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Sleep and 24-h activity rhythms in relation to cortisol change after a very low-dose of dexamethasone

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Summary The hypothalamic-pituitary-adrenal (HPA) axis plays an important role in sleep. Nevertheless, the association of sleep and its 24-h 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;

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0.206) after multivariate adjustment. These results indicated that the 24-h organization, duration and experience of sleep are all associated with the negative feedback control of the HPA axis.

1. Introduction

The hypothalamic-pituitary-adrenal (HPA) axis determines the stress response of humans as it regulates the release of cortisol by a negative feedback control (Dedovic et al., 2009). 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 (Buijs et al., 2003).

The HPA axis plays an important role in the regulation of sleep (Buckley and Schatzberg, 2005). However, research on the association of sleep parameters with cortisol secretion is not consistent (Elder et al., 2014). 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 (Zhang et al., 2011), and that cortisol levels in urine were not associated with objective sleep duration (Rao et al., 2013). In contrast, others have observed that self-reported sleep duration and disturbances were associated with the diurnal slope in cortisol secretion in the population (Kumari et al., 2009b). 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 (Minkel et al., 2014). 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 (Vgontzas et al., 2001). However, self-rated sleep was not related to cortisol levels after dexamethasone intake in a combined dexamethasone/corticotrophin-releasing-hormone (CRH) test (Hori et al., 2011), nor were sleep disorders (Lattova et al., 2011). Research has been complicated by the use of objective versus subjective measures of sleep in different studies (Rao et al., 2013). 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 (Buckley and Schatzberg, 2005). However, not much is known about the 24-h 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 (Lindholm, 2014), but it has also been proposed as a biomarker for psychiatric diseases (Arana et al., 1985). 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 (Ehlert et al., 2001). 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 (Huizenga et al., 1998b). 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 (Huizenga et al., 1998b). We specifically tested the level of cortisol after a very low-dose of dexamethasone controlled for baseline cortisol.

We explored whether the 24-h 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-h 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 (Pesonen et al., 2014). Lastly, we expected subjective sleep quality to be associated with the feedback of the HPA axis.

2. Methods

2.1. 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 (Hofman et al., 2013). 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.

2.2. Assessment of the very low-dose dexamethasone suppression test

For the very low DST participants were asked to collect a saliva sample at 0800 h at day 1, take a very low dose of dexamethasone (0.25 mg, PO) at 2300 h at day 1, and collect saliva again at 0800 h 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 min before the collection of the samples and to report the exact date and time of the sampling. Samples were stored at −80 °C 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).

2.3. Assessment of the 24-h activity rhythm and sleep

Actigraphy allowed us to measure the 24-h activity rhythm (Tobler and Borbely, 1993; De Souza et al., 2003) and to estimate sleep parameters objectively (Kushida et al., 2001). 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-s epochs (Kushida et al., 2001). Recordings had to consist of at least 96 h. All 24-h 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 h (standard deviation (SD): 14 h).

Activity rhythms were quantified using non-parametric indicators (Van Someren et al., 1996; Luik et al., 2013) to describe the rhythm without making strong assumptions about the shape of the rhythm (Van Someren, 2011). Two variables were calculated to assess the 24-h 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 (Ancoli-Israel et al., 2003). We used a validated algorithm (Kushida et al., 2001) to calculate total sleep time, sleep onset latency and wake after sleep onset using the actigraphy (van den Berg et al., 2008; Luik et al., 2013).

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).

2.4. 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 (Vreeburg et al., 2009; Luik et al., 2013). 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 (Fries et al., 1980). Cognitive status was measured with the Mini Mental State Exam (MMSE) (Folstein et al., 1975). We used the Center for Epidemiologic Studies-Depression (CES-D) scale to measure depressive symptoms (Radloff, 1977; Beekman et al., 1997). 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 (Buysse et al., 1989). 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 (Fogelholm et al., 2007). 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 18:00 h 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.

2.5. Statistical analyses

We assessed the associations of the 24-h 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-h 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-h rhythms and sleep with cortisol. We additionally analyzed the association of the 24-h 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-h activity rhythm and sleep indicators were winsorized at 4 standard deviations to the mean. We used a Box–Cox transformation (Box and Cox, 1964; Osborne, 2010) to obtain normally distributed values for interdaily stability ($\lambda = 7.0$), intradaily variability ($\lambda = 3.9$), actigraphic 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-h 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 (Schafer, 1999).

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-h activity rhythm	
Interdaily stability, score	0.80 ± 0.10
Intradaily variability, score	0.39 ± 0.11
Duration of actigraphy, h	137.72 ± 15.04
Actigraphic sleep	
Total sleep time, h	6.31 ± 0.88
Sleep onset latency, min	6.37 ± 2.44
Wake after sleep onset, min	68.67 ± 23.93
Self-rated sleep	
Total sleep time, h	6.77 ± 0.95
Sleep onset latency, min	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.

3. 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 14.7 mmol/L (SD: 8.65) in the morning before the intake of dexamethasone and 6.1 mmol/L (SD: 7.13) in the

Table 2 Correlations of cortisol in saliva, 24-h 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.

morning after the intake of 0.25 mg dexamethasone. Participants slept on average 6.3 h (SD: 0.88) according to the actigraphy, self-rated total sleep time was 6.8 h (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 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-h 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-h 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-h 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 ($B = -0.073$, 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).

Next, we studied whether sex and the intake of sleep medication, psycholeptics or psychoanaleptics modified the associations of the 24-h rhythm and sleep with cortisol after dexamethasone intake. Significant interaction effects of sex

Table 3 Associations of the 24-h 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-h 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**

Bold indicates $p < 0.05$. CI: confidence interval. 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.

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-h activity rhythm and sleep parameters with saliva cortisol before dexamethasone intake and after dexamethasone intake not adjusting for baseline cortisol (see Appendix, tables A1 and A2). 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 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.

4. Discussion

Our study demonstrated that a lower stability of the 24-h 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 (Hofman and Swaab, 2006), which can lead to unstable and fragmented rhythms and possibly disturbances in the cortisol rhythm (Clow et al., 2010). 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 (Buckley and Schatzberg, 2005). 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 (Arana et al., 1985). However, while depression has been related with more disturbed circadian rhythms (Germain and Kupfer, 2008), 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 (Ehlert et al., 2001) and job-related exhaustion (Menke et al., 2014). 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 (Kudielka and Wust, 2010). 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 (Buckley and Schatzberg, 2005). Thus, a less adaptive HPA axis could also lead to more unstable rhythms. Stability of the 24-h 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 (Vgontzas et al., 2001), or after experimental manipulation of sleep, i.e. after sleep deprivation (Minkel et al., 2014). Population-based studies have not found associations between objectively assessed sleep and cortisol (Zhang et al., 2011; Rao et al., 2013), 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 (Spath-Schwalbe et al., 1991), 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 (Kudielka and Wust, 2010), after prenatal stress (Painter et al., 2012) and in stress disorders (de Kloet et al., 2006). 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 (Steiger et al., 1989). In a previous population-based study, low waking salivary cortisol and a flatter slope in cortisol secretion were associated with fatigue (Kumari et al., 2009a). 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 (Ehlert et al., 2001) and job-related exhaustion (Menke et al., 2014).

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 (Kudielka and Kirschbaum, 2005). 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 0.25 mg 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-h 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 (Hunt et al., 1989; Lister and Sigalas, 2011). 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 (Ancoli-Israel et al., 2003). 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 (Remillard et al., 1993; Vreeburg et al., 2009). 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 (Buckley and Schatzberg, 2005). 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 (Reynolds et al., 1998). The susceptibility for this low dose of dexamethasone has also been suggested to be dependent on a distinct GR polymorphism (Huizenga et al., 1998a). Next, the timing of cortisol sampling was set at 08:00 h 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-h 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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Conflict of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2015.01.011.

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