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Tomorrow's Gonna Suck: Today's Stress Anticipation Predicts Tomorrow's Post-Awakening Cortisol Increase

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Abstract

The cortisol awakening response (a rapid rise in cortisol concentration shortly after awakening) is hypothesized to prepare the organism to cope with upcoming demands, suggesting a key role for anticipatory stress in its regulation. Yet, no thorough test of this hypothesis incorporating temporal dynamics of the underlying processes has been conducted so far. To address this gap in the literature, the present study investigated the effects of anticipated stress for the next day (assessed in the evening) on an estimate of the cortisol awakening response (assessed in the following morning). In an ambulatory assessment paradigm, 42 participants (69% female; mean age = 22.8, range = 18-30 years) completed 5 consecutive days of assessments in their daily lives, collecting saliva samples at awakening and 30 minutes later. Using hierarchical linear modeling, associations with anticipatory stress were examined separately on the within- and between-person level. In line with our expectations, anticipatory stress predicted the post-awakening cortisol increase on the within-person level, implying an elevated cortisol rise on days for which more stress than usual had been anticipated. In contrast, on the between-person level higher average anticipatory stress did not predict an increased cortisol rise. Taken together, the findings confirm a key role of anticipatory stress in the regulation of the cortisol awakening response on the within-person level.

Keywords: cortisol awakening response (CAR), anticipatory stress, ambulatory assessment

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1. Introduction

After awakening, cortisol levels in humans increase sharply with peak concentrations between 30 and 45 minutes post-waking (Pruessner et al., 1997). This so-called Cortisol Awakening Response (CAR) may represent a promising biomarker of health conditions (Boggero et al., 2017; Chida and Steptoe, 2009; Powell et al., 2013; Stalder et al., 2016), however, its physiological function has not yet been fully understood. One prominent idea regarding the role of the CAR concerns the preparation of the organism in anticipation of upcoming demands (e.g. Fries et al., 2009; Powell and Schlotz, 2012). In the following, we briefly summarize this hypothesis, review related empirical evidence and point towards two central aspects, which have been neglected in previous research.

Linking elevated CARs to subjective (and chronic) stress levels, Schulz and colleagues (1998) hypothesized that stronger increases in post-awakening cortisol levels reflect an increased need for energy, enabling the organism to cope with imminent demands. Later, Wilhelm and colleagues (2007) suggested that the CAR is associated with memory retrieval processes at awakening related to information about the self and orientation in time and space. On the basis of this neuroendocrine notion, Fries and colleagues (2009) proposed a link between memory processes concerning demand anticipation and the CAR. They suggested that prospective memory representations are activated at awakening, which enable orientation towards anticipated demands of the upcoming day thereby stimulating cortisol secretion. Further, arguing that the magnitude of the CAR may differ depending on the extent or intensity of anticipated demands they emphasized the adaptive function of the CAR. Summarizing these key points, Powell and Schlotz (2012) proposed the term *CAR anticipation hypothesis* to describe the CAR as an adaptive phenomenon, which is linked to neural activation at awakening in a dynamic fashion preparing the organism to deal with anticipated demands.

Some recent studies support the assumption that demand anticipation might be involved in the regulation of the CAR: For example, higher stress anticipation was linked to an increased CAR in a longitudinal single-case study (Stalder et al., 2010). Similarly, students showed a larger increase in the CAR on a day with a realistic mock examination (i.e., taking place at the same location and at the

same time as the official exam) compared to relaxation days (González-Cabrera et al., 2014). Using a controlled experimental design, Elder and colleagues (2018) provided more causal evidence on the importance of anticipation for the modulation of the CAR: Individuals expecting to participate in a competition the next day involving cognitively demanding tasks showed a significantly larger CAR than participants in the control condition expecting to perform relaxing activities. Taken together, these studies suggest a crucial role of the CAR in preparing individuals to cope with the challenges of the upcoming day. However, other studies yielded contradictory findings: Cropley and colleagues (2015) found work-related thoughts in the morning to be associated with a lower CAR and Powell and Schlotz (2012) reported no significant relation between anticipated stress for the upcoming day and the CAR. While college students showed larger increases in the CAR when stress was anticipated for the upcoming day, this association did not remain significant when controlling for sleep duration (Vargas and Lopez-Duran, 2014).

In sum, results are inconsistent and do not permit clear conclusions about the role of anticipatory stress in the regulation of the CAR. To enhance our understanding of these regulatory processes, two major issues need to be addressed: the time of measurement of anticipatory stress and the level of effects investigated. Provided that anticipatory stress was measured, the discussed studies assessed anticipatory stress on the same morning as the CAR, after awakening. However, according to the CAR anticipation hypothesis the magnitude of the CAR is linked to the activation of memory representations at awakening concerning the upcoming demands. As such, these representations need to be formed prior to the onset of the CAR, that is, prior to awakening. In other words, if stress anticipation shapes the CAR, it is reasonable to assume that underlying processes occur preceding the onset of the CAR. Consequently, to accurately reflect such temporal processes, anticipatory stress should be assessed before the rise in post-awakening cortisol occurs. As the CAR starts with the moment of waking up, anticipatory stress needs to be measured prior to awakening, that is, before going to sleep. The present study addresses this point by assessing anticipatory stress in the evening prior to the onset of the CAR, hence providing a more rigorous test of the CAR anticipation hypothesis.

Further, differences between persons do not necessarily correspond to differences within persons both conceptually and empirically (e.g., Hoffman and Stawski, 2009). Recently refined statistical methods such as multilevel modeling allow separating between-person from within-person effects in repeated measures designs. Indeed, parts of the inconsistency in empirical findings might be attributed to the different levels investigated (between- vs. within-person). Considering those studies analyzing within-person relations, results depict a clear trend: Higher anticipatory stress is associated with an elevated CAR (González-Cabrera et al., 2014; Stalder et al., 2010). Conversely, studies using single time-point measures provide inconclusive evidence: Some studies show greater anticipatory stress to be linked to an increased CAR (Elder et al., 2018) whereas others report a decreased CAR (Cropley et al., 2015) or no significant relation (Powell and Schlotz, 2012). However, analyses in these cross-sectional studies confound within- and between-person variance and thus, effects cannot be uniquely assigned to one level. To enhance our understanding of within- and between-person effects, the current study aims at disentangling both levels of analysis by employing a micro-longitudinal approach. On the basis of prior research, we expected that greater self-reported anticipatory stress in the evening would predict a more pronounced CAR the next morning on the within-person level.

2. Methods and Materials

2.1 Participants

Participants were recruited via an online platform at Heidelberg University advertising ongoing psychological studies. Individuals who expressed interest in the study received written information about the purpose and protocol of the investigation and completed an online questionnaire assessing eligibility. Eligibility criteria were: (1) Age between 18 and 40 years, (2) right-handedness, (3) no smoking, (4) lack of any interfering disorders, including chronic physical disease, psychiatric or neurological disease, and psychological disorders, (5) no permanent intake of medication, (6) no surgical intervention within the last 3 months, (7) no use of hormonal birth control and no pregnancy and/or breastfeeding for female participants, and (8) possession of a smartphone using the android operating system (version 4.0 or higher). The final sample consisted of 42 participants (69% female)

with the mean age of 22.8 years (*SD* = 3.25, range = 18-30 years). Most participants were German native speakers (88.1%). Non-native speakers had adequate German language skills to understand instructions and follow the study protocol. All participants provided written informed consent prior to the study procedures. The study was approved by the ethics committee of the Faculty of Behavioural and Cultural Studies at Heidelberg University and was carried out in accordance with the Declaration of Helsinki.

2.2 Procedures

The current study was part of a project investigating stress reactivity in a laboratory setting and during everyday life using ambulatory assessment. Interested participants filled in an online questionnaire in a first step to determine eligibility. Of the 60 participants who completed this assessment, 51 fulfilled all criteria and were invited to the second part of the study, which consisted of a laboratory based assessment using the Trier Social Stress Test (TSST; Kirschbaum et al., 1993), that is not part of the present analyses. Nine participants did either not schedule an appointment for the upcoming sessions or they scheduled an appointment but did not show up, yielding a final sample of 42 participants. At the end of the laboratory assessment, the movisensXS-app (version 1.0.0 and version 1.1.0, movisens GmbH, Karlsruhe, Germany), which was used for administering all questionnaires during the ambulatory assessment, was installed on participants' personal smartphones. Participants received (oral and written) information on how to proceed during the following five assessment days in their daily lives, and obtained the necessary equipment to collect the cortisol samples. They indicated the time at which they would get up on each of the following five days so that the movisensXS-app could be programmed to start the first survey of the day at the given time. This forced awakening procedure was chosen to ensure the collection of the first cortisol sample immediately upon awakening. In this context, the meaning of 'the moment of being awake' was explained precisely to the participants and the importance of adherence to the sampling protocol was emphasized. Since the research question addressed in the present study focusses on the daily life context, the following sections will provide a description of the procedure and measurements used in this ambulatory assessment phase.

Each ambulatory assessment day started with the so-called *morning survey*, which required participants to provide the first saliva sample, indicate the amount of minutes passed since waking up, and fill in several questionnaires. Completion of the morning survey took approximately 3 minutes. Participants were asked to refrain from eating, drinking, smoking and brushing their teeth within the next 30 minutes to prevent any influences on cortisol secretion in the post-awakening period. The morning survey determined the onset of four further surveys (further referred to as *saliva surveys*) with fixed timing throughout the day, which were scheduled 0.5, 2.5, 8 and 12 hours after the morning survey. During these surveys, participants collected saliva samples and responded to questionnaires along with control questions. Completion of the questionnaires and providing the sample took approximately 4 minutes. Additionally, participants received 15 prompts for further self-report data collection per day (*random surveys*), which occurred randomly within 14 hours after the morning survey (with the additional constraint that they were spaced at least 25 minutes apart). The random surveys consisted of a short questionnaire, which took less than 1 minute to complete. Before brushing their teeth and going to bed, participants were instructed to initiate the final assessment of the day. During this *evening survey*, participants again responded to several questionnaires and collected the last saliva sample. Completing the evening survey took approximately 7 minutes. Participants were instructed to store all saliva samples in their freezer or fridge until the end of the study. The day after their last daily assessment (i.e., six days after the TSST) all participants reported to the laboratory again, returned the cortisol samples and filled in a final set of questionnaires. Of the potential 210 morning assessments (42 participants x 5 days), 198 were filled in, corresponding to a compliance rate of 94.3%. Similar numbers were obtained for the saliva surveys (90.8% of potential 840 assessments), the random surveys (86.5% of potential 3150 assessments), and the evening surveys (98.6% of potential 210 assessments). Participants received 100€ compensation and – if they had completed more than 85% of all questionnaires in the ambulatory assessment phase – they obtained additional 15€.

2.3 Measurements

2.3.1 Cortisol Awakening Response

To assess cortisol, participants collected saliva samples using SaliCaps® (IBL, Hamburg, Germany). Participants accumulated saliva for 60 seconds before drooling it into the tube using a plastic straw. Labels on the tubes indicated the number of the test person, the assessment day (ranging from 1-5) and the sample within the day (ranging from 1-6). The CAR was estimated by calculating the difference between the cortisol concentration of the first and the second saliva sample. Thus, the estimated CAR reflects the change in cortisol levels from the first (awakening) to the second measurement (+30 min). Given the comprehensive study design (with 21 daily assessments), we did not follow the recommendation of using at least three saliva samples to assess the CAR (Stalder et al., 2016) to keep the burden for participants at a reasonable level. We utilized self-reports (instead of objective measurements) to determine awakening times. The app enabled objective monitoring of adherence to the sampling protocol via encoding the time point at which participants answered a survey and thus, received the instructions to collect the sample.

2.3.2 Anticipatory Stress

Stress anticipation of the next day was measured during the evening survey using an adapted version of the Anticipatory Stress Questionnaire (ASQ; Powell and Schlotz, 2012), which consisted of four items. The items were presented during the evening survey and responses were given on a seven-point Likert-Scale (1 = *I completely disagree*; 7 = *I completely agree*). The adapted ASQ comprises German translations of the following items: (1) “I expect tomorrow to be a stressful experience.”; (2) “I feel in control of those events I expect to occur tomorrow.”; (3) “I am confident I can cope with what challenges tomorrow will present.”; (4) “I am worried about how tomorrow will turn out.”. Item scores were averaged with reversed items (2 and 3) being recoded so that higher values represented greater anticipatory stress. Within-person internal consistency (within-person McDonald's ω; Geldhof et al., 2014) was .74 in this sample. The reliability on the between-person level (between-person McDonald's ω; Geldhof et al., 2014) was estimated as .92.

2.3.3 Covariates

Stress experience and sleep may relate to anticipatory stress as well as the CAR. Therefore, we included the daily stress level of the previous day (i.e., measured on the same day as stress anticipation) as well as sleep quality and sleep duration (measured on the same day as the CAR) as

covariates in the analysis. Current stress experience was assessed on each of the total of 21 measurements per day using one item ("At the current moment I feel stressed"). Answers were given on a visual analog scale (VAS) ranging from *not at all* (coded as 0) to *very* (coded as 100) with higher scores indicating an elevated current stress level. Daily stress levels were computed as the average of all current stress ratings during each assessment day. Sleep quality was assessed in the morning survey by asking participants how well they had slept and how restful their sleep had been on a VAS ranging from *very bad* to *very good* and from *not restful at all* to *very restful*, respectively. Item scores were averaged into one subjective sleep quality score per night and person with 0 representing the lowest and 100 the highest possible value. Higher scores indicated better subjective sleep quality. The within-person correlation of the two items was .64; the between-person correlation amounted to .80. Sleep duration was also measured during the morning survey. Participants indicated how long they had slept during the previous night. Answers referred to length in hours (decimal hours were allowed). Additional covariates included the cortisol level at awakening and sex. For an illustration of the different time points of time-varying measures used to predict the estimated CAR, please see Figure 1.

2.4 Cortisol Analysis

After participants had returned the saliva samples to the laboratory the samples were frozen at -80°C until analysis. Prior to analysis, samples were centrifuged at 3000g for 10 minutes. Salivary cortisol levels were measured in duplicate using an enzyme-linked immunosorbent assay (ELISA; RE52611, IBL International, Hamburg, Germany), at the biochemical lab at the Institute of Medical Psychology, University Hospital Heidelberg. We used the mean value (in nmol/L) of both measurements for all analyses. Cortisol samples showing red or blue discoloration were excluded from analysis as this indicates contamination of samples. The inter-assay coefficient of variation (CV) was 7.45%, and the intra-assay CV of all remaining cortisol samples (referring to all samples collected per day) was 6.58%. With regards to the estimated CAR, participants provided 200 out of the potentially available 210 CAR estimates. The exclusion of red or blue cortisol samples pertained to two CAR estimates. Repeated determination of one sample's cortisol concentration yielded values outside the measurable range (0.41-82.8 nmol/L). Therefore, the CAR estimated on the basis of this

sample was excluded from analysis. For statistical analyses, the first sample of the day was excluded if it was collected more than 15 minutes after (self-reported) awakening (n = 26) to ensure an accurate estimation of the CAR (Stalder et al., 2016) or if no data was available on how many minutes ago the participant had woken up (n = 11). This led to a final sample of 160 CAR estimates. Due to missing values on predictor variables, effective sample sizes can vary from model to model (see Table notes for details).

2.5 Statistical Analysis

We tested our hypothesis using multi-level models, also known as hierarchical linear models (HLM; Raudenbush and Bryk, 2002) to account for the nested data structure (observations nested within individuals). In order to distinguish within-person from between-person effects, the predictor as well as the covariates (except for sex) were centered using two different methods before being entered into the model (e.g., Hoffman and Stawski, 2009). First, all time-varying variables (anticipatory stress, previous day's daily stress, sleep quality, sleep duration, cortisol level at awakening) were centered on the person-mean. Second, person-level variables (the person-means of all time-varying variables) were centered on the grand-mean. On the person-level, we also included sex as a covariate (with value of 0 representing female sex). To test the study hypothesis, the estimated CAR was modeled as a function of both time-varying and person-level variables. In the first step, effects of all variables were modeled to be constant across participants resulting in a random-intercept-fixed-slope-model (Model 1). Next, the effects of time-varying predictors were allowed to vary across persons, resulting in a random-intercept-random-slope-model. Notably, a model with all five random slopes included failed to converge. We therefore removed random effects from the model in a stepwise approach. This resulted in a model including three random effects. As there are ten possibilities to include three (out of five possible) random effects in the model, we estimated the ten different versions of the model. Of those ten models, we report the best fitting model (determined as the model with the smallest Akaike Information Criterion; AIC) below (Model 2). Detailed results on the nine alternative models (Model a-i) can be found in Table A of the online supplemental material.

Models were compared using likelihood ratio tests for nested models as well as the AIC and variance explained (R^2; computation based on Xu, 2003). Graphical inspections of the residuals of the final model as well as descriptive statistics of skew and kurtosis (|both| < 1) indicated that distributional assumptions of the linear model were met. Analyses were performed using the nlme package (Pinheiro et al., 2017) in R (version 3.5.1 for Windows; R Core Team, 2018) as well as Mplus (version 8; Muthén and Muthén, 1998-2017).

3. Results

Table 1 depicts descriptive statistics on the between- and within-person level. All time-varying variables exhibited variation on both the between- and the within-level, with intra-class correlations ranging from .18 to .74. The estimated CAR ranged between -31.03 and 56.57 nmol/L with a mean of 7.89 nmol/L (*SD* = 5.94 nmol/L) across all days and persons. Correlations between variables were small or modest across and within persons, except for the correlation between the CAR estimates and the cortisol level at awakening on the within-person level, which amounted to r = -.52. On the between-person level, anticipatory stress correlated highly with daily stress (r = .59) and sleep quality (r = -.51).

3.1 Main Analysis

Comparing the random-intercept-fixed-slope-model (Model 1) to the model including random effects for anticipatory stress, sleep quality, and the cortisol level at awakening (Model 2) revealed a better model fit of the latter, $\chi^2(9) = 25.202$, p = .003. The respective parameter estimates of both models are presented in Table 2[1].

Referring to the upper panel of Table 2, labeled *fixed effects*, Model 2 revealed that participants showed an elevated CAR estimate on days for which they had anticipated higher stress

[1] Please note that our primary research hypothesis only regards the within-person effect of stress anticipation on the CAR estimate. The other tests reported in this section should be considered exploratory. Because of multiple testing, there is an overall increased probability for chance findings involving the effects of the other predictors / covariates in the model.

the night before, b = 1.884, 95% CI [0.173; 3.595]. This finding is in line with the CAR anticipation hypothesis. On the between-person level, there was no effect of anticipatory stress, b = 0.334, 95% CI [-1.896; 2.564]. That is, participants anticipating generally more stress (averaged over all days) did not show a more pronounced CAR estimate. Taken together, the findings support the CAR anticipation hypothesis on the within- but not on the between-person level (see Figure 2 for an illustration of the difference between the two levels investigated)[2]. Furthermore, model results indicate that yesterday's stress experience was also related to today's CAR estimate: Participants showed a larger CAR estimate when their previous day had been more stressful than usual (b = 0.200, 95% CI [0.061; 0.338]). Additionally, the cortisol level at awakening predicted the CAR estimate on the within- as well as on the between-person level. That is, on days with a higher cortisol concentration at awakening than usual participants displayed a blunted estimated CAR (b = -0.916, 95% CI [-1.182; -0.650]) and participants displaying generally higher cortisol levels at awakening were characterized by an attenuated CAR estimate (b = -0.384, 95% CI [-0.652; -0.116]). The lower panel of Table 2, labeled *random effects*, indicates that participants differed in the extent to which anticipatory stress, sleep quality, and the cortisol level at awakening affected the CAR estimates.

3.2 Sensitivity Analyses

To explore the stability of the results we conducted a series of sensitivity analyses. First, we re-estimated the final model excluding negative CAR estimates from the analysis. The CAR is supposed to reflect an increase in cortisol levels after awakening, and thus, negative CAR estimates may indicate measurement error or lack of compliance with instructions, potentially biasing the results. Second, we followed the recommendation of Stalder and colleagues (2016) to repeat the analysis only including cortisol samples collected within 5 minutes after awakening. Biased estimates of the CAR have been well established with a sampling delay of 15 minutes (see Stalder et al., 2016). For this reason, we had initially excluded samples collected later than 15 minutes after awakening.

[2] When including time as an additional predictor – either with a fixed or with a random slope – the model results did not change. In both models, time did not predict the CAR estimate.

However, the influence of smaller sampling delays on cortisol measurements is less well known. Thus, we intended to check whether the results of our final model also hold when only using very accurately collected samples. Third, we combined the two approaches and excluded cases with a sampling delay of more than 5 minutes as well as negative CAR estimates to test whether our results hold under very conservative restrictions. Finally, we tested whether the results were robust when excluding outliers using the ± 3 *SD*s criterion. This means that CAR estimates were excluded when the respective value was 3 *SD*s above or below the sample mean of the respective day[3]. Results of the sensitivity analyses can be found in Table 3.

Excluding negative CAR estimates (Model S1) did not change the pattern of results on the within-person level reported for the main analysis. However, the confidence interval of the between-person effect of the cortisol level at awakening contained the value of zero, b = -0.024, 95 % CI [-0.376; 0.329]. When including only cortisol samples collected within 5 minutes after awakening (Model S2), the effects on the between-person level remained unchanged compared to the main analysis. On the within-person level, however, previous day's stress no longer predicted the estimated CAR, b = 0.095, 95% CI [-0.042; 0.231]. This was also the case for the model additionally excluding negative CAR estimates (Model S3), b = 0.021, 95% CI [-0.132; 0.174]. Further, on the between-person level, the cortisol level at awakening did not predict the magnitude of the estimated CAR in this model, b = -0.004, 95 % CI [-0.321; 0.313]. Three CAR estimates were 3 *SD*s above or below the daily sample mean. Excluding these outliers (Model S4) did not change the model results reported in the main analysis. In all models (S1-S4), the 95% confidence intervals of the within-person effect of anticipatory stress did not include zero.

4. Discussion

To the best of our knowledge, this is the first study to investigate temporal dynamics of within-person anticipatory stress processes in participants' daily lives that are assumed to impact the

[3] There were no outliers on the within-person level (i.e., using the criterion of ± 3 pooled within-person *SD*s above the respective person mean).

magnitude of the CAR. Therefore, our work provides a more direct test of the CAR anticipation hypothesis. Furthermore, the adoption of a repeated measurement ambulatory assessment design across five days in combination with the use of multi-level modeling allowed a disaggregation of within- and between-person effects. In line with our expectations, results indicated that higher anticipatory stress was associated with a more pronounced CAR the next day, consistent with the CAR anticipation hypothesis on the within-person level. On the between-person level anticipatory stress was not linked to the magnitude of the CAR, suggesting no overall differences in the CAR between individuals with higher vs. lower average stress anticipation. Thus, results illustrate the importance of distinguishing within-person from between-person effects.

4.1 The Association Between Anticipatory Stress and the CAR on the Within-Person Level

On the within-person level, our results corroborate previous findings on the relationship between anticipatory stress and the CAR (González-Cabrera et al., 2014; Stalder et al., 2010) and imply a fundamental role of anticipation of stress and demands: A stronger increase in cortisol levels during the post-awakening period was observed, when more stress than usual had been anticipated for this day (indicated by the participants the night before). The robustness of this finding was further corroborated by the sensitivity analyses: When excluding cases, which might have biased the results (i.e., days with negative CAR estimates, days with sampling delay, or outliers), the within-person effect of anticipatory stress remained significant. Of note, in most of these models there were less observations available than in the main analysis (the number of observations ranged from 89-127 in the sensitivity analyses). Fewer observations reduce the power of the analyses and might also lead to instable parameter estimation, which may explain the slight change in the pattern of effects seen in some of the models (S1-S4). In this context of instable estimates, the fact that the within-person effect of anticipatory stress remained significant in all models supports the stability of the results.

Our study provides support for the CAR anticipation hypothesis, which proposes that the CAR prepares the organism to deal with upcoming challenges and that the CAR is adaptive, that is, its magnitude differs depending on the extent or intensity of anticipated demands (e.g., Fries et al., 2009; Powell and Schlotz, 2012). A higher CAR potentially reflects an increased mobilization of energy,

which, in turn, might help the organism to cope with the anticipated demands (Adam et al., 2006). Hence, the present results suggest an important role for stress anticipation in the modulation of the CAR. Collecting information on what types of stressors were anticipated could provide interesting insights on potential moderating mechanisms. Specific characteristics of anticipated demands may further affect the CAR. For example, in their meta-analysis Dickerson and Kemeny (2004) found stressors characterized by social-evaluative components (where performance can be judged by others) or uncontrollability (where behavioral responses do not influence the outcome of the situation) to elicit greater stress responses than non-evaluative or controllable stressors. Accordingly, investigating the magnitude of the CAR as a function of different characteristics of anticipated stressors might present a promising avenue for future research. In addition, there is evidence that not only the anticipation of adverse outcomes but also of positive events influences the magnitude of the CAR: Children showed an increased CAR on days when they anticipated getting a present compared to control days (Bäumler et al., 2014). Hence, the CAR may be modulated by anticipation processes in general.

In addition to stressor types, future research should also consider to study psychological processes that might play an important role for the anticipation-CAR link: Anticipating stressful events might help the individual to take preparatory actions and as such facilitate proactive as well as anticipatory coping (see Neupert et al., 2018). Proactive coping refers to efforts or strategies undertaken to prevent the occurrence of a stressor (Aspinwall and Taylor, 1997) whereas anticipatory coping refers to efforts and strategies to minimize the possible negative consequences of an upcoming stressful event (Folkman and Lazarus, 1985). Accordingly, anticipation of upcoming demands could improve coping with the respective stressor when it occurs. In line with this assumption, Neupert and Bellingtier (2018) showed that younger adults experienced less negative affect in reaction to home stressors (but not other stressor types) when an increased amount of home stressors had been anticipated.

With regard to the adaptive role of the CAR, it is of note that the present sample consisted of healthy young adults who reported to anticipate relatively minor everyday life stressors. In other populations, however, an increased CAR in reaction to more intense stress anticipation may not

necessarily be adaptive. Furthermore, based on the Allostatic Load Model (McEwen, 2017) it might be expected that chronic or repeated stress anticipation and resulting increases in the CAR might lead to adaptation processes with reduced central nervous feedback sensitivity of the HPA axis and long-term alterations in these processes. As such, the adaptive role of the CAR could wear off under conditions of chronic stress anticipation. This might be the case in, for example, individuals with major depression, a condition that has been characterized by chronically elevated cortisol levels (Plotsky et al., 1995). In reaction to negative events or stressors, participants with depression did not show significant elevations of cortisol compared to healthy controls in a study by Peeters and colleagues (2003), suggesting that depressed individuals show altered neuro-endocrinological processes. As a consequence, a more pronounced CAR in response to stress anticipation may not necessarily reflect an adaptive process in this population.

4.2 The Association Between Anticipatory Stress and the CAR on the Between-Person Level

Persons anticipating more stress in general did not display elevated CARs. Thus, with regard to between-person effects the present results are in line with another study investigating the CAR anticipation hypothesis in the daily life context that also did not find a reliable association (Powell and Schlotz, 2012). However, our results do not match previous findings from an experimental study in a controlled laboratory setting which reported a positive relationship between anticipatory stress and the CAR (Elder et al., 2018). This might indicate that effects established in a laboratory setting do not necessarily transfer to the daily life context. Nevertheless, when comparing our between-person effect (or the lack thereof) to these findings it must be noted that analyses in both studies (Elder et al., 2018; Powell and Schlotz, 2012) confounded within-person and between-person variance, given the cross-sectional nature of these studies. Therefore, it is not possible to attribute the results of these studies uniquely to the between-person level. Accordingly, more research disentangling both levels is needed to draw firm conclusions about between-person relations of anticipatory stress and the CAR. In addition, it is also important to carefully interpret the finding on the between-person level in our study. Our sample consisted of 42 participants and thus, with respect to the between-person level statistical power was limited. A larger sample might be needed to detect small to medium between-

person effects. For this reason, findings regarding the between-person analyses need to be interpreted very cautiously and we do not intend to draw strong conclusions from these particular results obtained in our study.

4.3 Strengths and Limitations

Using a micro-longitudinal approach in everyday life allowed clarification of underlying temporal dynamics regarding stress anticipation and the CAR in an ecologically valid setting. In combination with the statistical approach, the longitudinal design permitted to separate within- from between-person effects. Furthermore, several sensitivity analyses confirmed the stability of the findings from our main analyses and therefore support the robustness of the within-person effect of anticipatory stress on the CAR. To the best of our knowledge, the present study was the first to provide a test of the CAR anticipation hypothesis on the within-person level using stress anticipation measured prior to the onset of the CAR as predictor of the CAR. As such, the present investigation extends studies with an experimental focus on anticipatory stress (Elder et al., 2018) or naturally occurring stressors (González-Cabrera et al., 2014), which did not include measures of subjective stress anticipation. Above this, the present study addressed the temporal dynamics of the transmission processes (cf. Cropley et al., 2015; Powell and Schlotz, 2012; Stalder et al., 2010; Vargas and Lopez-Duran, 2014). In this regard, the present work provides valuable information that enhances our understanding of the function of the CAR.

Nevertheless, the findings should be interpreted in light of several limitations: First, concerning the number of saliva samples, it is recommended to use at least three samples in the post-awakening period to accurately capture the CAR as peak levels can occur within 45 minutes after awakening (Stalder et al., 2016). Thus, peak levels of cortisol secretion were possibly missed in the present study as we used only two samples within 30 minutes after awakening to assess the CAR. The number of required samples was restricted to a minimum in order to avoid a decrease in participants' motivation to adhere to the (rather burdensome) study protocol. We therefore collected only two samples to estimate the CAR, following the procedures used in past research (e.g., Adam et al., 2006; Griefahn and Robens, 2008; Wetherell et al., 2015). However, we expect that a possibly inaccurate

assessment of the CAR might have reduced the power of the statistical analyses, thus – if anything – resulting in an underestimation of effects of anticipated stress on the CAR.

Second, the lack of objective tools to assess the time of awakening constitutes another limitation of the present study. The expert consensus guidelines (Stalder et al., 2016) recommend objective monitoring of awakening to guarantee an accurate assessment of the CAR. We did not implement objective tools (e.g., actigraphy) and thus, we cannot completely rule out the possibility that participants did not collect the first sample immediately upon awakening. However, the app administering the questionnaires and instructions for saliva collection was programmed to start the first assessment of the day at the same time when the participant's alarm would go off, which should then, in turn, lead to accurate sampling immediately upon awakening. The timing of the alarm was set by the participants on the day prior to the ambulatory assessment phase. Nevertheless, participants may have woken up prior to the planned wake-up time. Therefore, we also relied on participant's self-reports to exclude days on which they had woken up more than 15 minutes prior to filling in the morning survey and thus, collecting the first sample. Because participants received detailed instructions for sample collection each time sampling was required, it seems unlikely that participants postponed the sample collection but answered the questionnaires. Still, due to the lack of objective monitoring the estimates of the CAR might have been assessed with less precision, which might explain the presence of 18 (out of 131) negative CAR estimates, potentially indicating method bias (see Stalder et al., 2016)[4].

[4] The timing of cortisol sampling in relation to awakening is *the* most central point in the interpretation of the CAR. In this context, one could argue that a description like "post-awakening cortisol slope" should be used instead of the term "CAR". When referring to the operationalization of the construct CAR we decided to use terms indicating that our measure reflects an estimate of the construct and not the CAR itself (i.e., "post-awakening cortisol increase" or "CAR estimate"). However, when referring to the theoretical level, that is, to the construct "CAR", we retained the term "CAR" for two reasons: First of all, the theoretical framework of our study is formed by empirical work on demand anticipation and the cortisol increase after awakening. Not all of the studies that we refer to implemented objective tools to assess the time point of awakening. Nevertheless, they all use the term "CAR". To embed our findings in the context of these studies and to provide a useful interpretation of our results, we consider it important to use the same terminology to facilitate the integration of our findings with previous literature. Further, the forced awakening approach increased the likelihood of sample collection immediately upon awakening.

Finally, including only young and healthy adults restricted the representativeness of the sample and thus the generalizability of the results. Larger and more heterogeneous samples would allow for more thoroughly investigating (a) the generalizability of the findings beyond a student population, (b) factors potentially moderating the anticipation-CAR association, and (c) the between-person association (or lack thereof) of stress anticipation and the CAR.

5. Conclusions

Taken together, this study provides compelling evidence for the role of anticipated demands in the modulation of the CAR. Our findings point towards a key role of the CAR in preparing the organism to cope with challenges of the upcoming day and thus proved evidence consistent with the CAR anticipation hypothesis (Powell and Schlotz, 2012) on the within-person level. Future research could investigate whether the CAR differs depending on the characteristics or quality of anticipated stressors. Above this, these results can lay the ground for investigating the clinical relevance of the CAR and further adaptation processes in the anticipation of substantially stressful events. Finally, the current results clearly point to the dissociation of within- and between-person effects. We therefore encourage the use of study designs and statistical approaches enabling the segregation of effects.

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Table 1

Descriptive Statistics.

						Correlations						
	Mean	Range	Between *SD*	Within *SD*	ICC	Sex[a]	CAR[b]	Cortisol Level at Awakening[b]	Anticipatory Stress	Daily Stress	Sleep Quality	Sleep Duration[c]
Sex[a]	0.31	0 - 1	0.46			-						
CAR[b]	7.89	-31.03 - 56.57	5.94	8.81	.31	-.16	-	-.52***	-.09	.25** *	.18	.01
Cortisol Level at Awakening[b]	11.36	0.92 - 71.05	5.06	7.25	.33	.30	-.07	-	.06	-.11	-.19	-.01
Anticipatory Stress	3.01	1.00 - 6.75	0.85	1.00	.42	.10	.31	.10	-	.06	-.13	.06
Daily Stress	26.77	0.63 - 83.48	14.71	8.83	.74	-.09	.24	.21	.59***	-	-.06	.10
Sleep Quality	57.55	1.5 - 98.5	9.59	16.45	.25	.23	-.22	.12	-.51*	-.27	-	.38***
Sleep Duration[c]	7.22	2 - 14	0.71	1.52	.18	.30*	-.35	.08	-.26	-.09	.39	-

Note. Between-person correlations are depicted below the diagonal, within-person correlations are shown above the diagonal. CAR = Estimated Cortisol Awakening Response; ICC = Intra-class Correlation Coefficient.

[a]0 = female; 1 = male. [b]in nmol/L. [c]in hours.

*$p \leq .05$; ***$p \leq .001$.

Table 2

Multilevel Models of Anticipatory Stress Predicting the Estimated CAR.

	Model 1		Model 2	
	Fixed Effects			
	Est.	95% CI	Est.	95% CI
Intercept	**7.259**	**[4.785; 9.734]**	**7.112**	**[4.849; 9.375]**
Within lag Anticipatory Stress	**1.831**	**[0.341; 3.322]**	**1.884**	**[0.173; 3.595]**
Within lag Daily Stress	**0.261**	**[0.101; 0.420]**	**0.200**	**[0.061; 0.338]**
Within Sleep Quality	0.087	[-0.015; 0.189]	0.116	[-0.020; 0.253]
Within Sleep Duration	-0.338	[-1.386; 0.711]	0.290	[-0.598; 1.177]
Within Cortisol Level at Awakening	**-0.621**	**[-0.810; -0.431]**	**-0.916**	**[-1.182; -0.650]**
Between Anticipatory Stress	0.588	[-2.209; 3.384]	0.334	[-1.896; 2.564]
Between Daily Stress	0.149	[-0.008; 0.306]	0.070	[-0.062; 0.203]
Between Sleep Quality	0.028	[-0.171; 0.228]	0.010	[-0.147; 0.167]
Between Sleep Duration	-1.132	[-3.435; 1.170]	-0.516	[-2.406; 1.373]
Between Cortisol Level at Awakening	**-0.340**	**[-0.673; -0.007]**	**-0.384**	**[-0.652; -0.116]**
Sex[a]	1.454	[-3.458; 6.366]	0.310	[-3.812; 4.432]
	Random Effects (Variances)			
Intercept	25.102		27.121	
Within lag Anticipatory Stress	-		11.001	
Within sleep quality	-		0.092	
Within cortisol level at awakening	-		0.242	
Residual	49.489		23.112	
	Model Fit Parameters			
AIC	949.652		942.45	
R^2	.421		.729	

Note. Table depicts point estimates. The word lag indicates that the respective predictor was measured the day prior to the assessment of the CAR. For better clarity, parameters are printed in bold, if the confidence interval does not include the value zero. Computation of R^2 based on Xu (2003). Number of participants = 41; total number of observations = 131; Est. = Estimate; CI = Confidence Interval; AIC = Akaike Information Criterion.

[a]0 = female; 1 = male.

Table 3

Multilevel Models of Anticipatory Stress Predicting the Estimated CAR Based on Specific Subsamples.

	Model S1		Model S2		Model S3		Model S4	
	Fixed Effects							
	Est.	95% CI	Est.	95% CI	Est.	95% CI	Est.	95% CI
Intercept	**8.424**	**[6.428; 10.420]**	**5.855**	**[3.553; 8.158]**	**7.847**	**[5.847; 9.846]**	**6.957**	**[4.821; 9.093]**
Within lag Anticipatory Stress	**1.915**	**[0.167; 3.663]**	**2.619**	**[0.486; 4.752]**	**2.642**	**[0.359; 4.924]**	**1.524**	**[0.169; 2.880]**
Within lag Daily Stress	**0.164**	**[0.013; 0.316]**	0.095	[-0.042; 0.231]	0.021	[-0.132; 0.174]	**0.154**	**[0.009; 0.299]**
Within Sleep Quality	0.086	[-0.057; 0.229]	0.065	[-0.086; 0.217]	-0.007	[-0.160; 0.146]	0.128	[-0.005; 0.260]
Within Sleep Duration	0.080	[-0.862; 1.022]	**0.858**	**[0.023; 1.693]**	0.861	[-0.001; 1.723]	0.189	[-0.680; 1.058]
Within Cortisol Level at Awakening	**-0.650**	**[-1.046; -0.253]**	**-1.143**	**[-1.472; -0.813]**	**-0.646**	**[-1.053; -0.238]**	**-0.665**	**[-0.862; -0.467]**
Between Anticipatory Stress	1.414	[-0.728; 3.556]	0.394	[-1.559; 2.347]	1.644	[-0.104; 3.392]	0.574	[-1.665; 2.813]
Between Daily Stress	0.038	[-0.079; 0.154]	0.053	[-0.067; 0.173]	0.017	[-0.085; 0.119]	0.064	[-0.070; 0.198]
Between Sleep Quality	0.078	[-0.058; 0.214]	0.019	[-0.128; 0.166]	0.102	[-0.022; 0.226]	0.039	[-0.120; 0.197]
Between Sleep Duration	-0.447	[-2.146; 1.251]	-0.297	[-1.975; 1.380]	-0.413	[-1.855; 1.028]	-0.736	[-2.648; 1.176]
Between Cortisol Level at Awakening	-0.024	[-0.376; 0.329]	**-0.525**	**[-0.752; -0.297]**	-0.004	[-0.321; 0.313]	**-0.441**	**[-0.705; -0.176]**
Sex[a]	1.387	[-2.416; 5.191]	1.858	[-1.913; 5.629]	0.917	[-2.372; 4.206]	-0.128	[-4.189; 3.932]
	Random Effects (Variances)							
Intercept	14.583		26.382		10.953		22.193	
Within Anticipatory Stress	10.708		19.249		24.295		2.700	
Within sleep quality	0.082		0.105		0.923		0.081	

Within cortisol level at Awakening	0.225	0.311	0.345	0.096
Residual	21.861	13.347	11.718	24.935

Note. Table depicts point estimates. Model S1 represents the model including only positive cortisol awakening reactions (number of participants = 40; total number of observations = 113); model S2 denotes the model including only samples collected within 5 minutes after awakening (number of participants = 40; total number of observations = 100); model S3 represents a modification of model S2 by additionally excluding negative cortisol awakening reactions (number of participants = 39; total number of observations = 89); in model S4 outliers were excluded from the analysis (number of participants = 41 ; number of observations = 127). The word lag indicates that the respective predictor was measured the day prior to the assessment of the CAR. For better clarity, parameters are printed in bold, if the confidence interval does not include the value zero. Est. = Estimate; CI = Confidence Interval.
[a]0 = female; 1 = male.

Online Supplemental Material

Table A

Parameter Estimates for 9 Multilevel Models Predicting the Estimated CAR with Random Effects on Different Predictors.

	Model a		Model b		Model c		Model d		Model e	
	Fixed Effects									
	Est.	95% CI	Est.	95% CI	Est.	95% CI	Est.	95% CI	Est.	95% CI
Intercept	**7.229**	**[4.723; 9.735]**	**7.732**	**[5.182; 10.282]**	**7.613**	**[5.073; 10.152]**	**7.365**	**[4.827; 9.903]**	**7.747**	**[5.206; 10.288]**
Within lag Anticipatory Stress	**2.088**	**[0.432; 3.744]**	**2.127**	**[0.635; 3.618]**	**1.923**	**[0.667; 3.180]**	**2.025**	**[0.371; 3.679]**	**1.914**	**[0.626; 3.202]**
Within lag Daily Stress	**0.221**	**[0.051; 0.391]**	**0.234**	**[0.042; 0.425]**	**0.218**	**[0.040; 0.396]**	**0.234**	**[0.042; 0.427]**	**0.216**	**[0.017; 0.415]**
Within Sleep Quality	0.052	[-0.035; 0.139]	0.097	[-0.009; 0.203]	0.110	[-0.010; 0.230]	0.066	[-0.025; 0.156]	0.107	[-0.004; 0.218]
Within Sleep Duration	0.033	[-0.895; 0.960]	-0.006	[-0.944; 0.932]	-0.001	[-0.926; 0.925]	-0.212	[-1.320; 0.895]	-0.225	[-1.173; 0.723]
Within Cortisol Level at Awakening	**-0.810**	**[-1.040; -0.580]**	**-0.532**	**[-0.706; -0.357]**	**-0.635**	**[-0.842; -0.429]**	**-0.636**	**[-0.806; -0.465]**	**-0.493**	**[-0.682; -0.304]**
Between Anticipatory Stress	0.160	[-2.448; 2.768]	0.344	[-2.335; 3.022]	0.492	[-2.223; 3.207]	0.157	[-2.562; 2.877]	0.669	[-2.100; 3.438]
Between Daily Stress	0.091	[-0.059; 0.240]	0.131	[-0.023; 0.286]	0.132	[-0.019; 0.284]	0.117	[-0.037; 0.270]	0.150	[-0.006; 0.306]
Between Sleep Quality	-0.028	[-0.207; 0.152]	-0.020	[-0.209; 0.169]	0.038	[-0.158; 0.234]	-0.022	[-0.210; 0.166]	0.034	[-0.165; 0.233]
Between Sleep Duration	-0.273	[-2.321; 1.775]	-0.677	[-2.912; 1.557]	-1.160	[-3.491; 1.171]	-0.409	[-2.556; 1.739]	-1.384	[-3.741; 0.974]

Between Cortisol Level at Awakening	-0.241	[-0.551; 0.068]	-0.246	[-0.560; 0.069]	**-0.389**	**[-0.702; -0.076]**	-0.224	[-0.544; 0.097]	-0.326	[-0.649; -0.003]
Sex[a]	1.514	[-3.090; 6.119]	0.379	[-4.402; 5.159]	-0.146	[-4.993; 4.701]	1.242	[-3.505; 5.989]	0.063	[-4.845; 4.971]
	Random Effects (Variances)									
Intercept	32.949		35.718		34.278		32.864		32.948	
Within lag Anticipatory Stress	7.925		5.110				6.818			
Within lag Daily Stress	0.059		0.123		0.068		0.110		0.118	
Within Sleep Quality			0.028		0.045				0.026	
Within Sleep Duration							0.863		0.000	
Within Cortisol Level at Awakening	0.071				0.057					
Residual	31.101		26.506		28.471		31.933		30.524	
	Model Fit Parameters									
AIC	945.247		946.572		950.074		951.019		954.218	
R^2	.636		.690		.667		.626		.643	

	Model f		Model g		Model h		Model i	
	Fixed Effects							
	Est.	95% CI	Est.	95% CI	Est.	95% CI	Est.	95% CI
Intercept	**6.813**	**[4.385; 9.242]**	**7.200**	**[4.691; 9.709]**	**7.416**	**[4.927; 9.905]**	**7.312**	**[4.869; 9.755]**

Within lag Anticipatory Stress	**2.081**	**[0.339; 3.822]**	1.586	[-0.008; 3.180]	**2.010**	**[0.655; 3.365]**	**2.132**	**[0.841; 3.423]**
Within lag Daily Stress	**0.204**	**[0.054; 0.355]**	**0.263**	**[0.111; 0.414]**	**0.226**	**[0.037; 0.414]**	**0.269**	**[0.115; 0.422]**
Within Sleep Quality	0.085	[-0.005; 0.176]	**0.126**	**[0.013; 0.239]**	0.062	[-0.033; 0.156]	0.124	[0.001; 0.248]
Within Sleep Duration	-0.118	[-1.251; 1.015]	-0.166	[-1.153; 0.821]	-0.269	[-1.241; 0.703]	0.014	[-1.045; 1.072]
Within Cortisol Level at Awakening	**-0.885**	**[-1.164; -0.605]**	**-0.598**	**[-0.793; -0.404]**	**-0.663**	**[-0.882; -0.445]**	**-0.756**	**[-1.029; -0.484]**
Between Anticipatory Stress	-0.066	[-2.585; 2.453]	0.265	[-2.404; 2.935]	0.449	[-2.340; 3.239]	0.859	[-1.774; 3.492]
Between Daily Stress	0.086	[-0.061; 0.232]	0.125	[-0.030; 0.280]	0.132	[-0.024; 0.289]	0.135	[-0.016; 0.286]
Between Sleep Quality	-0.031	[-0.204; 0.141]	-0.009	[-0.196; 0.177]	0.020	[-0.179; 0.218]	0.052	[-0.138; 0.243]
Between Sleep Duration	-0.343	[-2.298; 1.612]	-0.682	[-2.870; 1.506]	-1.011	[-3.308; 1.287]	-1.389	[-3.638; 0.861]
Between Cortisol Level at Awakening	-0.245	[-0.537; 0.047]	-0.273	[-0.591; 0.045]	-0.307	[-0.637; 0.022]	**-0.401**	**[-0.706; -0.097]**
Sex[a]	2.022	[-2.461; 6.506]	1.242	[-3.533; 6.017]	1.167	[-3.678; 6.013]	0.640	[-4.117; 5.397]
				Random Effects				
Intercept	29.532		30.268		28.945		29.452	
Within lag Anticipatory Stress	8.663		4.036					
Within lag Daily Stress					0.084			
Within Sleep Quality			0.024				0.048	
Within Sleep Duration	1.563		0.000		0.000		0.740	

Within Cortisol Level at Awakening	0.172		0.033	0.148
Residual	33.607	39.572	37.137	32.150
			Model Fit Parameters	
AIC	951.277	960.048	956.029	956.651
R^2	.607	.537	.565	.624

Note. Table depicts point estimates. The word lag indicates that the respective predictor was measured the day prior to the assessment of the CAR. For better clarity, parameters are printed in bold, if the confidence interval does not include the value zero. Computation of R^2 based on Xu (2003). Number of participants = 41; total number of observations = 131; Est. = Estimate; CI = Confidence Interval; AIC = Akaike Information Criterion.
[a]0 = female; 1 = male.

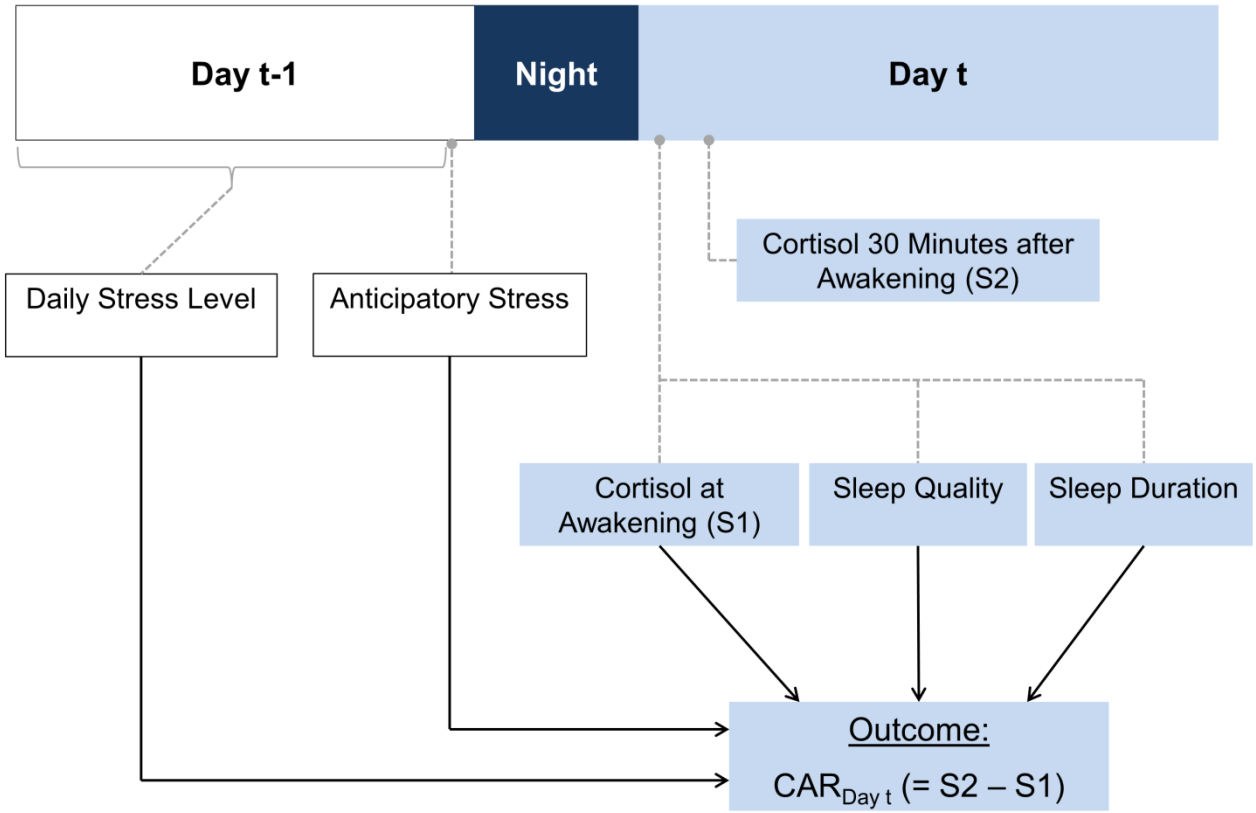

Figure 1. Overview of the different times of measurement of the variables in daily life used to predict the CAR. The dependent variable (CAR) on day t was estimated as the difference between the first and the second measurement on day t. It was predicted by stress anticipation and stress level of the day before, i.e., of day t-1. Additional predictors included the cortisol level at awakening (S1) as well as sleep quality and sleep duration of the last night. These predictors were measured on the same day as the CAR, i.e., on day t. Sex was also included as a predictor but is not depicted in the figure.

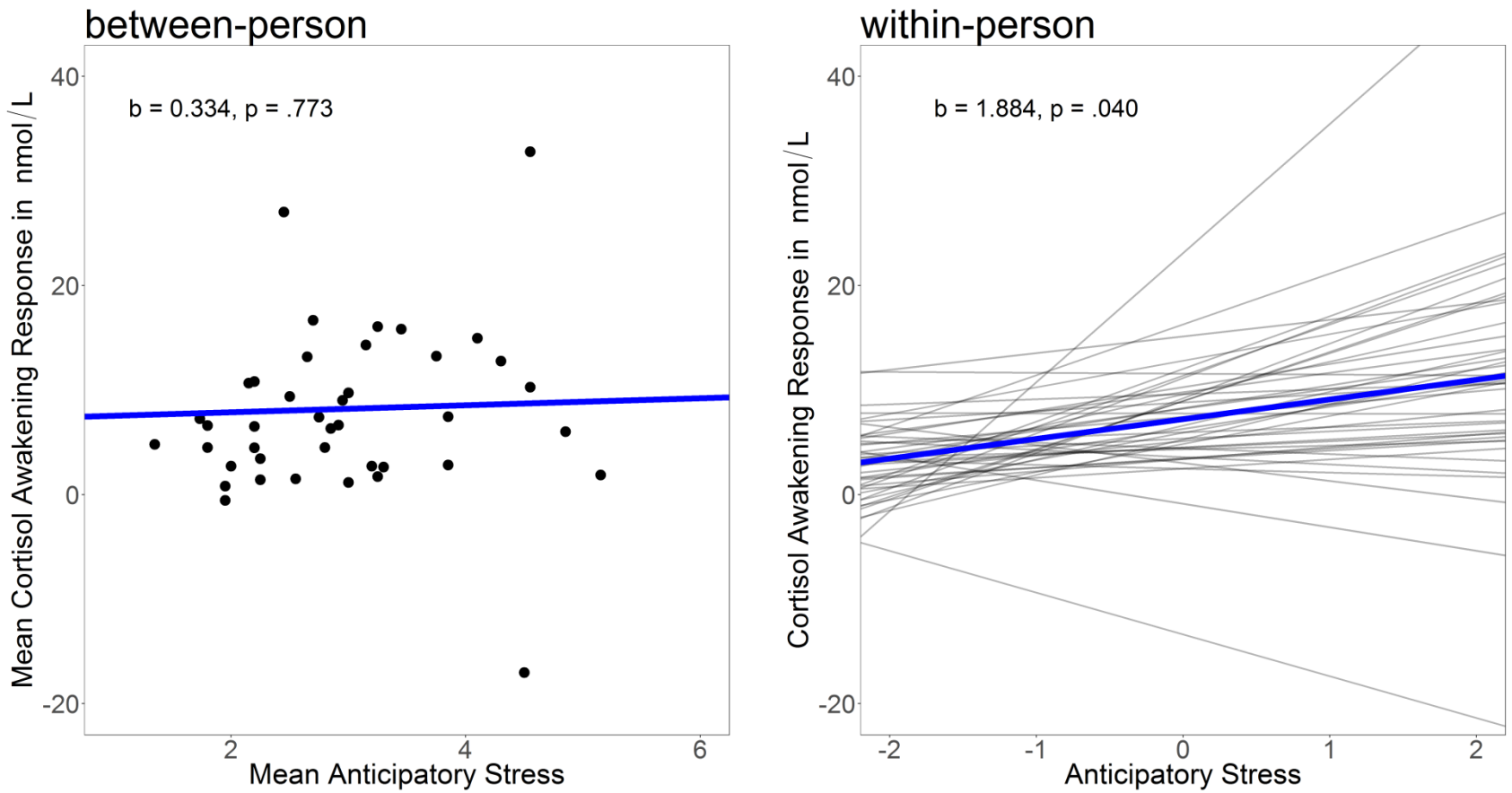

Figure 2. Left-hand side: Scatterplot of between-person relations of anticipatory stress and the estimated cortisol awakening response (averaged across the study period) as well as model predicted between-person relationship of anticipatory stress and the estimated cortisol awakening response. Abscissa shows the raw scores of anticipatory stress (range: 1-5). Right-hand side: Spaghetti plot of average (thick) and person-specific (thin) regression lines for the estimated cortisol awakening response as a function of anticipatory stress. Abscissa represents the deviation from the person mean in anticipatory stress.