of the rhythm, sleep duration and a poor perceived sleep quality are related to enhanced negative feedback of the HPA axis, which was tested by measuring cortisol levels after the intake of a very low-dose of dexamethasone (0.25 mg). Only the stability of the rhythm was also related to cortisol levels before dexamethasone intake.

In **chapter 4** the first results of a polysomnography (PSG) sleep study in the Rotterdam Study are reported. Alterations in rapid eye movement (REM)-sleep have been consistently related to depression in clinical studies, but evidence from population-based studies has been limited. The results reported in **Chapter 4.1** suggest that REM-density is a marker of depressive symptoms in the general population and that associations of REM-sleep are modified by the use of medication. In **chapter 4.2** I assess the interrelation of sleep apnea, depressive symptoms and fatigue. Our results suggest that sleep apnea and depressive symptoms are not related, although both relate to fatigue. Severe fatigue also obscures the association of sleep apnea and depressive symptoms. The interrelation between sleep apnea, depressive symptoms and fatigue should be carefully assessed when diagnosing and treating sleep apnea.

Chapter 5 reviews the main findings of this thesis, it discusses methodological topics, describes clinical implications and does suggestions for further research.

Samenvatting

We brengen ongeveer een derde deel van ons leven slapend door, maar nog altijd is er veel onbekend over slaap (**Hoofdstuk 1**). Slaap is waarschijnlijk het gevolg van de interactie van twee processen, de slaapbehoefte en het circadiaans ritme. Het circadiaans ritme is het bij benadering 24-uur durende dag-nacht ritme wat ons lichaam volgt. Zowel de slaapbehoefte als het circadiaans ritme zijn essentieel voor ons slaap-waak ritme. Slaap wordt meestal gemeten met polysomnografie. PSG bestaat uit een veelvoud van electrofysiologsche metingen waarmee we onder andere de hersenactiviteit meten. De hersenactiviteit verandert niet alleen tussen slapen en waken, maar ook tijdens onze slaap. PSG is een zeer uitgebreid onderzoek, waardoor het niet geschikt is voor langere perioden. We gebruiken daarom niet PSG om het 24-uurs ritme te meten, maar actigrafie. Bij een actigrafie-onderzoek dragen deelnemers ongeveer een week lang een soort horloge. Dit horloge meet beweging, waardoor de 24-uurs organisatie van het bewegingsritme kan worden vastgesteld. Problemen in slaap en het 24-uurs ritme komen zowel solitair als in combinatie met andere ziekten en problemen voor. Vooral neuropsychiatrische problemen zijn vaak gerelateerd aan verstoringen in de slaap en het 24-uurs ritme. Het doel van dit onderzoek is het beschrijven van de variatie in 24-uurs ritmes en slaap in volwassenen en ouderen in de algemene populatie, en hoe deze variatie samenhangt met neuropsychiatrische problemen en stoornissen.

In **hoofdstuk 2** beschrijf ik factoren die samenhangen met het 24-uurs bewegingsritme in de deelnemers van de Rotterdam Studie. **Hoofdstuk 2.1** rapporteert hoe demografische kenmerken, leefstijl en slaap geassocieerd zijn met het 24-uurs ritme. Een oudere leeftijd is niet alleen geassocieerd met een meer stabiel, maar ook met een meer gefragmenteerd ritme. Ouderen lijken dus meer rigide te zijn in hun gedrag, terwijl het vermogen om langer in een actieve of non-actieve staat te blijven beperkt is. Een minder gezonde leefstijl, bijvoorbeeld door een hogere BMI of door roken, is ook gerelateerd aan meer verstoringen in het 24-uurs ritme. Slaap kenmerken, wanneer bepaald met actigrafie, zijn gerelateerd aan het 24-uurs ritme, maar deze constructen zijn geen proxy voor elkaar, ze hebben dus elk een unieke waarde in wetenschappelijk onderzoek. Problemen in 24-uurs ritmes kunnen de gezondheid ernstig verstoren. In **hoofdstuk 2.2** worden de effecten van verstoorde 24-uurs ritmes op mortaliteit gerapporteerd. Zowel meer gefragmenteerde als onstabiele ritmes verhogen de kans op vervroegde mortaliteit, onafhankelijk van leeftijd of leefstijl. Waarschijnlijk reflecteren verstoringen in het 24-uurs ritme veranderingen in de biologische klok, die mogelijk een indicatie voor een slechte gezondheid zijn.

De samenhang van het 24-uurs ritme en slaap met neuropsychiatrische problemen is beschreven in **hoofdstuk 3**. Als eerste rapporteer ik in **hoofdstuk 3.1** de associaties van het 24-uurs ritme en slaap met cognitie. Cognitie verandert niet alleen met de leeftijd, maar kan

ook verslechteren door verstoringen in slaap en het 24-uurs ritme. De resultaten laten zien dat slaap voornamelijk gerelateerd is aan geheugentaken, terwijl het 24-uurs ritme vooral gerelateerd is aan taken die de executieve functies en snelheid testen. In **hoofdstuk 3.2** worden de associaties van het 24-uurs ritme en slaap met de twee veelvoorkomende psychiatrische stoornissen, depressie en angst, beschreven. Depressie, en in mindere mate angst, zijn nauw verweven met slaap, en lijken samen een vicieuze cirkel te vormen. De relatie van het 24-uurs ritme met depressie en angst is minder veelvuldig onderzocht. In onze studie is de fragmentatie van het 24-uurs ritme gerelateerd aan depressie en angst, terwijl slaap, wederom bepaald met behulp van actigrafie, niet gerelateerd is aan depressie en angst. Hoe mensen hun slaap ervaren, de zogenaamde subjectieve slaapkwaliteit, is wel gerelateerd aan depressie en angst. Het lijkt dus zo te zijn dat niet slaap, maar het 24-uurs ritme en de ervaring van slaap belangrijk zijn voor depressie en angst. Een vergelijkbaar patroon wordt gezien in **hoofdstuk 3.3**. In dit hoofdstuk heb ik de samenhang tussen het 24-uurs ritme en slaap met het functioneren van de hypothalamus-hypofyse-bijnier as getest. Het functioneren van de hypothalamus-hypofyse-bijnier as is onderzocht door het cortisol niveau in het speeksel te bepalen, zowel voor als na de inname van een zeer lage dosis dexamethason (0.25 mg). De resultaten lieten zien dat een verstoord 24-uurs ritme, een korte slaapduur en een slechte slaapkwaliteit gerelateerd waren aan een versterkte negatieve terugkoppeling in de hypothalamus-hypofyse-bijnier as, maar dat alleen een verstoord 24-uurs ritme gerelateerd was aan cortisol voor en na de inname van dexamethason

In **hoofdstuk 4** beschrijf ik de eerste resultaten van het PSG onderzoek in de Rotterdam Studie. Eerder onderzoek heeft laten zien dat veranderingen in REM-slaap gerelateerd zijn aan depressie in de klinische populatie, maar er is weinig bewijs voor deze associaties in de algemene populatie. In **hoofdstuk 4.1** laat ik zien dat REM-dichtheid, de dichtheid waarmee snelle oogbewegingen plaatsvinden in de REM-slaap, een marker is voor het hebben van depressieve symptomen in de algemene bevolking. Deze associaties worden beïnvloed door het gebruik van medicatie. In **hoofdstuk 4.2** wordt de relatie tussen slaap apneu, depressie en vermoeidheid beschreven. De resultaten laten zien dat slaap apneu en depressie niet gerelateerd zijn in onze studie, maar dat beiden wel gerelateerd zijn aan vermoeidheid. Ernstige vermoeidheid beïnvloedt ook de relatie tussen slaap apneu en depressie. Dit benadrukt het belang van het beoordelen van depressie bij slaap apneu.

In **hoofdstuk 5** worden de algemene bevindingen van dit onderzoek beschreven, wordt de methodologie bediscussieerd en worden suggesties gedaan voor de klinische praktijk en het wetenschappelijk onderzoek.

Chapter 7

About the author

PhD portfolio

List of publications

Acknowledgements

About the author

Annemarie Luik was born on March 30, 1984 in Apeldoorn, the Netherlands. After finishing her pre-university education at the Rodenborch College in Rosmalen in 2003, she started to study Psychology at the Radboud University in Nijmegen. In 2004, she additionally started taking classes in Religion Studies. In 2006, she finished her Bachelor of Arts in Religion Studies, and in 2007 she finished her Bachelor of Science in Psychology (specialization Neuropsychology). After a clinical internship at the Jeroen Bosch Hospital, 's-Hertogenbosch and a research internship at the Sleep and Performance Research Center of the Washington State University, Spokane (USA) under the supervision of Prof. Dr. Hans van Dongen, she obtained her Master of Science in Psychology (cum laude). After earning her degree, she started working clinically at the St Anna Hospital in Geldrop. In 2009, she earned her Masters' degree in Religion Studies (specialization Interreligious Management) and continued to work as a junior psychologist at Kempenhaeghe, a center of expertise for Epileptology, Sleep Medicine and Neurocognition.

In October 2010 she started the work presented in this thesis at the department of Epidemiology under the supervision of Prof. Dr. Henning Tiemeier and Prof. Dr. Eus van Someren (Netherlands Institute for Neuroscience of the Netherlands Royal Academy of Arts and Sciences, Amsterdam and the VU University and Medical Center, Amsterdam). As part of this PhD-project she obtained a Master of Science in Health Sciences (Clinical Epidemiology) from the Netherland Institute for Health Sciences (NIHES) in August 2012. In 2014 she got awarded with a Sleep Research Society Merit-Based Award for her contributions to the SLEEP 2014 conference, Minneapolis (USA).

From November 2014 she started working as a research associate for the Arthritis Research UK Centre for Epidemiology of the Institute of Inflammation and Repair at the University of Manchester (UK). Her work at the institute focuses on circadian rhythms, sleep and inflammatory diseases.

PhD portfolio

	Year	Workload (ECTS)
General courses		
- Biomedical writing	2012	4.0
Specific courses (e.g. Research school, Medical Training)		
Nihes Master of Science - Clinical Epidemiology		
- Clinical Epidemiology	2010	5.7
- Courses for the Quantitative Researcher	2010	1.4
- Medical Demography	2011	1.1
- Psychiatric Epidemiology	2011	1.1
- Repeated Measurements in Clinical Epidemiology	2011	1.4
- Principles of Research in Medicine	2011	0.7
- Clinical Decision Analysis	2011	0.7
- Methods of Public Health Research	2011	0.7
- Pharmaco-Epidemiology	2011	0.7
- Markers and Prognostic Research	2011	0.7
- The Practice of Epidemiologic Analysis	2011	0.7
- Missing Values in Clinical Research	2011	0.7
- History of Epidemiologic Ideas	2011	0.7
- Case-control Studies	2011	0.7
- Study Design	2011	4.3
- Biostatistical Methods I: Basic Principles	2011	5.7
- Methodologic Topics in Epidemiologic Research	2012	1.4
- Biostatistical Methods II: Popular Regression Models	2012	4.3
- Genome Wide Association Analyses	2012	1.4
Seminars and workshops		
- Wetenschapsknooppunt 'Het familiealbum'	2011	0.2
- Seminars Department of Epidemiology	2010-2014	4.0
- Oxford SCNi Circadian Rhythm and Sleep Summer School	2014	1.4

Presentations

	Year	Workload
- Poster presentation: *'Objectively assessed activity rhythms and sleep in a large population-based cohort of the elderly'* at SLEEP 2012, Boston	2012	1.0
- Oral presentation: *'Depression and anxiety disorders in relation to sleep-wake patterns in the elderly'* at CTR, November 2012, Amsterdam	2012	1.0
- Oral presentation: *'The association of 24-hour activity rhythms and sleep with cognitive functioning: A population-based study'* at SLEEP 2014, Minneapolis	2014	1.0
- Poster presentation: *'The association of depressive symptoms with polysomnographic assessed sleep and self-rated sleep: A population-based study'* at SLEEP 2014, Minneapolis	2014	1.0
- Poster presentation: *'24-Hour activity rhythms and sleep in population-based study of middle-aged and elderly persons'* at SCNi Sleep and Circadian Neuroscience Summerschool, Oxford	2014	1.0

(Inter)national conferences

	Year	Workload
- Najaarssymposium NSWO, Nieuwegein	2011	0.3
- Najaarssymposium NSWO, Sint Michielsgestel	2012	0.3
- SLEEP 2012, Boston	2012	1.2
- CTR meeting November 2012, Amsterdam	2013	0.3
- Najaarss Symposium NSWO, Eindhoven	2014	0.3
- SLEEP 2014, Minneapolis	2014	1.2

Teaching	**Year**	**Workload (ECTS)**
Supervising practicals and excursions, Tutoring		
- Tutor Principles of Research in Medicine	2013	1.0
Supervising Master's theses		
- Tutor Master thesis 'Clinical Neuropsychology'	2014	1.0
Other		
- Lectures Wetenschapsknooppunt (primary schools)	2012-2014	1.0

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'There is a time for many words, and there is also a time for sleep (Homer, The Odyssey).