Polysomnography-estimated sleep and the negative feedback loop of the hypothalamic-pituitary-adrenal (HPA) axis

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ABSTRACT

Background: Sleep and stress are highly interrelated. To improve our understanding of the role of sleep in functioning of the negative feedback loop of the stress system, we assessed the association between sleep and functioning of the hypothalamic-pituitary-adrenal (HPA) axis in a population-based sample.
Methods: This study included 403 participants (mean age: 62.4 ± 5.0 years, 55% women) of the population-based Rotterdam Study. Between 2012 and 2014, sleep was assessed with polysomnography. Functioning of the negative feedback loop of the HPA axis was estimated by measuring cortisol levels before and after the intake of a very low dose of dexamethasone (0.25 mg) on average 0.9 ± 37.8 days after the polysomnography. We used linear regression analyses adjusted for multiple confounders and performed sensitivity analyses in a sample excluding those with clinically relevant depressive symptoms and using psychoactive medicine, and a sample excluding non-suppressors.
Results: Short N2 sleep (adjusted difference = 0.005, 95%CI = 0.002;0.009), long N3 sleep (adjusted difference = −0.007, 95%CI = −0.010;−0.003), and short sleep onset latency (adjusted difference = 0.006, 95%CI = 0.001;0.011) were associated with an enhanced response to dexamethasone, but the association of sleep onset latency did not survive multiple testing correction. Associations remained similar after excluding those with clinically relevant depressive symptoms and those using psychoactive medicine or exclusion of non-suppressors.
Conclusions: This study suggests that more slow wave sleep is particularly associated with a stronger suppression of cortisol within the negative feedback loop of the HPA axis. These findings provide further support that slow wave sleep is important for health.

1. Introduction

The hypothalamic-pituitary-adrenal (HPA) axis is part of the stress system and helps us to respond to all sorts of commonly occurring stressors for example by the secretion of cortisol (Dedovic et al., 2009). Rising cortisol levels induce physiological changes that are required for the body to respond to a stressor, but at the same time a negative feedback loop inhibits further secretion of cortisol by the HPA axis (Dedovic et al., 2009). Correct and well-balanced functioning of this negative feedback loop is required for a healthy response to stress, as both a diminished negative feedback and an enhanced negative

feedback of cortisol have been associated with negative health outcomes (Zorn et al., 2017; Kumari et al., 2010). Previous literature reported a diminished negative feedback to be associated with major depressive disorder (Zorn et al., 2017) and an enhanced negative feedback has been associated with cardiovascular disease and schizophrenia (Zorn et al., 2017; Arana et al., 1985; O'Connor et al., 2021).

Sleep is long thought to be associated with functioning of the stress system (Zorn et al., 2017; Bao et al., 2017; Kalmbach et al., 2018). Poor sleep has been reported to induce dysregulation of the stress system (van Dalfsen and Markus, 2018). Studies have for example shown that actigraphy-estimated habitual sleep is related to the negative feedback

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loop and that patients with insomnia also show altered functioning of the HPA axis (Riemann et al., 2010; Luik et al., 2015). Vice versa, acute stressors and long-term stress are also thought to cause poor sleep (Kalmbach et al., 2018; van Dalfsen and Markus, 2018; Hall et al., 2015). However, the evidence is typically mixed, in part because specific aspects of sleep have been associated differently with functioning of the HPA axis. For example, sleep deprivation and insomnia have been associated with hyperactivity of the HPA axis (Buckley and Schatzberg, 2005), but excessive daytime sleepiness has been associated with a diminished response of the HPA axis (van Dalfsen and Markus, 2018). Although these findings might in part be explained by differences in methodology, they could also indicate that aspects of sleep relate differently to the HPA axis (van Dalfsen and Markus, 2018).

The gold standard for the assessment of sleep is polysomnography (PSG) (Rundo and Downey Iii, 2019; Iber, 2007). PSG enables a thorough assessment of multiple aspects of sleep (American Academy of Sleep, 2007) and has been successfully applied in population-based cohorts. So far, research investigating the association between PSG-measured sleep and functioning of the HPA axis was however mainly done in experimental settings, with only one exception to our knowledge (van Dalfsen and Markus, 2018). Yet, observational studies are required to assess the association of sleep and the stress system in a habitual setting, which may be more representative for daily life.

The functioning of negative feedback loop of the HPA axis has been successfully studied in larger samples with a very low dose dexamethasone suppression test (Direk et al., 2016). In this test, where dexamethasone acts as a pharmacological stressor, cortisol levels are compared before and after the intake of a very low dose of dexamethasone (0.25 mg). Dexamethasone suppresses the secretion of cortisol via Adrenocorticotropic Hormone (ACTH) (Direk et al., 2016), a decrease in cortisol level is therefore expected after the intake of dexamethasone. The difference between the cortisol level before and after dexamethasone is therefore an indicator of the functioning of the negative feedback loop of the HPA axis. If the decrease in cortisol is larger, the suppression of cortisol is stronger, indicating an enhanced negative feedback of the HPA axis. If the decrease in cortisol is smaller, the suppression is less, indicating a diminished negative feedback loop.

In the current study, embedded in the population-based Rotterdam Study, we aimed to estimate the cross-sectional association between PSG-measured sleep and the functioning of the negative feedback loop of the HPA axis in a population-based sample of middle-aged and older adults. In line with previously observed hyperactivity of the HPA axis in patients with insomnia (Hall et al., 2015), we hypothesized that indicators of poor PSG-measured sleep are cross-sectionally associated with an enhanced response of the negative feedback loop of the HPA axis, indicated by a stronger suppression of cortisol after dexamethasone intake.

2. Methods

2.1. Participants and design

We included participants from the population-based Rotterdam Study cohort of middle-aged and elderly inhabitants of Rotterdam, the Netherlands. The cohort was set up in 1990 with the main aim to examine neurological, cardiovascular, psychiatric, and other chronic non-communicable diseases. Details of the study design have been described by Ikram and colleagues (Ikram et al., 2020).

Between 2012 and 2014, a random subsample of 1728 participants was invited for a 1-night ambulant PSG at a regular research center visit, 929 agreed. After excluding participants with unusable recordings because of device malfunctioning (n = 17), insufficient data (n = 8), or recording failure (n = 5), valid PSG data was obtained for 899 participants. No other exclusion criteria were used. Of the 899 participants with a valid PSG, 828 participants were invited to participate in a very low dose dexamethasone suppression test and 656 agreed during the

same research center visit. After exclusion of those with incomplete data or insufficient saliva volumes (n = 58), those with a time difference between samples deviating more than 3 h from the specified 24 h (n = 10), and those who were prescribed corticosteroids (Anatomical Therapeutic Chemical Classification code H02: oral, inhalation or dermal) in the year before the dexamethasone suppression test or who self-reported use of corticosteroids at the time of data collection (n = 185), valid cortisol data was available for 403 participants.

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The Rotterdam Study has been entered into the Netherlands National Trial Register (NTR; www.trialregister.nl) and into the WHO International Clinical Trials Registry Platform (ICTRP; www.who.int/ictrp/network/primary/en/) under shared catalogue number NTR6831. All participants provided written informed consent to participate in the study and to have their information obtained from treating physicians.

2.2. Assessment of sleep

Sleep was measured with a 1-night ambulant PSG at the participants' home using six electroencephalography (EEG) derivations (F3/A2, F4/A1, C4/A2, C4/A1, O1/A2, O2/A1). Eye movement was monitored using a bilateral electrooculography (EOG) and heart rate was monitored using electrocardiography (ECG). Lastly, oximetry was used to measure the oxygen saturation in the blood and respiratory belts on the abdomen and chest, a nasal pressure sensor and an oronasal thermal sensor were used to detect the airflow and respiratory effort in the thorax and abdomen. Set-up followed the standard American Academy of Sleep Medicine guidelines (American Academy of Sleep, 2007). Participants were instructed to spend the night as normal as possible and without restrictions on bedtimes, use of alcohol, coffee or medication. Additionally, participants were asked to press a button to mark the time of when they intended to go to sleep and when they got out of bed.

A registered PSG technologist analyzed the recordings to determine sleep following the American Academy of Sleep Medicine guidelines (American Academy of Sleep, 2007). Total sleep time indicated the total time scored as sleep during the night. Sleep efficiency indicated the proportion of time in bed spent sleeping (100% x (total sleep time/ time in bed), where time in bed was defined as the time between time to bed and get-up time in hours). Sleep onset latency indicated the time between time to bed and sleep start. Wake after sleep onset indicated the total time scored as wake between sleep start and sleep. Additionally, we determined the duration for each of the sleep stages, rapid eye movement (REM) sleep and three stages of non-rapid eye movement (NREM) sleep (N1, N2, N3).

Spectral power and spindles in the C3/A2 derivation were calculated with PRANA software (PhiTools, Strasbourg, France) (Zoubek et al., 2007). We performed spectral analysis using 4–s epochs with 50% overlap, averaged over 30–s epochs. To calculate the spectral power, band-pass filtering (0.125–128 Hz) and automated removal of artefacts were applied. Absolute spectral power was calculated in the delta (0.75–4.50 Hz), theta (4.50–8.50 Hz), alpha (8.50–15.50 Hz), beta (15.50–22.50 Hz) and gamma (22.50–40.00 Hz) frequency bands.

2.3. Assessment of the negative feedback loop of the HPA axis

We estimated the functioning of the negative feedback loop of the HPA axis with a very low dose dexamethasone suppression test (Direk et al., 2016). In this test, we compared cortisol levels before and after intake of a very low dose of dexamethasone (0.25 mg). Dexamethasone activates the negative feedback loop of the HPA axis, and therefore suppresses cortisol release. A stronger suppression of cortisol by the negative feedback loop (i.e. lower levels of cortisol after intake of dexamethasone) indicates an enhanced response of the negative

feedback loop of the HPA axis. Vice versa, a weaker suppression of cortisol by the negative feedback loop (i.e. higher levels of cortisol after intake of dexamethasone) indicates a diminished response of the negative feedback loop of the HPA axis. Participants received oral and written instructions on how to collect saliva samples using Salivette sampling devices (Sarstedt, Nümbrecht, Germany), emphasizing the importance of specific timing of sampling. On day 1, participants were instructed to collect saliva at 8 a.m. and take a very low dose of dexamethasone (orally, 0.25 mg) at 11 p.m. On day 2, participants were instructed to collect saliva again at 8 a.m. Additionally, participants were asked to write down the exact time of dexamethasone intake and saliva sampling. The salivettes were stored at − 80 °C, until samples were analyzed at the laboratory of Biopsychology, Technical University of Dresden, Germany. Cortisol concentrations in saliva were measured in nmol/l using liquid chromatography-mass spectrometry (LCMS; IBL Hamburg, Hamburg, Germany), which is the current golden-standard (El-Farhan et al., 2017). Intra-assay (9%) and inter-assay (14%) co-efficients of variation indicated a valid measurement (Calvi et al., 2017). The lower limit of detection was 0.1 nmol/liter. None of our participants had a saliva sample with cortisol levels below the detection limit.

2.4. Other variables

Age, sex, and educational level were assessed upon inclusion of participants in the Rotterdam Study. Educational level was based on highest education achieved, classified as primary education (primary), lower/intermediate general education or lower vocational education (low), intermediate vocational education or higher general education (middle), or higher vocational education or university (high). Employment, alcohol intake, smoking behavior, depressive symptoms, use of psychoactive medicine, diabetes mellitus, and average wake-up time were assessed during the home interview. Employment was classified as a binary variable, based on whether the participant had paid work. Alcohol intake was assessed by self-reported intake of alcoholic beverages in units and recalculated to average grams of alcohol per day. Smoking behavior was classified as never, former, or current smoker. Depressive symptoms were assessed using the Center for Epidemiologic Studies Depression scale (CES-D) (Beekman et al., 1994). Anxiety symptoms were assessed using the Hospital Anxiety and Depression Scale (Zigmond and Snaith, 1983). Use of psychoactive medicine (Anatomical Therapeutic Chemical Classification code: N05 Psycholeptics or N06 Psychoanaleptics) was based on self-report. Diabetes mellitus was based on medical record data. Average wake-up time was assessed using the question "At what time do you usually wake up?" from the Pittsburgh Sleep Quality Index. Body mass index (kg/m^2) was calculated after measuring height and weight on calibrated scales at the research center without heavy clothing and shoes. As an indicator of sleep apnea we used the apnea—hypopnea index (AHI), which was calculated as the number of apneas and hypopneas per hour of sleep (American Academy of Sleep, 2007). Apneas were defined as an airflow reduction of $\geq$ 90% of baseline for $\geq$ 10 s and a hypopnea was defined as an airflow reduction of $\geq$ 30% of baseline for $\geq$ 10 s and a desaturation of $\geq$ 3% from baseline or an arousal (Iber, 2007).

2.5. Statistical analyses

Descriptive statistics were presented as number with percentage for categorical variables and mean with standard deviation (SD) for numerical data. Differences in level of cortisol before and after intake of dexamethasone were assessed with a paired *t*-test. Cortisol levels before and after dexamethasone were log-transformed, as they were not normally distributed. Missing values for all covariates were less than 10% and handled using multiple imputation (MICE R package (Buuren and Groothuis-Oudshoorn, 2010) with 5 imputed datasets); pooled statistics are presented (Rubin, 2004). To correct for multiple testing, we used false discovery rate (FDR) to calculate adjusted p-values based on 8

determinants (Benjamini and Hochberg, 1995). R version R 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria, www.R-project. org) was used for all analyses.

We used cross-sectional linear regression models to estimate the association of sleep with (1) cortisol before dexamethasone and (2) response to dexamethasone corrected for cortisol before dexamethasone. All associations were studied in three models to improve our insight in potentially confounding factors. A sex-age adjusted model (model 1), a model additionally adjusted for education, employment, alcohol intake, smoking behavior, depressive symptoms score, anxiety symptoms score use of psychoactive medicine, wake-up time, and body mass index, (model 2), and a model additionally adjusted for prevalence of diabetes mellitus and AHI (model 3). For sleep stage durations (N1, N2, N3, and REM), all models were additionally adjusted for total sleep time. As exploratory analyses, we additionally estimated the association between absolute spectral power and response to dexamethasone.

We performed four sensitivity analyses. First, short total sleep time can have a substantial impact on sleep estimates, specifically the duration of different sleep stages. We therefore reran our analyses excluding participants with a total sleep time below 5 h, as this excluded the 10% with the shortest sleep duration. Second, to assess whether the associations found were explained by those not responding to dexamethasone (non-suppressors), we reran analyses excluding participants for whom cortisol levels did not drop after their dose of dexamethasone. Third, to ensure that the observed associations were not only driven by those with clinically relevant depressive symptoms, we repeated our analyses after exclusion of participants indicating clinically relevant depressive symptoms at baseline (CES-D $\geq$ 16) and those indicating use of psychoactive medicine. Last, because functioning of the HPA axis has been reported to differ for men and women (Goel et al., 2011), we reran our analyses stratified for sex.

3. Results

A total of 403 participants were included with a mean age of 62.4 $\pm$ 5.0 years and 55% women (Table 1). Cortisol levels after dexamethasone intake (mean 4.0 $\pm$ 10.8) were significantly lower than cortisol levels before dexamethasone intake (mean 11.5 $\pm$ 8.6, t = 13.76, p < 0.001). Those who refused to participate in the PSG study were slightly older (t = 2.12, p = 0.035), more often women (χ^2 = 5.62, p = 0.018), and lower educated (χ^2 = 11.52, p = 0.009) than those who participated. Participants who refused to participate in the dexamethasone suppression test study were more often women (χ^2 = 38.66, p < 0.001) than those who participated.

3.1. Sleep and the cortisol response to dexamethasone

No PSG-measured indicators of sleep were associated with cortisol before the intake of dexamethasone after adjustment for confounders (Supplementary Table 1). Short N2 sleep (adjusted mean difference = 0.005, 95%CI = 0.001;0.009) and long N3 sleep (adjusted mean difference = −0.006, 95%CI = −0.010;−0.003) were associated with an enhanced response to dexamethasone after confounder adjustment (Model 2, Table 3). further adjustment for diabetes mellitus and AHI, showed that a short sleep onset latency was also associated with an enhanced response to dexamethasone (adjusted mean difference = 0.006, 95%CI = 0.001;0.012), but this association did not hold after multiple testing correction. No other sleep estimates were associated with response to dexamethasone (Table 3). In addition, there was no association between absolute spectral power and response to dexamethasone (Table 4).

We performed four sensitivity analyses to assess the robustness of the results. First, after exclusion of participants with less than 5 h of sleep (remaining N = 360), results did not change substantially, with the exception of increased effect estimates for sleep efficiency (Supplementary Table 2). Second, after exclusion of non-suppressors (remaining

Table 1

Characteristics of the study population, Rotterdam study. N = 403.

	N (%)	Mean ± SD
Demographics		
Age (years)		62.4 ± 5.0
Women	222 (55%)	
Employed	216 (56%)	
Education		
Primary	33(8%)	
Low	135(33%)	
Intermediate	107(27%)	
High	127(32%)	
Health Indicators		
Body mass index (kg/m^2)		27.3 ± 4.6
Alcohol (glasses/day)		0.5 ± 0.9
Smoking		
Never smoker	140(35%)	
Former smoker	205(51%)	
Current smoker	58(14%)	
Diabetes mellitus	47 (12%)	
Apnea-Hypopnea Index (events/hour)[a]		13.8 ± 12.9
Depressive symptoms[b,c]		3(1–7)
Anxiety symptoms[c,d]		2 (1–4)
Use of psychoactive medicine	51 (13%)	
Cortisol		
Cortisol before dexamethasone (nmol/L)[c]		9.4 (6.0–14.4)
Sampling time day 1 (clock time)		7:59 ± 0:43
Cortisol after dexamethasone (nmol/L)[c]		1.9(0.7–4.7)
Sampling time day 2 (clock time)		7:52 ± 0:39
Time between sleep and cortisol assessment (days)		0.9 ± 37.8*

For categorical variables the absolute number (%) is indicated, for numeric variables the mean ± SD. Missing values: education (n = 1, 0.2%), alcohol (n = 27, 4.2%), diabetes mellitus (n = 1, 0.2%), apnea-hypopnea index (n = 36, 8.9%).

*Please note that the cortisol assessment could be completed before and after polysomnography, allowing both negative and positive values for this variable. Overall, it showed a normal distribution.

  [a] Assessed based the apnea hypopnea Index using polysomnography data.
  [b] Assessed using the Center for Epidemiologic Studies Depression scale.
  [c] Median and Interquartile Range.
  [d] assessed using the Hospital Anxiety and Depression Scale.

Table 2

Sleep in the study population. N = 403.

	Mean ± SD
Global sleep	
Total sleep time (min)	379.7 ± 62.9
Sleep efficiency (%)	81.2 ± 10.4
Sleep onset latency (min)	20.6 ± 23.0
Wake after sleep onset (min)	68.5 ± 43.3
Sleep stage duration (min)	
N1	48.5 ± 24.5
N2	200.0 ± 49.8
N3	51.7 ± 38.2
REM	79.7 ± 24.4
Absolute spectral power (µV^2/Hz)[a]	
Alpha	12.1 ± 8.6
Beta	3.3 ± 2.3
Gamma	2.5 ± 2.1
Delta	135.7 ± 117.4
Theta	20.9 ± 13.4

  [a] Spectral EEG data was available for 359 (89%) of the participants.

N = 363) short sleep onset latency (adjusted mean difference = 0.005, 95%CI = 0.000;0.010) and long N3 sleep (adjusted mean difference = −0.004, 95%CI = −0.008;−0.001), but not N2 sleep, were associated with enhanced response to dexamethasone. However, these results did not hold after multiple testing correction (Supplementary Table 3). After

exclusion of participants with clinically relevant depressive symptoms (CES-D ≥ 16), clinically relevant anxiety symptoms (HADS ≥ 8), and those who self-reported the use psychoactive medicine (remaining N = 327), results did also not change substantially. A short N2 sleep (adjusted mean difference = 0.005, 95%CI = 0.001;0.009) and long N3 sleep (adjusted mean difference = −0.006, 95%CI = −0.010;−0.002) were also associated with enhanced response to dexamethasone in this smaller sample (Supplementary Table 3). After stratification for sex, effect sizes did not differ substantially between men and women. However, the association between long N3 sleep and enhanced response to dexamethasone (adjusted mean difference = −0.008, 95%CI = −0.013;−0.003) remained significant in women only (Supplementary Table 4).

4. Discussion

This study suggests a cross-sectional association of short N2 sleep and long N3 sleep with an enhanced response to dexamethasone (i.e. lower levels of cortisol after intake), indicating a stronger suppression of cortisol within the negative feedback loop of the HPA axis. There was no significant association of other sleep parameters, including spectral power, and response to dexamethasone. Results remained similar after excluding those who did not respond to the very low dose of dexamethasone, those with clinically relevant depressive symptoms and those who reported to use psychoactive medicine. No differences between men and women were found.

Well-balanced functioning of the stress system is important for optimal health. Both diminished (i.e. higher levels of cortisol after intake) and enhanced (i.e. lower levels of cortisol after intake) negative feedback have been associated with negative health outcomes (Zorn et al., 2017; Kumari et al., 2010). Within our study population of middle-aged and elderly adults, we observed an association of short N2 sleep and long N3 sleep with an enhanced response to dexamethasone, suggesting a stronger suppression of cortisol by the negative feedback loop of the HPA axis, similar as seen in stress-related disorders (Zorn et al., 2017; Arana et al., 1985). These findings are in line with previous literature which reports an association between more slow wave sleep (N3) and low cortisol levels (Steiger, 2002; Lavie, 2001). Because an increase in slow wave sleep is likely at the expense of reduction in other sleep stages, we hypothesize that the association of short N2 sleep with enhanced response to dexamethasone might be explained by prolonged slow wave sleep. As slow wave sleep is thought to be particularly important to maintain cognitive functioning and neuronal plasticity (Nicolaides et al., 2000; Rasch and Born, 2013), our observed association between more slow wave sleep and enhanced response of the HPA axis might suggest that the positive effects of slow wave sleep are even more widespread across the brain. However, more slow wave sleep can also indicate a heightened sleep pressure (Lavie, 2001; Retey et al., 2005), which might indicate a sleep deprivation. Sleep deprivation has been related to increased cortisol levels and stress during the day (Leproult and Van, 2010). Exclusion of those with short sleep in our sample, albeit not necessarily an indicator of sleep deprivation, did not change our results. Lastly, we note that although we found an association of more N3 sleep, we did not find an association between delta spectral power and response to dexamethasone, even though delta waves are a key feature of N3 sleep (Nayak and Anilkumar, 2019). This might be explained by the age of our study population, delta power is significantly reduced with increasing age (Schwarz et al., 2017), even within the N3 stage.

Our study also suggested a potential association of short sleep onset latency with enhanced response to dexamethasone. This is in line with a previous study, which suggested that difficulties initiating sleep are associated with a delayed cortisol peak (Steiger, 2002). Additionally, psychological stress and difficulty falling asleep are important complaints of those with insomnia disorder (Riemann et al., 2010). According to the hyperarousal model, heightened stress, or hyperarousal,

Table 3

The cross-sectional association of polysomnography-estimated sleep with response to a very low dose of dexamethasone. N = 403.

	Model 1				Model 2			
	Adjusted mean difference (95%CI)	Standardized adjusted mean difference	p-value	Corrected p-value	Adjusted mean difference (95%CI)	Standardized adjusted mean difference	p-value	Corrected p-value
Global sleep								
Total sleep time (min)	0.001 (−0.001;0.003)	0.044	0.47	0.85	0.001 (−0.001;0.003)	0.072	0.28	0.45
Sleep efficiency (%)	0.000 (−0.011;0.012)	0.004	0.95	0.95	0.000 (−0.013;0.013)	-0.001	0.99	0.99
Sleep onset latency (min)	0.005 (−0.001;0.010)	0.106	0.08	0.21	0.005 (0.000;0.010)	0.118	0.06	0.16
Wake after sleep onset (min)	-0.001 (−0.004;0.002)	-0.039	0.53	0.85	-0.001 (−0.004;0.002)	-0.040	0.57	0.76
Sleep stage duration (min)								
N1	0.001 (−0.004;0.006)	0.028	0.67	0.89	0.004 (−0.002;0.009)	0.087	0.24	0.45
N2	0.004 (0.001;0.007)	0.203	0.017	0.07	0.005 (0.002;0.009)	0.257	0.005 *	0.020
N3	-0.005 (−0.009;−0.002)	-0.193	0.005 *	0.040	-0.006 (−0.010;−0.003)	-0.241	0.001 *	0.008
REM	0.000 (−0.006;0.007)	0.006	0.94	0.95	-0.001 (−0.008;0.006)	-0.035	0.69	0.79

Abbreviations: CI, Confidence Interval; Effect estimates were obtained using cross-sectional linear regression models, adjusted for cortisol before DST, sex, and age (Model 1), or adjusted for cortisol before DST, sex, age, education, employment, BMI, smoking, alcohol, time difference, depressive symptoms, anxiety symptoms, and psychoactive medicine (Model 2).

* P-value remained significant (< 0.05) after correcting for multiple testing, using FDR.

Table 4

The cross-sectional association of polysomnography-estimated absolute spectral power with response to a very low dose of dexamethasone. N = 359.

	Model 1				Model 2			
	Adjusted mean difference (95%CI)	Standardized adjusted mean difference	p-value	Corrected p-value	Adjusted mean difference (95%CI)	Standardized adjusted mean difference	p-value	Corrected p-value
Absolute spectral power (μV^2/Hz)[a]								
Alpha	-0.007 (−0.023;0.010)	-0.056	0.43	0.72	-0.005 (−0.022;0.013)	-0.039	0.62	0.62
Beta	0.008 (−0.053;0.069)	0.018	0.80	0.93	0.023 (−0.045;0.091)	0.052	0.51	0.62
Gamma	0.003 (−0.060;0.065)	0.006	0.93	0.93	0.042 (−0.031;0.115)	0.089	0.26	0.62
Delta	-0.001 (−0.002;0.000)	-0.130	0.07	0.33	-0.001 (−0.003;0.001)	-0.096	0.37	0.62
Theta	-0.008 (−0.019;0.002)	-0.109	0.13	0.33	-0.008 (−0.020;0.005)	-0.102	0.24	0.62

Abbreviations: CI, Confidence Interval; Effect estimates were obtained using cross-sectional linear regression models, adjusted for cortisol before DST, sex, and age (Model 1), or adjusted for cortisol before DST, sex, age, education, employment, BMI, smoking, alcohol, time difference, depressive symptoms, anxiety symptoms, and psychoactive medicine (Model 2). None of the P-values was significant remained significant (< 0.05) after correcting for multiple testing, using FDR. a EEG data was available for 359 (89%) of the participants contributing to the associations with PSG-estimated sleep. a alpha (range: 8.50–15.50), beta (range: 15.50–22.50 Hz), gamma (range: 22.50–40.00 Hz), delta (range: 0.75–4.50 Hz), and theta (range: 4.50–8.50 Hz).

is a key factor in maintaining insomnia. However, we only found this association after additional correction for diabetes mellitus and AHI. Hyperarousal might thus have a potentially stronger impact on cortisol response in those without these disorders. Potentially, diabetes mellitus and sleep apnea already lead to such a dysregulation of cortisol levels (Joseph and Golden, 2017), that sleep onset latency does not add on to this effect.

Two of the most common explanations for the association of sleep with altered response of the HPA axis are chronic stress and a shared underlying psychopathology. Chronic stress, for example induced by work or family problems, is associated with heightened cortisol levels (Mizoguchi et al., 2003) and less slow wave sleep (Kim and Dimsdale, 2007). Equally, depressive symptoms are also associated with a dysregulated HPA axis (Zorn et al., 2017; Gaffey et al., 2016) and poor sleep, such as less slow wave sleep and difficulties with falling asleep (Murphy and Peterson, 2015; Newson and Thiagarajan, 2019). This way, chronic stress or underlying psychopathology might be a common cause explaining the changes in sleep as well as functioning of the negative

feedback loop of the HPA axis. Our findings did not substantially change after adjusting for depressive symptoms, anxiety symptoms and use of psychoactive medication, nor after exclusion of participants with these complaints. This suggests that the association between sleep and response of the HPA axis is independent of these forms of psychopathology.

When interpreting our results several limitations need to be taken into account. First, we were not able to draw conclusions on temporality or causality because repeated measurements were not available. Second, participation in the PSG study and the dexamethasone suppression test study was optional. Participants agreeing to participate are typically healthier, potentially inducing selection bias. Third, our study population consisted of middle-aged and elderly participants. Therefore our results might not be generalizable to other age groups. Fourth, despite PSG being the gold standard to assess sleep, a first night effect might have made our measurements less representative for habitual sleep (Byun et al., 2019). Fourth, environmental light is an important factor that could affect both sleep and cortisol levels. Unfortunately we were

not able to obtain valid data on environmental light and therefore could not take this into account. Last, in our study we did not observe substantial changes in effect estimates between men and women. Potentially, this is due to limited power in these analyses, which is why we recommend further research into potential sex-specific associations. Nevertheless, having different PSG sleep measures and the very low dose dexamethasone suppression tests in a relatively large population-based sample is unique in the field and allowed us to assess associations between sleep and the stress system with objective measures in the general population.

Altogether, in our study population of middle-aged and elderly we demonstrated an association of short N2 sleep and long N3 sleep with an enhanced response to dexamethasone over time, independent of indicators of psychopathology. These results suggest that duration of slow wave sleep, and potentially needing a longer time to fall asleep, might play a role in altered functioning of the stress system.

Author disclosures

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CRediT authorship contribution statement

Maud de Feijter: Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Athanasia Katimertzoglou:** Conceptualization, Data curation, Methodology, Writing – review & editing. **Jitske Tiemensma:** Methodology, Writing – review & editing. **M. Arfan Ikram:** Data curation, Investigation, Project administration, Resources, Supervision, Writing – review & editing. **Annemarie I. Luik:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2022.105749.

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