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Poor night's sleep predicts following day's salivary alpha-amylase under high but not low stress

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ABSTRACT

Although sleep is linked to physiological stress systems like the autonomic nervous system (ANS), research is still limited regarding night-and-day interactions between nocturnal sleep characteristics, stress, and diurnal parameters of salivary alpha-amylase (sAA) as a surrogate marker of ANS activity. Fifty healthy university students rated their chronic stress burden and completed two five-day periods of ecological momentary assessment – under everyday conditions of both low stress (beginning of semester) and high stress (final examination preparation). Participants collected saliva six times daily and reported on the previous night's sleep (quality, latency, duration, disturbances) immediately after awakening. Additionally, a sub-sample wore actigraphs recording 'time in bed'. In contrast to previous assumptions, poor sleep predicted lower sAA awakening values, more decreased awakening responses, and steeper diurnal slopes the following day only under high stress, but not under low stress. Diurnal sAA parameters did not predict the following night's sleep characteristics. The sAA profile does not seem to be sensitive to everyday occurring sleep variations, but rather seems to be an indicator of more prolonged stress induced ANS dysregulation.

1. Introduction

Both sleep and stress-responsive physiological systems are subject to bio-regulatory processes that are aligned both temporally and functionally. Thus, activity of the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis is, next to the stress-responsive capacity of these systems, characterized by 24-h oscillations that are closely related to the sleep-wake cycle and controlled by a circadian clock located in the suprachiasmatic nucleus (Hastings et al., 2003). Additionally, it has been shown that acute as well as chronic stress lead to impairments in subjective sleep quality as well as polysomnographically measured sleep structure (Akerstedt et al., 2007; Hall et al., 2015). Some findings furthermore indicate that higher evening arousal, reflected in increased neuroendocrine levels, lead to sleep impairments (e.g. Rodenbeck et al., 2002, investigating blood cortisol levels, a marker of HPA axis activity). Therefore, it is generally assumed that acute and chronic stress impairs sleep via neuroendocrine activation ("arousal hypothesis" - for an overview see Van Reeth et al., 2000). Moreover, recent studies showed that poor sleep leads to heightened ANS activity (van Leeuwen et al., 2018) as well as reactivity (Massar et al., 2017). Moreover, a recent review suggests that poor sleep is not only associated with heightened HPA acitivity levels, but also with a heightened HPA stress reactivity (van Dalfsen and Markus, 2018). Therefore, poor sleep seems to further affect ANS and HPA axis activity and therefore influences the adaptive physiological response to everyday challenges (as has also been suggested by Meerlo et al., 2008).

Whereas the literature on the effects of stress on sleep via the HPA axis, and the effects of sleep on the HPA axis (e.g. using salivary cortisol (sCort) as the primary end product of the HPA axis as a biomarker), is relatively abundant (for review see van Dalfsen and Markus, 2018; Van Reeth et al., 2000), research regarding the interaction between stress, ANS, and sleep is still limited (Kageyama et al., 1998). Although sleep loss or disrupted sleep has been shown to affect the ANS, with corresponding increases in heart rate and blood pressure and a decrease in parasympathetic activity (van Leeuwen et al., 2018; Zhong et al., 2005), assessment of cardiovascular parameters in the field is fraught with

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methodological problems (Berntson et al., 1997). Salivary measures could avoid these problems (like device failures or time-consuming second-to-second analyses) but non-cardiovascular indicators of the ANS remain under-investigated.

Secretion of salivary alpha-amylase (sAA) has been suggested to be a surrogate marker of ANS activity and a possible indicator of sympathetic activation (Nater and Rohleder, 2009) that might overcome the above-mentioned methodological problems. Increased sAA activity has been reported following experimentally manipulated acute sleep restriction (O'Leary et al., 2015) and total sleep deprivation (Seugnet et al., 2006), suggesting that sAA may represent a measure of increased sleep pressure. Whether diurnal alterations in sAA activity, in turn, predict sleep has not yet been investigated in this context. Furthermore, Pajcin and colleagues (2017) found that diurnal sAA profiles were unaffected by sleep deprivation, showing that results are not unequivocal.

Ecological momentary assessment (EMA) studies collecting data repeatedly across multiple measurement time points in real-life situations (for more information see, e.g., Ebner-Priemer and Trull, 2009) are required to investigate reciprocal interactions between diurnal sAA parameters and nocturnal sleep characteristics in everyday life. To the best of our knowledge, only two ecologically valid studies have examined the relationship between sAA and different sleep characteristics. Nater et al. (2007) utilized an EMA approach and found a marked diurnal sAA profile with a pronounced decrease in the first 30 min after awakening and a steady increase across the day. While the alpha-amylase awakening response (AAR) was independent of quality and duration of the previous night's sleep, it was slightly higher in subjects who used an alarm clock instead of waking up on their own (Nater et al., 2007). Van Lenten and Doane (2016) also used an EMA design, but additionally obtained objective measures of sleep using actigraphy. Their results suggested that worse subjective sleep quality over the past month was related to higher sAA waking levels, whereas actigraphy-measured sleep characteristics (such as duration, duration variability, efficiency) were not related to sAA (Van Lenten and Doane, 2016). Therefore, it might be concluded that diurnal sAA parameters are not sensitive to variation in sleep in the short term, and that more prolonged changes in sleep (e.g., in insomnia) or external influences that impair sleep may be required to alter diurnal sAA parameters. As mentioned earlier, one such influence that leads to poor sleep might be an increase in stress level. Concerning possible interaction effects between sleep and stress, and the role of sAA, one study found that children with lower sleep efficiency showed higher sAA levels across the Trier Social Stress Test (Räikkönen et al., 2010). Another study showed a decrease of waking sAA levels due to a stress-reducing sleep-related intervention in cancer survivors (Lipschitz et al., 2013). However, there is also one study showing no differences between on-call and off-call nights on daily sAA levels and profiles in firefighters and emergency service personnel (Hall et al., 2017). Effects of stress and sleep on sAA in an adult population in the context of a naturalistic setting have not yet been investigated.

Proceeding from the aforementioned findings, in the present study, we also used an EMA design with subjective and objective measures of sleep and saliva sampling several times a day. However, the number of days was extended (from 3 or 4 to 10), the self-reported sleep variables were assessed each morning directly after awakening (whereas Van Lenten and Doane (2016) retrospectively examined subjective sleep variables via questionnaire), and the presence of everyday stress was quasi-experimentally manipulated (whereas Van Lenten and Doane (2016) considered typical weekdays). We hypothesized that characteristics of nocturnal sleep are unrelated to the following day's parameters of sAA in the case of lower stress, whereas poor sleep predicts increased sAA under higher stress. We further hypothesized that increased diurnal sAA parameters predict the following night's sleep characteristics (either for the better or for the worse).

2. Material and methods

Data were gathered as part of larger study on the role of everyday life stress in students. For a detailed description, see Doerr et al. (2015). In the current analyses, we focused on previously unpublished data on sleep and sAA.

2.1. Sample

Participants were recruited via university student mailing lists or notices on campus. Inclusion criteria were as follows: German language proficiency, age 18–35 years, BMI $< 30\,kg/m^2$, no psychiatric or medical illness, smoking < 5 cigarettes / week, no drug use or intake of medication affecting neuroendocrine or autonomic functions (except for hormonal contraceptives); for women: no pregnancy, no breast feeding, regular menstrual cycle (Strahler et al., 2017). Five subjects of the initial sample ($N = 55$) had to be excluded retrospectively due to dropout ($n = 3$), device failures ($n = 1$), or incomplete data with more than 50% missing values in the exam condition ($n = 1$); thus, data of 50 participants were included in the final statistical analyses. All individuals provided written informed consent and were compensated with 50 EUR or course credits. The study protocol was approved by the institutional Ethics Committee (Department of Psychology, University of Marburg, Germany).

2.2. Procedure

Using a quasi-experimental within-subject design with varying stress intensity, data were collected twice for five consecutive days each, during the first weeks of the semester (lower stress, control condition) and during the preparation for final examinations within the last weeks of the semester (higher stress, exam condition). Previous analyses confirmed that the reported stress level was higher during the exam condition (Doerr et al., 2015). Following the initial check for eligibility, participants were instructed to fill out questionnaires online and were then familiarized with the handling of a pre-programmed iPod touch® (iDialogPad, G. Mutz, Cologne, Germany). During both five-day periods of assessment, the first iPod entry of the day had to be triggered by the participants themselves directly upon awakening. The subsequent time-based entries were prescheduled for 30 min after awakening, 10 a.m., 2 p.m., 6 p.m., and 9 p.m. Each time after having answered the self-report questions, participants collected saliva using pre-labeled saliva vials. A sub-sample was additionally equipped with an actigraph ($n = 43$, of which $n = 29$ during both conditions) according to availability, because the number of devices was limited.

2.3. Measures

2.3.1. Sleep

Self-reported sleep: Participants rated their previous night's subjective sleep quality each morning directly upon awakening on a visual analogue scale from '0' (very bad) to '100' (very good). Furthermore, they estimated sleep latency (in minutes), sleep duration (total sleep time, TST, minutes from bedtime to awakening minus sleep latency), as well as how often sleep was disrupted by awakening in the night or early in the morning (0x, 1x, 2x, 3x, ≥3x). The items were based on the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989) and adapted for use in an EMA design. Thus, in our study, self-reported bad sleep quality, long estimated sleep latency, short sleep duration, and/ or sleep disruptions due to awakenings indicate 'poor sleep'.

Actigraphy-measured sleep: Participants were equipped with a triaxial accelerometer on their non-dominant wrist (SOMNOwatch®, SOMNOmedics Randersacker, Germany), which recorded activity by one count each second. A predefined algorithm in the supplied software, integrating information about intensity of light and movement, calculated time in bed (TIB).

2.3.2. Stress

Self-reported chronic stress: The 12-item Screening Scale of the Trier Inventory for the Assessment of Chronic Stress (SSCS) (Schulz et al., 2004) assesses chronic stress experiences in the past three months on a 5-point Likert scale ranging from 'never' to 'very often'. The SSCS was completed online once during the control condition to capture students' general chronic stress burden and to control for influences on short-term sAA-sleep associations. For the interpretation of the SSCS score, the test manual provides a conversion table (from raw scores to T-scores) based on a norm sample (Schulz et al., 2004).

Salivary alpha-amylase: Participants collected saliva samples using the SaliCap® system (IBL, Hamburg, Germany) via passive drool (not swallowing for 2 min and transferring the accumulated saliva to a vial using a straw) to eliminate effects of chewing or sucking on sAA activity (Strahler et al., 2017). Furthermore, participants were instructed to refrain from snoozing, brushing their teeth as well as drinking caffeinated beverages or eating during the first 30 min after awakening. They also indicated if they woke on their own or by an alarm clock. After saliva sampling, the pre-labeled vials were stored at as cool a temperature as possible (participants' freezers or fridges). At the end of each assessment period, the tubes were stored at −20 °C until analysis. sAA activity was measured using a kinetic colorimetric test and reagents obtained from Roche (Fa. Roche Diagnostics, Mannheim, Germany). Inter- and intra-assay variance was below 10%. Based on the raw scores, the following parameters of sAA were analyzed: awakening value, awakening response (AAR = delta of awakening value and the 30 min post-awakening value), evening value, diurnal slope (linear change across the day considering time since awakening; time points: 10 a.m., 2 p.m., 6 p.m., 9 p.m.).

2.4. Statistical analyses

Two-level hierarchical linear models (HLM 7, Scientific Software International Inc., Lincolnwood, IL) were conducted with assessment day at level 1 (variables: condition (control/exam), alarm clock (no/yes), sleep characteristics, sAA parameters) nested within persons at level 2 (variable: SSCS sum score). All χ^2 tests for the sleep and sAA outcomes were statistically significant ($ps < .05$); this indicates variance by person and therefore justifies the use of hierarchical linear modeling. At level 2, the intercept (β_0) was additionally modeled as a function of the control variables 'age', 'sex', and 'BMI'. As no associations were observed with outcome variables, the control variables on level 2 were not included in the final models (see notes below tables). For the analyses regarding the effect of sAA parameters on the following night's sleep characteristics that were assessed the next day's morning, sAA parameters (diurnal slope, evening value) were time-lagged, i.e., shifted one row forward in the data matrix by the syntax command x_lag = LAG(x)). As a measure of effect size, we calculated "*Pseudo-R^2* = (σ^2reference model - σ^2final model) / σ^2reference model", where the reference model is the final model excluding the predictor in question. Therefore, *Pseudo-R^2* quantifies the proportional reduction in within-person residual variance by the predictor in question. There is some debate in how to estimate effect sizes in hierarchical linear models because a variable can cause reduction of residual variance at one level, but increases at another level. However, this does not necessarily rule out that both models (reference and final) are valid statistical models for the observation at hand (see Snijders and Bosker, 1994). *Pseudo-R^2* measures are common and generally seen as relatively easily comprehensible and useful data analytic tools if constructed and interpreted carefully (Singer and Willett, 2003). After computing the aggregated models, we separately analyzed for each predictor variable an interaction model including condition as well as the interaction term of condition x predictor to test whether the association between predictor and outcome differed between the two conditions.

Missing values at level 2 were imputed by mean substitution, whereas missing values at level 1 were deleted pairwise by the HLM program when running analyses for the final models and listwise when calculating the reference model. We used the standardized final estimation with robust standard errors when reporting results. For all analyses, unstandardized coefficients (UC) were presented, *p*-values of < .05 were considered significant, and two-tailed *p*-values were reported if not otherwise specified.

3. Results

3.1. Sample characteristics

The sample consisted of 31 women and 19 men with a mean age of 23.3 years (*SD* = 3.2) and normal weight (BMI: *M* = 21.9, *SD* = 2.4). The chronic stress level measured by the SSCS (referring to the three months before the control condition) was high compared to the norm sample (*M* = 27.3, *SD* = 8.4, T-score = 63).

3.2. Sleep and salivary alpha-amylase depending on sub-acute stress

3.2.1. Sleep characteristics and stress condition

Descriptive analyses of sleep characteristics under both conditions are depicted in Table 1. The mean subjective sleep quality was 65.9 (*SD* = 21.4) and did not differ between the control condition (*M* = 66.9, *SD* = 22.4) and the exam condition (*M* = 64.8, *SD* = 20.5), *t*(433) = 1.00, *p* = .318. Sleep latency was longer in the exam condition (*M* = 15.5, *SD* = 20.0) than in the control condition (*M* = 1.4, *SD* = 6.8), *t*(277) = -10.12, *p* < .001; *Pseudo-R^2* = .32 in the aggregated model (see Table 3). The mean sleep duration was 441.8 minutes (*SD* = 92.6) and was comparable between the control condition (*M* = 445.3, *SD* = 95.2) and the exam condition (*M* = 438.1, *SD* = 89.9), *t*(453) = .83, *p* = .407. Though only to a small degree, sleep was more often disrupted in the control condition (*M* = .8, *SD* = 1.2) than in the exam condition (*M* = .5, *SD* = .8), *t*(381) = 3.21, *p* < .01, *r* = 0.16. The mean actigraphy-measured TIB was 534.9 min (*SD* = 156.7) and

Table 1
Descriptive statistics of salivary alpha-amylase and sleep variables comparing control and exam condition (N = 50).

	M (SD)		
	Control condition	Exam condition	*t*
Salivary alpha-amylase (U/ml)			
Awakening value	57.16 (71.64)	73.15 (79.11)	−1.940
Awakening response	−16.01 (72.10)	−21.65 (82.12)	.651
Evening value	91.65 (114.11)	96.24 (99.21)	−.416
Diurnal slope	.08 (.35)	.05 (.11)	.936
Sleep (self-report)			
Sleep quality	66.89 (22.36)	64.83 (20.46)	.999
Sleep latency	1.40 (6.78)	15.54 (20.03)	−10.117***
Sleep duration	445.34 (95.17)	438.14 (89.92)	.830
Sleep disruptions	.85 (1.19)	.54 (.78)	3.209**
Time in bed (actigraphy)[a]	546.41 (147.14)	522.60 (165.99)	1.252

Note. M = mean, SD = standard deviation. Awakening response was calculated as delta of awakening value and the 30 min post-awakening value. Evening value was assessed at 9 p.m. Diurnal slope was calculated as the linear change across the day considering time since awakening from the following time points: 10 a.m., 2 p.m., 6 p.m., 9 p.m. Items assessing self-reported sleep characteristics were assessed as follows: 'sleep quality' on a visual analogue scale from '0' (very bad) to '100' (very good), 'sleep latency' in minutes, 'sleep duration' in minutes from bedtime to awakening minus sleep latency, frequency of 'sleep disruptions' 0x/1x/2x/3x/ > 3x .
*$p < .05$, **$p < .01$, ***$p < .001$.
[a] Sub-sample (n = 43, of which n = 29 during both conditions).

Table 2
Hierarchical Linear Models Predicting the Following Day's Parameters of Salivary Alpha-Amylase (Level 2: N = 50) Using Restricted Maximum Likelihood.

	Awakening value		Awakening response		Diurnal slope	
Fixed effects	*UC*	*SE*	*UC*	*SE*	*UC*	*SE*
Intercept	**65.04*****	17.11	11.36	25.47	.04	.03
Level 2:						
Chronic stress (SSCS)[a]	1.75	1.02	−.10	1.27	−.00	.00
Level 1:						
Condition (control vs. exam)[b]	21.26	11.83	**−18.80***	9.37	.00	.01
Sleep quality (self-report)	.59	.30	−.66	.37	.00	.00
Sleep latency (self-report)	−.22	.24	−.03	.30	.00	.00
Sleep duration (self-report)	−.01	.06	−.00	.06	−.00	.00
Sleep disruptions (self-report)	.03	3.53	.10	3.95	−.00	.01
Time in bed (actigraphy)	−.06	.03	.03	.03	.00	.00
Random effects	*SD*	*VC*	*SD*	*VC*	*SD*	*VC*
Intercept	54.93	**3017.24*****	46.70	**2180.65*****	.07	.00
Sleep quality (self-report)	.41	**.17****	1.02	**1.04*****	.00	.00
Sleep latency (self-report)	.19	**.04****	.31	**.10***	.00	.00
Sleep duration (self-report)	.12	**.01***	.10	.01	.00	.00**
Sleep disruptions (self-report)	6.83	**46.63***	6.34	**40.14***	.01	.00
Time in bed (actigraphy)	.13	**.02*****	.07	**.00*****	.00	.00

Note. UC = unstandardized coefficient, SE = standard error, VC = variance component, SD = standard deviation, SSCS = Screening Scale for Chronic Stress. Salivary alpha-amylase in U/ml. Awakening response was calculated as delta of awakening value and the 30 min post-awakening value. Diurnal slope was calculated from the following time points: 10 a.m., 2 p.m., 6 p.m., 9 p.m. Items assessing self-reported sleep characteristics were assessed as follows: 'sleep quality' on a visual analogue scale from '0' (very bad) to '100′ (very good), 'sleep latency' in minutes, 'sleep duration' in minutes from bedtime to awakening minus sleep latency, frequency of 'sleep disruptions' 0x/1x/2x/3x/ > 3x . Covariates: alarm clock (level 1), sex (level 2); results not reported.
*$p < .05$; **$p < .01$; ***$p < .001$.
[a] SSCS items were assessed on a 5-point Likert scale from 'never' to 'very often'.
[b] Control = 0; Exam = 1.

did not differ between the control condition (M = 546.4, SD = 147.1) and the exam condition (M = 522.6, SD = 166.0), $t(269) = 1.25$, p = .212).

3.2.2. Salivary alpha-amylase parameters and stress condition

Descriptive analyses of sAA parameters under both conditions are depicted in Table 1. The mean sAA awakening value was 73.1 (SD = 79.1) in the exam condition and 57.2 (SD = 71.6) in the control condition. The mean sAA evening value was 96.2 (SD = 99.2) in the exam condition and 91.6 (SD = 114.1) in the control condition. The sAA diurnal slope ranged between -.2 and .5 (M = .0, SD = .1) in the exam condition and between -.3 and 3.6 (M = .1, SD = .3) in the control condition. None of the sAA parameters significantly differed between the two conditions ($ps \geq .05$ in t-tests). However, when considering the aggregated model in Table 2, the decrease of the AAR was more pronounced in the exam condition (control condition: M = -16.0, SD = 72.1, exam condition: M = -21.6, SD = 82.1, $Pseudo\text{-}R^2$ = .01).

3.3. Alpha-amylase and its relation to the previous and the following night's sleep

3.3.1. Sleep predicting the following day's alpha-amylase

None of the characteristics of nocturnal sleep (subjective sleep quality, sleep latency, sleep duration, sleep disruptions, TIB) predicted the following day's parameters of sAA in the aggregated models (see Table 2). However, in the exam condition, worse subjective sleep quality was associated with a lower sAA awakening value the following

Table 3
Hierarchical Linear Models Predicting the Following Night's Sleep Characteristics (Level 2: N = 50) Using Restricted Maximum Likelihood.

	Self-report[c]								Actigraphy	
	Sleep quality		Sleep latency		Sleep duration		Sleep disruptions		Time in bed	
Fixed effects	*UC*	*SE*	*UC*	*SE*	*UC*	*SE*	*UC*	*SE*	*UC*	*SE*
Intercept	**67.22*****	3.70	.98	.65	**438.40*****	15.17	**.95*****	.19	**539.99*****	27.81
Level 2:										
Chronic stress (SSCS)[a]	−.68	.38	.04	.08	−1.12	1.20	.02	.01	.06	1.50
Level 1:										
Condition (control vs. exam)[b]	−6.82	3.53	**10.62*****	1.45	6.81	11.89	.03	.17	−11.02	30.39
Alpha-amylase diurnal slope	−19.00	18.38	−11.45	9.72	16.21	89.55	−1.01	1.42	−422.82	251.59
Alpha-amylase evening value	.03	.04	.03	.01	−.26	.14	−.00	.00	.20	.38
Random effects	*SD*	*VC*	*SD*	*VC*	*SD*	*VC*	*SD*	*VC*	*SD*	*VC*
Intercept	13.63	**185.76*****	2.81	**7.88****	52.92	**2800.66*****	.68	**.46****	30.07	904.28
Alpha-amylase diurnal slope	27.28	744.14	37.41	**1399.41***	126.65	16040.45	4.76	22.69	268.54	72116.64
Alpha-amylase evening value	.08	.01	.05	.00	.20	.04	.00	.00	.66	.44

Note. UC = unstandardized coefficient, SE = standard error, VC = variance component, SD = standard deviation, SSCS = Screening Scale for Chronic Stress. Salivary alpha-amylase in U/ml. Evening value was assessed at 9 p.m. Covariate: alarm clock (level 1); results not reported.
*$p < .05$; **$p < .01$; ***$p < .001$.
[a] SSCS items were assessed on a 5-point Likert scale from 'never' to 'very often'.
[b] Control = 0; Exam = 1.
[c] Items assessing self-reported sleep characteristics were assessed as follows: 'sleep quality' on a visual analogue scale from '0' (very bad) to '100' (very good), 'sleep latency' in minutes, 'sleep duration' in minutes from bedtime to awakening minus sleep latency, frequency of 'sleep disruptions' 0x/1x/2x/3x/ > 3x .

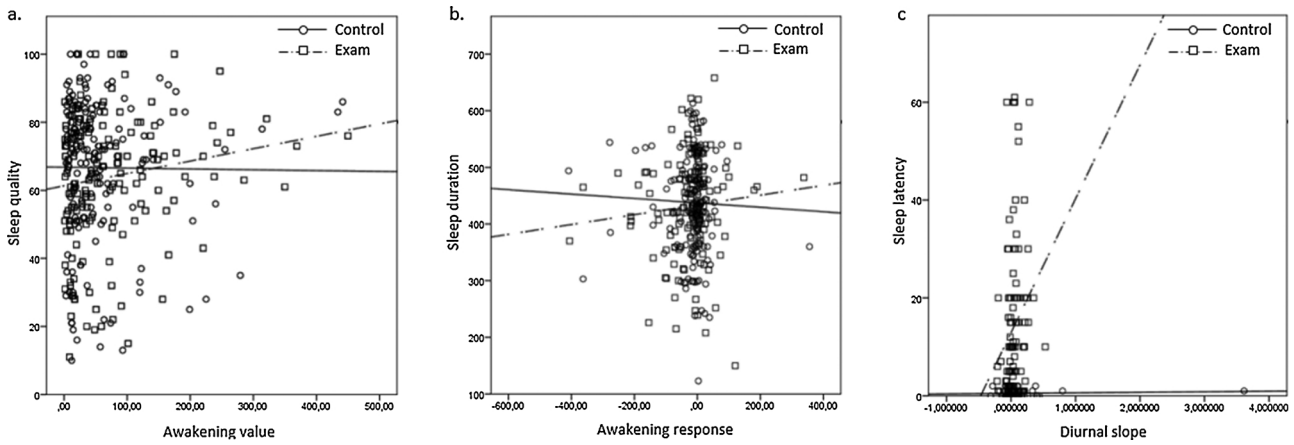

Fig. 1. Interaction models including condition (control vs. exam) as well as the interaction term of condition x predictor (a) sleep quality (dependent variable: salivary alpha-amylase awakening value), (b) sleep duration (dependent variable: salivary alpha-amylase awakening response), (c) sleep latency (dependent variable: salivary alpha-amylase diurnal slope). Salivary alpha-amylase in U/ml. Awakening response was calculated as delta of awakening value and the 30 min post-awakening value. Diurnal slope was calculated from the following time points: 10 a.m., 2 p.m., 6 p.m., 9 p.m.. Items assessing self-reported sleep characteristics were assessed as follows: 'sleep quality' on a visual analogue scale from '0' (very bad) to '100' (very good), 'sleep duration' in minutes from bedtime to awakening minus sleep latency, 'sleep latency' in minutes.

day, whereas in the control condition, the variables were only marginally correlated, with inverse tendency (interaction term "condition x subjective sleep quality": $UC = .57$, $SE = .27$, $p < .05$, $Pseudo\text{-}R^2 = .08$). Similarly, in the exam condition, a shorter sleep duration was associated with a more decreased AAR the following day, whereas in the control condition, the variables were only marginally correlated, with inverse tendency (interaction term "condition x sleep duration": $UC = .16$, $SE = .07$, $p < .05$, $Pseudo\text{-}R^2 = .17$). In the exam condition, a longer sleep latency was stronger associated with a steeper sAA diurnal slope than in the control condition (interaction term "condition x sleep latency": $UC = .01$, $SE = .00$, $p < .05$, $Pseudo\text{-}R^2 = .07$). The interactions mentioned above are illustrated in Fig. 1. The use of an alarm clock had no effect on the sAA parameters in the aggregated models.

3.3.2. Alpha-amylase predicting the following night's sleep

None of the sAA parameters (diurnal slope, evening value) predicted characteristics of the following night's sleep in the aggregated models (see Table 3). The same was true for the interaction models, where the sAA predictor variables likewise had no effect on sleep characteristics – independently of stress condition.

4. Discussion

This is the first study to show that under quasi-experimentally manipulated stress (exam condition) in everyday life, poor nocturnal sleep (worse subjective sleep quality, short sleep duration, long sleep latency) predicted lower sAA awakening values, more decreased AAR, and steeper sAA diurnal slopes the following day. Under lower stress, sleep and the following day's parameters of sAA were unrelated. This was observed irrespective of students' general chronic stress burden. Furthermore, diurnal sAA parameters (diurnal slope, evening value) did not predict the following night's sleep characteristics.

Within-person findings about the apparent unrelatedness of nocturnal sleep and the next day's sAA measures under normally stressful everyday life conditions are in accordance with previous EMA research (Nater et al., 2007; Van Lenten and Doane, 2016). The association found by Van Lenten and Doane (2016) between retrospectively reported bad sleep quality during the past month and an increased sAA awakening value during three typical workdays was the opposite in our stress condition, in which poor sleep quality predicted a lower sAA awakening value the following day. Nater et al. (2007), on the other hand, also found no association between sleep quality and following day's awakening sAA value (comparable to our findings in the control condition). Therefore, associations between sleep quality and sAA awakening value might differ depending on 1) the level of sleep assessment (within vs. between persons), 2) the time frame (long-term vs. short-term), and 3) the overall stress level (heightened vs. typical week). From these three studies, it seems that sleep quality that was measured referring to a larger time frame (and between persons) has a negative association with sAA morning values, whereas a positive association can be observed on a day-to-day (within-person) basis, particularly under high-stress conditions. More research is clearly warranted to substantiate these assumptions.

On the other hand, another characteristic of poor sleep, namely a long sleep onset latency, predicted a steeper sAA diurnal slope (reflecting a greater increase in ANS activity) the following day – particularly in our stress condition. This, in turn, is different from the findings of Van Lenten and Doane (2016), who found no association of sAA slope with any sleep parameter. Again, the effect we found might only occur under high-stress conditions. Therefore, it seems likely that stress level is a moderator of the association between sleep and sAA (and therefore the ANS) which might indicate a process of physical adaptation to high stress. Again, more research using quasi-experimental designs in everyday life should be implemented to further investigate these preliminary findings.

We further investigated the reverse effect, namely of sAA parameters on the following night's sleep. Neither the sAA diurnal slope nor the evening value predicted nocturnal sleep characteristics. This is contrary to the assumption of O'Leary and colleagues (2015), who interpreted the increased sAA after acute sleep restriction as an indicator of sleep pressure, which should rather result in longer and deeper sleep for the purpose of recovery. Eventually, our finding that sleep was less often disrupted in the exam condition supports the idea that more restful sleep is needed under stress. However, given that, on average, sleep was reported to be disrupted less than one time per night in each condition (0.5 in the exam condition, 0.8 in the control condition), our sample was probably less impaired than that of O'Leary and colleagues' which makes findings not directly comparable.

Taken together, we propose that sAA diurnal parameters stand out due to their relative stability (Out et al., 2013) and that the relative independence of momentary factors that might influence sAA activity speaks for the usefulness of sAA diurnal profiles for the assessment of long-term changes in ANS activity. The AAR, for example, has been related to various disease states and stress-related disorders in other studies (Schumacher et al., 2013). This, again, might suggest that high

stress levels might be important for altering processes reflected by sAA. Therefore, other indicators of sympathetic activity (like heart rate or blood pressure) might be more appropriate for the assessment of short-term sleep consequences – although, as mentioned above, assessment of cardiovascular parameters in the field is problematic from a methodological standpoint (Berntson et al., 1997), which is why we decided to measure sAA. As there is still much to learn about this sympathetic marker, it is important to collect more information of its usefulness in different designs and contexts. Our findings might inform future research that might incorporate a comparison between long- and short-term associations between sAA and health outcomes such as sleep in the same sample in a larger-scale study.

One major strength of the present study is the high ecological validity due to the EMA approach, which results in numerous intraindividual observations and therefore enhances statistical power and reliability. On the other hand, internal validity may be a critical issue due to potentially confounding effects of unassessed variables – even though we controlled for a variety of potential confounders. Although we investigated a healthy and not a clinical sample, variance in the investigated variables might have been restricted due to the fact that the chronic stress level measured in the control condition was already high compared to the norm. This might also be one reason why students' general chronic stress burden has not been shown to be a significant predictor beyond everyday stress. Since a sample of university students was examined, results have to be interpreted with keeping in mind the limited generalizability according to sample specifics like younger age, higher socioeconomic status, and physiological as well as psychological characteristics (e.g., reactivity to stress). Although this limitation is to some extent outweighed by the increased ecological validity of testing a clearly defined real-life stressor (i.e. exams) in this sample, our findings should be confirmed in a more representative sample of the total population. Additionally, we referred to the suggestion of Maas and Hox (2005) of having a sample size of at least 50 to validly detect effects in multilevel models. However, our study may have been under-powered to detect associations between sAA and actigraphy-measured sleep given our relatively small sample size of these analyses on level 2 (Maas and Hox, 2005), although the sample size was comparable to other EMA studies.

5. Conclusions

Our study is the first to examine day-and-night interactions between multiple components of sleep and sAA as an under-investigated indicator of the ANS, while considering the impact of stress. Our findings now give further insights into the stress-related dysregulation of autonomic functioning as well as the sleep-wake cycle and thus provide further hints for important research questions in this field (e.g., which sAA abnormalities occur during poor sleep in stressful periods or what long-term consequences sAA abnormalities have on health and what are the underlying mechanisms). Knowledge about physiological and psychological effects of stress is crucial to prevent detrimental health consequences.

Author contributions

KK conducted statistical analyses, completed literature searches and drafted the manuscript. JMD, JS, NS, AL, and UMN designed and conducted the study. All authors provided critical revisions and approved the final version of the manuscript before submission.

Conflicts of interest

The authors declare no conflict of interest.

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