Variability in Sleep-Efficiency-Related Cortisol Awakening Response and Its Association with Trait Anxiety and Psychological Resilience

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Abstract

The cortisol awakening response (CAR) is closely linked to individual mental health. Traditional research has employed multi-day averages as an indicator of CAR to explore this relationship; however, due to the influence of state-dependent factors (e.g., sleep) on CAR, research conclusions have been highly inconsistent. To address this issue, the present study utilizes CAR variability across multiple days as a novel metric for quantifying CAR, and examines trait anxiety and psychological resilience as key variables reflecting mental health status, to investigate the relationship between CAR variability and mental health under conditions where sleep efficiency is controlled or manipulated. Experiment 1, conducted in a natural sleep context, reduced CAR variability by enhancing the stability of sleep efficiency, and found a significant positive correlation between CAR variability during natural sleep and trait anxiety scores, demonstrating that smaller CAR variability in a stable environment is associated with lower levels of trait anxiety. Experiment 2 increased CAR variability through the manipulation of total sleep deprivation, and found a significant positive correlation between CAR variability before and after sleep deprivation and psychological resilience, indicating that greater CAR variability in a changing environment is associated with higher levels of psychological resilience. These findings suggest that CAR variability is a reliable physiological indicator of mental health. Considering CAR variability across multiple days is crucial for understanding how individuals adapt to daily stressors and challenges, and can provide novel perspectives and a basis for promoting mental health and designing effective intervention strategies.

Full Text

Preamble

Self-Check Report for *Acta Psychologica Sinica* Submission

1. Please list up to three innovative contributions of this study in the form of "Research Highlights," not exceeding 200 words total.

Acta Psychologica Sinica aims to publish cutting-edge psychological research that is "both scientifically excellent and of particularly broad interest and significance." Studies with only minor incremental contributions, those that do not attempt to open new areas of inquiry or propose unique and innovative perspectives, or those that merely investigate algorithms or techniques without addressing clear psychological questions, have low acceptance probability and are recommended for submission elsewhere.

1) For the first time, this study employs variability in the cortisol awakening response (CAR) across multiple days as a quantitative metric, breaking through traditional approaches that use CAR means and providing a novel perspective.

2) By manipulating sleep efficiency, the study examines its effects on CAR variability and its associations with psychological resilience/trait anxiety, revealing the complex interplay between mental health and physiological responses.

3) Through two controlled experimental conditions—natural sleep and total sleep deprivation—the study innovatively validates hypotheses about how environmental changes affect CAR variability and mental health.

2. Have you used the same data from previously submitted or published articles in this study? If yes, please attach the articles for review. (We do not approve of authors publishing multiple articles with the same variables from one dataset, nor do we approve of splitting a series of related studies into multiple publications.)

3. Non-experimental, non-intervention studies in management, clinical, personality, and social fields that rely solely on self-report (questionnaires) need to check for common method bias. What methods did you use to control or demonstrate that such bias would not affect the validity of your conclusions? What measures were taken? (For literature on common method bias, see: http://journal.psych.ac.cn/xlkxjz/CN/abstract/abstract894.shtml) Studies based on cross-sectional data with only self-reports, measured merely in convenience samples, are easy to conduct but typically lack innovative value and have low acceptance probability.

Answer: This study is experimental in nature. Both experiments employed quasi-experimental and true experimental designs, respectively, not solely ques-

tionnaire methods.

4. Did you report and analyze effect sizes (e.g., Cohen' s d for t-tests, ^{2} or ^{2}p for ANOVA, standardized regression coefficients)? (Many studies mechanically report effect sizes without necessary analysis or explanation, such as whether the effect size is small, medium, or large, or its theoretical/applied significance.) (Searching "effect size calculator" on Google yields many convenient apps. For explanations of effect sizes in Chinese, see: http://journal.psych.ac.cn/xlkxjz/CN/abstract/abstract1150.shtml; in English, see: http://www.uccs.edu/lbecker/effect-size.html) Did you report 95% CIs for statistical analyses? (e.g., 95% CI for differences, 95% CI for correlation/regression coefficients) For calculations and plotting of confidence intervals, see https://thenewstatistics.com/itns/esci/

5. Please state the planned sample size and actual sample size. If they differ, please provide the reason. Low statistical power due to insufficient sample sizes has been a widespread problem in psychological research. We recommend explaining your rationale for sample size calculation in the Methods section. Sample size should be determined based on a well-justified effect size and desired power, with the calculation software or program reported. For rationale and practices regarding sample size planning, see https://osf.io/5awp4/

Answer: This study used G*Power software for sample size calculation. Based on previous research findings, the effect size was set at 0.5, α at 0.05, and 1-β at 0.8. The planned sample size was 26 participants. The final actual sample size was 28 for Experiment 1 and 40 for Experiment 2.

6. (Question about p-value reporting thresholds and Bayesian factors - appears incomplete in original)

7. To ensure completeness of data reporting, if any data were excluded from statistical analysis, was this reported in the text? What were the reasons? How would the results change if this data were included? How were missing data handled in statistical analysis? When using scales, were any individual items deleted? Why? How would results change if these items were included? Were there any measured items or variables not reported? Why? Please indicate where in the paper this information appears.

Answer: This study excluded data from a very small number of participants during statistical analysis because these participants failed to comply with sampling protocols, resulting in obvious anomalies or missing data. Detailed information can be found in the "Participants" section of the Methods. All scales used were original versions without item deletion. Specific scale information is provided in the appendices.

8. Are any experimental materials, scales, or questionnaires that have not undergone peer review and validation attached at the end of the document for review? If not, please state the reason. If this

article is published, are you willing to share these materials with other researchers?

Answer: This study did not use any experimental materials, scales, or questionnaires that have not undergone peer review and validation.

9. This journal requires authors to provide raw data. Please select one option:

c) Raw data and programs have been shared on the Psychological Science Data Bank (https://psych.scidb.cn/) ( )

d) If not available, please explain the reason or provide relevant proof.

10. Is your study a clinical intervention or laboratory experiment? Yes (√) No ( )

If yes, please provide the preregistration number. If no, please explain why. This study adopted an exploratory research approach at the design stage, aiming to investigate for the first time the relationship between CAR variability across multiple days and mental health. In this field, relevant literature is relatively scarce, so we hoped to explore possible associations between variables through a series of experimental data. Due to the lack of relevant theoretical foundation and prior data, the initial design was relatively open, making explicit hypothesis preregistration impossible.

Note: Clinical interventions or laboratory experiments should be preregistered before data collection. Other experimental studies are also encouraged to preregister. Preregistration requires stating all research hypotheses and their support, as well as detailed experimental/intervention procedures. This journal' s preregistration website is https://os.psych.ac.cn/preregister (see the journal website' s "Download Center" for instructions) or https://osf.io/ or https://aspredicted.org/. If your study is preregistered, it will significantly increase acceptance probability. For the importance of preregistration, see https://osf.io/5awp4/

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The Relationship between Variability in Cortisol Awakening Response Associated with Sleep Efficiency and Its Correlation with Trait Anxiety and Psychological Resilience

Abstract

The Cortisol Awakening Response (CAR) is closely associated with individual psychological health. Traditional studies have used the average value of CAR across multiple days as an indicator to explore this relationship. However, because CAR is influenced by state-dependent factors (e.g., sleep), research conclusions have been highly inconsistent. In response, this study introduces variability in CAR across multiple days as a novel metric for quantifying CAR and examines its relationship with psychological health under controlled/manipulated sleep efficiency conditions, using trait anxiety and psychological resilience as key variables reflecting mental health status.

Experiment 1 reduced CAR variability by increasing sleep efficiency stability under natural sleep conditions and found a significant positive correlation between natural sleep CAR variability and trait anxiety scores, indicating that smaller CAR variability in stable environments is accompanied by lower trait anxiety levels. Experiment 2 increased CAR variability through total sleep deprivation manipulation and found a significant positive correlation between CAR variability before and after sleep deprivation and psychological resilience, indicating that greater CAR variability in changing environments is accompanied by higher psychological resilience levels. These results demonstrate that CAR variability is a reliable physiological indicator of psychological health. Considering CAR variability across multiple days is important for understanding how individuals adapt to daily life stressors and challenges, and can provide new perspectives and evidence for promoting mental health and designing effective intervention strategies.

Keywords: Cortisol Awakening Response, Variability, Sleep Efficiency, Trait Anxiety, Psychological Resilience

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The Cortisol Awakening Response (CAR), as part of the circadian activity of the stress hormone cortisol, refers to the peak in cortisol levels that occurs 30-45 minutes after awakening, increasing by 50% to 160% within one hour (Clow et al., 2004; Pruessner et al., 1997). Understanding CAR' s role in mental health may contribute to providing biomarkers for the diagnosis and treatment of psychological disorders, leading researchers to pay particular attention to the relationship between CAR and mental health. Some studies emphasize that high CAR levels may reflect positive psychological characteristics, as CAR serves as a resource whose increase helps individuals cope with future challenges or tasks (Adam et al., 2006; Xiong et al., 2021). Conversely, stress adaptation theory suggests that chronic life stress and stimuli may lead the body to gradually adjust to reduce excessive physiological activation (McEwen & Stellar, 1993), which may manifest as reduced CAR. Within this theoretical framework, low CAR levels may be viewed as a manifestation of stress adaptation, reflecting the body' s adjustment to reduce long-term physiological burden. These contradictory perspectives are particularly prominent in a meta-analysis conducted by Chida and Steptoe (2009). In some studies, negative psychological characteristics such as fatigue and burnout are associated with reduced CAR (Shea et al., 2007; Therrien et al., 2008); in other studies, positive psychological health characteristics are also associated with reduced CAR (Steptoe et al., 2007; Wüst et al., 2000). Even for the same mental health variable (depression), some studies associate it with increased CAR while others associate it with decreased CAR (Johnson et al., 2008; Therrien et al., 2008).

Notably, previous research has typically used the average value of CAR across multiple days as an indicator to explore the relationship between CAR magnitude and mental health. This analytical approach is primarily based on earlier studies that considered CAR a stable biomarker with high stability across multiple days (Pruessner et al., 1997). However, as research has progressed, an increasing number of factors have been found to significantly influence CAR, such as sleep characteristics (Stalder et al., 2016). Research has found that individuals who awaken earlier typically have higher CAR (Edwards et al., 2001); shorter sleep duration is associated with higher CAR (Kumari et al., 2009; Wüst et al., 2000); and compared to control groups, individuals experiencing a full night of sleep deprivation show significant CAR blunting with delayed peak points (Vargas & Lopez-Duran, 2020). Additionally, evidence suggests that poorer sleep quality is associated with lower CAR (Lasikiewicz et al., 2008). Thus, specific sleep characteristics are important variables affecting CAR levels, and day-to-day changes in CAR may reflect the body' s flexible regulation of stress levels and adaptation to different sleep characteristics. Therefore, using only average metrics to characterize CAR may fail to capture this adaptive change, leading to biased research conclusions.

As one of the important products of the rhythmic activity of the hypothalamic-pituitary-adrenal (HPA) axis that regulates stress responses and hormonal balance, CAR exhibits fluctuation. The fluctuation of CAR across multiple days is defined as CAR variability, quantified using the standard deviation of CAR indi-

cators across multiple days. Compared to traditional approaches that consider the average CAR across multiple days, analyzing CAR variability better captures the dynamic and fluctuating nature of individual physiological responses. Under conditions of similar daily environments and stable expected stress levels, smaller CAR variability indicates good HPA axis adaptation to environmental stress and relatively stable psychological states, thereby reflecting good mental health (Pruessner et al., 1997). On the other hand, significant changes in environment and internal stress require individuals' HPA axes to flexibly adjust cortisol release to adapt to these changes. Therefore, under conditions of significant day-to-day environmental changes, greater CAR variability reflects the HPA axis' s flexible adaptation to different stress levels, an adaptability that is a marker of psychological resilience and coping ability, helping individuals maintain long-term mental health (Adam et al., 2006).

Among the many variables reflecting individual mental health, trait anxiety and psychological resilience are two important concepts that play key roles in how individuals cope with stress, adapt to change, and maintain mental health. Trait anxiety refers to a stable personality trait where individuals tend to experience anxiety, reflecting persistent and generalized stress responses when facing potential threats (Spielberger et al., 2017). Psychological resilience refers to an individual' s ability to recover strength when facing adversity, involving adaptability and recovery capacity after experiencing stress, challenges, or failure (Masten, 2001). Trait anxiety and psychological resilience reflect individuals' abilities to cope with stress and adversity from two different dimensions. Trait anxiety emphasizes individuals' sensitivity to stress and possible negative adaptation patterns, while psychological resilience highlights individuals' recovery strength and positive adaptation strategies. Examining both variables simultaneously provides a more comprehensive perspective for understanding how individuals psychologically cope with life challenges.

In summary, this study attempts to use CAR variability as an indicator reflecting the circadian activity of the stress hormone cortisol to analyze the relationship between CAR variability and trait anxiety/psychological resilience. CAR variability is influenced by sleep characteristics (Stalder et al., 2016). Sleep efficiency, one of the main sleep characteristics, refers to the proportion of actual sleep time relative to total time in bed (Wolpert, 1969). Compared to other sleep characteristics, sleep efficiency more comprehensively reflects the effectiveness of an individual' s sleep and is an important factor affecting CAR variability. Therefore, it is necessary to consider the role of sleep efficiency when calculating CAR variability indicators. Since individuals' circadian rhythms of stress hormones generally exhibit stability (Selmaoui & Touitou, 2003), if sleep efficiency changes are small, individuals' CAR variability will also be smaller. Conversely, if sleep efficiency shows large fluctuations, individuals' CAR variability will also show greater fluctuation. To test these hypotheses, Experiment 1 used a natural sleep observation method to reduce CAR variability by increasing sleep efficiency stability. In this context, smaller CAR variability reflects positive mental health characteristics, specifically higher psychological resilience scores

and lower trait anxiety scores. Experiment 2 used sleep deprivation methods to increase CAR variability by reducing sleep efficiency stability. In this context, greater CAR variability better reflects positive mental health characteristics, with individuals showing higher psychological resilience scores and lower trait anxiety scores.

Experiment 1: CAR Variability and Its Relationship with Trait Anxiety/Psychological Resilience under Natural Sleep Observation Conditions

2.1 Research Purpose

Under natural sleep observation conditions, this experiment reduced CAR variability by increasing sleep efficiency stability and explored its relationship with trait anxiety/psychological resilience.

2.2.1 Participants

To ensure sufficient statistical power to detect expected effects, sample size was calculated using G*Power 3.1. *Based on previous research findings showing medium to large effect sizes between CAR and trait anxiety/psychological resilience (Kudielka and Kirschbaum, 2005; Pruessner et al., 1999), and given that CAR variability better captures the dynamic changes of cortisol across multiple days than traditional CAR averages and may therefore be more closely related to trait anxiety/psychological resilience, the expected effect size was set at 0.5. The significance level α was set at 0.05, and statistical power 1-β was set at 0.8. According to Pearson correlation analysis, G*Power calculations indicated that at least 26 participants were needed to ensure detection of correlation effects. Considering potential participant attrition, this experiment recruited 30 healthy university students as paid volunteers through online advertisements. Due to failure to comply with CAR collection protocols (Stalder et al., 2016, 2022), data from 2 participants were excluded, resulting in a final sample of 28 participants, including 15 females (M = 20.33, SD = 1.72) and 13 males (M = 19.69, SD = 2.06). Since different phases of the menstrual cycle may affect CAR in females (Wolfram et al., 2011), female participants were confirmed to be in the luteal phase through questionnaire screening to avoid interference with experimental conclusions. All participants were free from psychiatric, neurological, or sleep disorders, were not taking psychotropic or glucocorticoid medications, and did not abuse alcohol or other substances.

2.2.2 Mental Health Questionnaires

Trait Anxiety Inventory (TAI): This scale assesses individuals' long-term, trait anxiety levels (Spielberger & Diaz-Guerrero, 1971). This study used the Chinese version revised by Ye Renmin (Ye Renmin & TomRocklin, 1988), consisting of 20 items covering various aspects of anxiety symptoms. Each item

uses a 4-point Likert scale ranging from 1 (“almost never”) to 4 (“almost always”). Higher total scores indicate higher trait anxiety levels. The Cronbach' s α for this questionnaire in this study was 0.897.

Connor-Davidson Resilience Scale (CD-RISC): This scale assesses individuals' adaptability and recovery capacity when coping with stress, challenges, and adversity (Connor & Davidson, 2003). This study used the Chinese version of the CD-RISC scale (Yu Xiao-nan & Zhang Jian-xin, 2007), consisting of 25 items, each using a 5-point Likert scale ranging from 0 (“not true at all”) to 4 (“true nearly all the time”). Higher scale scores indicate better psychological resilience. The Cronbach' s α for this questionnaire in this study was 0.87.

2.2.3 Sleep Data Collection

Objective sleep data were collected using Actigraph wristwatches (Phillips Respironics, Inc.). This device is an accelerometer-based unit worn on the dominant wrist, designed to objectively monitor sleep-wake cycles and physical activity levels. Data collection and preliminary analysis were completed using ActiLife sleep analysis software (Version 6.13, MiniMitter/Philips Respironics). The following sleep indicators were calculated: Time in Bed (TIB), the total duration from when participants attempted to fall asleep until final awakening; Wake After Sleep Onset (WASO), the total duration of all awakening periods between initial sleep onset and morning awakening; and Total Sleep Time (TST), calculated by subtracting WASO from TIB. Finally, objective Sleep Efficiency (SE) was calculated as the percentage of actual sleep time relative to time in bed using the formula SE = (TST / TIB) $\times$ 100%.

Participants' subjective sleep data were recorded through sleep diaries, which included: (1) time of closing eyes to prepare for sleep last night; (2) time required to fall asleep; and (3) time of morning awakening. Based on this information, the following sleep indicators were calculated: Total Bed Time (TBT), calculated as the time of morning awakening minus the time of closing eyes to prepare for sleep last night; and Total Sleep Time (TST), calculated as TBT minus the time required to fall asleep. Finally, subjective Sleep Efficiency (SE) was calculated as the percentage of actual sleep time relative to total bed time using the formula SE = (TST / TBT) $\times$ 100%.

2.2.4 Salivary Cortisol Collection and Analysis

Participants were required to collect four saliva samples on each collection day at 0, 30, 45, and 60 minutes post-awakening. Additionally, participants were not allowed to eat, drink beverages (including those containing alcohol, caffeine, or juice), brush teeth, rinse mouth, or smoke before completing daily sample collection. Saliva samples were collected using specific collection devices (Salivette, SARSTEDT, Germany). During each collection, participants needed to pour the cotton swab from the collection tube directly into their mouth, chew for 1 minute, then spit the cotton swab back into the collection tube and

close the lid. Throughout the collection process, contact with hands had to be avoided to prevent contamination. After each collection, the collection tube needed to be placed in a medication monitoring system (MEMSCap™ Medication Event Monitoring System, MWV Switzerland Ltd.) to record objective collection times. After saliva samples were collected, all samples were stored in a -20°C freezer before analysis.

Cortisol concentrations were determined using enzyme-linked immunosorbent assay (ELISA, IBL-Hamburg, Germany) to assess participants' circadian cortisol activity. Before analysis, cortisol data were cleaned and extreme values were processed to meet statistical analysis assumptions. First, cortisol data units were converted from g/dl to nmol/l. Since cortisol data typically show a positively skewed distribution, square root transformation was applied (Miller & Plessow, 2013; Schlotz, 2011), followed by normality testing to ensure data conformed to normal distribution. Extreme values were defined as data points more than three standard deviations from the mean and were replaced using the corresponding upper and lower 5th percentiles (Schlotz, 2011).

2.2.5 Experimental Procedure

Before the formal experiment began, participants needed to schedule a face-to-face meeting with the experimenter at the laboratory, a design proven effective for improving participant compliance with sample collection protocols (Stalder et al., 2016, 2022). During the meeting, the experimenter first introduced participants to the sleep wristwatch wearing method and precautions. Throughout the experimental period, participants were required to prepare for sleep before midnight and maintain 7-9 hours of adequate and regular sleep; going to sleep or waking up too early or too late was prohibited. Additionally, participants needed to continuously wear the sleep wristwatch on their dominant hand, keeping it close to the skin; it could not be removed without special circumstances and was uniformly collected by the experimenter after the experiment. Participants were then informed about saliva collection procedures and precautions. Participants needed to choose any three appropriate weekdays within a given week for saliva sample collection. The selected collection days needed to be as close as possible to participants' daily life states, with no major stressful events before or after collection days to control for differences between weekends and weekdays and the impact of stress factors on CAR (Stalder et al., 2016). After each saliva sample collection, participants also needed to record the specific collection time; the accuracy of collection time would affect participants' final compensation. After the meeting, participants completed relevant demographic and mental health questionnaires.

During the formal data collection period, the experimenter would schedule times with participants before each collection day to provide and collect experimental materials and confirm whether participants understood and complied with experimental precautions. Behaviors not meeting experimental requirements and unexpected events were recorded, and after assessing their impact on data qual-

ity, data were either excluded or retained. Additionally, participants needed to promptly complete sleep diaries sent by the experimenter at a scheduled time (8:00) each morning.

2.2.6 Data Analysis

All data were statistically analyzed using SPSS 27.0 software. Before ANOVA, data were tested for normality and homogeneity of variance. Considering that repeated measures data may violate sphericity assumptions, Greenhouse-Geisser correction was applied to adjust degrees of freedom for variables not meeting sphericity assumptions to correct statistical inference bias resulting from assumption violations. When ANOVA results were significant, Bonferroni method was used for post-hoc multiple comparisons. Specifically, collection day (Day 1, Day 2, and Day 3) was used as the independent variable, and one-way repeated measures ANOVA was used to test day-to-day differences in sleep efficiency. For raw cortisol data, collection time point (0, 30, 45, and 60 minutes) and collection day (Day 1, Day 2, and Day 3) were used as independent variables, and repeated measures ANOVA was used to test main effects and interactions of collection time point and day differences. Subsequently, among the four daily collection time points, the mean increase (Mnlnc) was calculated using the average cortisol level at the last three time points relative to the first time point, and the range (max-min) of cortisol levels across the four time points was used as the daily CAR indicator (Stalder et al., 2016). Collection day (Day 1, Day 2, and Day 3) was used as the independent variable, and one-way repeated measures ANOVA was used to test day-to-day differences in CAR.

Finally, the day-to-day standard deviations of CAR mean increase (Mnlnc) and range (max-min) were used as indicators of CAR variability. Before correlation analysis, CAR variability indicators and questionnaire scores were tested for normality to ensure they met prerequisites for product-moment correlation. Since age and gender have been found to affect CAR (Kudielka & Kirschbaum, 2005; Oskis et al., 2009; Pruessner et al., 1999; Stroud et al., 2002), age and gender were set as covariates when calculating partial correlations between CAR variability indicators and trait anxiety/psychological resilience. Additionally, the mean CAR across the three days was calculated, and after confirming normality, its partial correlation with trait anxiety/psychological resilience was calculated to compare differences in associations with mental health indicators between CAR variability and CAR averages.

2.3.1 Day-to-Day Differences in Sleep Efficiency

Descriptive statistics for objective and subjective sleep efficiency across the three natural days are shown in Table 1 . Repeated measures ANOVA results found no significant differences in objective sleep efficiency across the three collection days, $F(2, 54) = 0.57$, $p = 0.61$; similarly, no significant differences were found in subjective sleep efficiency across the three collection days, $F(2, 54) = 0.03$, $p = 0.97$.

2.3.2 Day-to-Day Differences in CAR

Daily cortisol levels at each collection time point are shown in Figure 1 Figure 1: see original paper. Repeated measures ANOVA results indicated a significant main effect of collection time point, $F(2.04, 54.98) = 19.81$, $p < 0.001$, with an effect size of $^{2} = 0.42$. This indicates that 42% of the total observed variation in cortisol changes can be explained by collection time point. This result emphasizes the significant impact of time on cortisol level changes after morning awakening, potentially revealing physiological mechanisms of how cortisol rapidly adjusts post-awakening. Further post-hoc multiple comparisons showed that cortisol levels at 30 minutes post-awakening were significantly higher than at awakening ($p < 0.001$, 95% CI: 0.26-0.88), at 45 minutes post-awakening ($p = 0.01$, 95% CI: 0.03-0.35), and at 60 minutes post-awakening ($p < 0.001$, 95% CI: 0.29-0.79). The main effect of collection day was not significant, $F(2, 54) = 1.99$, $p = 0.147$, and the interaction between collection day and collection time point was not significant, $F(3.72, 100.36) = 0.59$, $p = 0.66$.

Daily CAR indicators (Mnlnc, max–min) across the three natural days are shown in Figure 2 Figure 2: see original paper. Repeated measures ANOVA found no significant differences in either daily indicator across the three collection days, F_{Mnlnc}(5, 54) = 0.24, p = 0.78; F_{max}-min(5, 54) = 1.83, p = 0.17.

2.3.4 Correlation Analysis between CAR and Trait Anxiety/Psychological Resilience

Correlations between CAR variability, CAR averages, and trait anxiety/psychological resilience are shown in Table 2 . Results showed that after controlling for age and gender, CAR variability calculated based on Mnlnc was significantly positively correlated with trait anxiety, $r = 0.42$, $p = 0.03$. The scatter plot of residuals for these two variables after controlling for covariates is shown in Figure 2. These results indicate that smaller CAR variability is accompanied by lower trait anxiety scale scores. The correlation between CAR variability and psychological resilience scores was not significant. Additionally, when using CAR averages as an indicator, no significant correlations were found with either trait anxiety or psychological resilience scores.

Table 2 Pearson Correlations between Different CAR Calculation Indicators and Trait Anxiety/Psychological Resilience Scales

CAR Indicator	CAR Variability	CAR Average
	Mnlnc	max–min
Trait Anxiety	0.42*	
Psychological Resilience		

Note: Significance (two-tailed): $p < 0.05$.*

2.4 Discussion

Similar CAR response patterns appeared across the three collection days, with cortisol levels peaking at approximately 30 minutes post-awakening and then gradually declining until reaching levels not significantly different from awakening levels by 60 minutes post-awakening. This CAR response pattern is consistent with previous research. The rise in cortisol levels at 30 minutes post-awakening and subsequent decline may reflect rapid HPA axis response and regulatory mechanisms. This pattern suggests that HPA axis adaptation to morning awakening is rapid, and once adaptation to morning challenges is completed, cortisol levels fall back, reflecting HPA axis regulation of daily rhythms (Pruessner et al., 1997).

Second, results found that participants had small variations in sleep efficiency across the three natural sleep conditions, and their CAR on the following mornings also showed small variability. This may indicate a potential association between stable sleep patterns and HPA axis function. The HPA axis is the primary neuroendocrine system responding to stress, helping the body adapt to stress through the release of hormones such as cortisol. Sleep is physiologically linked to the HPA axis because sleep is a critical period for the body to restore HPA axis activity. High-quality sleep may help maintain HPA axis homeostasis, while sleep disorders may lead to HPA axis dysfunction (Vgontzas et al., 2001). Therefore, stable sleep efficiency may reflect good HPA axis regulatory function, which may further manifest as CAR stability.

Importantly, the study found that under three similar natural sleep conditions, lower individual CAR variability was accompanied by lower trait anxiety scale scores. As one of the physiological indicators of stress response, CAR variability may reflect individuals' ability to cope with environmental stress. Smaller CAR variability indicates relatively stable daily physiological responses upon awakening, possibly meaning they can more effectively manage and adapt to daily life stress. This stable coping mechanism is associated with higher mental health levels and lower anxiety levels (Kuhlman et al., 2020). Additionally, stable CAR may reflect good coordination between psychological and physiological regulatory mechanisms. When individuals can effectively manage psychological stress and reduce the impact of negative emotions and anxiety, this psychological stability may be manifested by reducing fluctuations in physiological stress responses, thus showing smaller CAR variability (Scher et al., 2010).

However, the significant positive correlation between CAR variability and trait anxiety was only observed when using Mnlnc as the daily CAR calculation indicator. When using max-min as the daily CAR indicator, the correlation was not significant. This difference may stem from the different biological meanings behind the two daily CAR indicators. Mnlnc primarily reflects the overall upward trend of cortisol levels after awakening. This indicator can be viewed as a measure of individuals' sensitivity and intensity of response to awakening as a daily physiological process. In contrast, max-min reflects the fluctuation range

of cortisol levels within a certain period after awakening, i.e., the amplitude of CAR fluctuations. This indicator provides information about individual physiological response variability and can be viewed as an indicator of individuals' stress system regulatory capacity and adaptability (Clow et al., 2004). These two indicators may reflect different aspects of the body' s stress response to the awakening process, revealing different physiological regulatory mechanisms and stress coping strategies. In Experiment 1, where participants were in natural sleep conditions, Mnlnc, as an indicator reflecting the overall upward trend of CAR, may better capture individuals' physiological regulatory capacity in relatively stable environments. The consistency of this upward trend may be related to how effectively individuals manage daily stress and emotional fluctuations, thus showing a significant correlation with trait anxiety.

In addition to the above results, Experiment 1 did not observe a significant correlation between CAR variability and psychological resilience. In addition to possible limitations from the relatively small sample size, this may be because under similar natural sleep conditions, individuals lacked obvious external stressor stimulation, and psychological resilience may not have played a significant role, thus no significant association was found between psychological resilience and CAR variability. Based on this, Experiment 2 increased CAR variability through sleep deprivation experimental manipulation and further analyzed the relationship between CAR variability and trait anxiety/psychological resilience.

Experiment 2: The Relationship between CAR Variability and Trait Anxiety/Psychological Resilience under Sleep Deprivation Conditions

3.1 Research Purpose

Using sleep deprivation methods to increase CAR variability by reducing sleep efficiency stability and exploring its relationship with trait anxiety/psychological resilience.

3.2.1 Participants

Experiment 2 also used G*Power 3.1 for sample size calculation with the same parameters as Experiment 1 (effect size = 0.5, α = 0.05, 1-β = 0.8), based on Pearson correlation analysis. Combined with G*Power calculation results and potential sample attrition, Experiment 2 recruited 42 healthy university students as paid volunteers through online advertisements. Due to failure to comply with CAR collection protocols (Gröschl, 2008; Jelkmann, 2001), data from 2 participants were excluded, resulting in a final sample of 40 participants, including 20 females (M = 20.6, SD = 1.60) and 20 males (M = 20.9, SD = 1.80). Female participants were confirmed to be in the luteal phase through questionnaire screening. All participants were free from psychiatric, neurological, or sleep disorders, were not taking psychotropic or glucocorticoid medications, and did not abuse alcohol or other substances.

3.2.2 Mental Health Questionnaires

Same as Experiment 1.

3.2.3 Sleep Deprivation

Total Sleep Deprivation (TSD) was used to manipulate sleep efficiency, creating a special experimental condition completely different from natural sleep conditions. Total sleep deprivation refers to a state where individuals have no sleep for a certain period. Under this condition, individuals are prevented from entering any form of sleep, including all sleep stages and rapid eye movement (REM) sleep.

This experiment was approved by the ethics review committee. All participants were fully informed about the research purpose, procedures, and potential risks, and voluntarily signed informed consent forms.

3.2.4 Salivary Cortisol Collection and Analysis

Experiment 2 used liquid chromatography-mass spectrometry (LC-MS) technology to quantitatively analyze cortisol levels in participants' saliva samples. This technology was selected for its high sensitivity and specificity, making it particularly suitable for detecting low-concentration hormones in biological samples (Gröschl, 2008; Jelkmann, 2001).

Before analysis, saliva samples were centrifuged (5000 rpm, 4°C, 10 minutes). The supernatant was extracted and filtered through a membrane to remove large molecular impurities. The liquid chromatography system used for analysis was equipped with a reversed-phase chromatography column. The mobile phase consisted of aqueous and organic phases, with gradient elution to optimize separation efficiency. Mass spectrometry detection used an electrospray ionization (ESI) source operated in positive ion mode. Multiple reaction monitoring (MRM) transitions for cortisol were set to specific precursor and product ions to ensure analytical specificity and sensitivity. Mass spectrometry parameters such as collision energy and ion source temperature were optimized to obtain optimal signal intensity. Cortisol levels were quantified using external standard methods by comparing the response of cortisol in samples with the response of a known concentration standard curve. Data processing and quantitative analysis were performed using specific mass spectrometry software.

3.2.5 Experimental Procedure

In Experiment 2, the content of the initial meeting, questionnaire completion, and materials and specific manipulation procedures used on the first two collection days were the same as in Experiment 1. The difference was that before participating in the TSD experiment, participants would coordinate timing with the experimenter, during which they were informed that on the experimental day, they were not allowed to sleep or nap during the day and must strictly wear

the sleep wristwatch without removing it arbitrarily. The experiment began at 10:00 PM, when participants needed to arrive at the laboratory and complete a full night of sleep deprivation. Throughout the deprivation period, natural light was completely blocked using blackout curtains, and only constant-power LED lights were kept on in the laboratory. Two experimenters participated throughout the entire process to ensure participants did not sleep or nap; if experimenters found participants napping, they would quickly wake them. During the experiment, participants needed to maintain stable emotions; intense stimulating activities and consumption of beverages containing alcohol or caffeine were prohibited. Between 7:00 and 8:00 AM the next morning, participants were required not to eat, drink beverages, brush teeth, rinse mouth, or smoke. At 8:00, 8:30, 8:45, and 9:00, participants were reminded by experimenters to collect saliva samples. After collection, saliva samples and sleep wristwatches were uniformly collected and stored.

3.2.6 Data Analysis

All data were statistically analyzed using SPSS 27.0 software. Same as Experiment 1, data were tested before ANOVA to meet statistical analysis prerequisites. For variables not meeting sphericity assumptions, Greenhouse-Geisser correction was applied to adjust degrees of freedom. After significant ANOVA results, Bonferroni method was used for post-hoc multiple comparisons. Specifically, collection time point (0, 30, 45, and 60 minutes) and collection day (Day 1, Day 2, and Deprivation Day 3) were used as independent variables, and repeated measures ANOVA was used to test main effects and interactions of collection time point and day differences. Subsequently, mean increase (Mnlnc) and range (max–min) were also used as daily CAR indicators, with collection day (Day 1, Day 2, and Deprivation Day 3) as the independent variable, and one-way ANOVA was used to test day-to-day differences in CAR. In Experiment 2, CAR variability was calculated as the standard deviation of CAR calculated from the mean CAR under the first two normal sleep days and CAR after sleep deprivation. After normality testing, partial correlations between CAR variability and trait anxiety/psychological resilience were analyzed after controlling for age and gender.

3.3.1 Day-to-Day Differences in CAR

Cortisol levels at each collection time point are shown in Figure 3 Figure 3: see original paper. The interaction between collection time point and collection day was significant, $F(4.51, 171.43) = 7.08$, $p < 0.001$, with an effect size of $^{2} = 0.16$. This indicates that 16% of the total variation in cortisol changes was explained by the interactive influence of collection time point and collection day. Results showed that cortisol level changes were not only affected by single factors but also related to the interaction between these two factors. Further simple effects analysis showed that on Natural Day 1 and Natural Day 2, participants' cortisol levels peaked at approximately 30 minutes post-awakening and then gradually

declined. However, after experiencing total sleep deprivation, no significant differences were found among the four collection time points. Daily CAR indicators (Mnlnc, max-min) are shown in Figure 3(b). Results showed significant main effects of collection day for both daily CAR indicators, F_{Mnlnc}(2, 76) = 12.09, p < 0.001, ^{2} = 0.24; F_{max}-min(2, 76) = 10.84, p < 0.001, ^{2} = 0.22. These results indicate that Mnlnc and max-min showed considerable variability across different collection days, and a large portion of this variation could be explained by changes in collection day. Further multiple comparison results showed no significant differences in CAR between the first two collection days, but CAR on both of these days was significantly higher than on the third day after sleep deprivation.

Figure 3. Tests of Differences in Cortisol and Daily CAR Indicators

Note: Significance (two-tailed): p < 0.05; **p < 0.01;** *p < 0.001.*

3.3.2 Correlation Analysis between CAR and Trait Anxiety/Psychological Resilience

After controlling for age and gender, correlations between CAR variability and trait anxiety/psychological resilience are shown in Table 3 . Results showed that CAR variability calculated based on max-min was significantly correlated with the psychological resilience scale, r = 0.36, p = 0.03. The residual scatter plot of this CAR variability indicator and psychological resilience after controlling for covariates is shown in Figure 4 [Figure 4: see original paper]. These results indicate that greater CAR variability before and after sleep deprivation is accompanied by higher psychological resilience scores.

Table 3 Pearson Relationships between Different CAR Calculation Indicators and Trait Anxiety/Psychological Resilience Scale Scores

CAR Indicator	CAR Variability	CAR Average
	Mnlnc	max-min
Trait Anxiety		
Psychological Resilience		0.36*

Note: Significance (two-tailed): p < 0.05.*

Figure 4. Residual Scatter Plots of CAR Variability Indicators with Psychological Resilience/Trait Anxiety

3.4 Discussion

Experiment 2 results showed that sleep deprivation caused significant CAR blunting the next day, consistent with previous research findings (Minkel et al., 2012). Prolonged wakefulness and sleep deprivation may weaken the HPA axis response to awakening stimuli, leading to reduced cortisol release upon

awakening, i.e., CAR blunting. This effect may occur because the HPA axis enters a relatively inhibited state after overactivation, temporarily reducing its sensitivity to awakening stimuli (Vargas & Lopez-Duran, 2020).

Correlation analysis results showed a positive correlation between CAR variability after sleep deprivation and psychological resilience levels, indicating that individuals with higher psychological resilience levels showed higher CAR variability after sleep deprivation. Psychological resilience emphasizes individuals' adaptability and recovery capacity when coping with stress, challenges, and adversity (Connor & Davidson, 2003). Individuals with high psychological resilience can more effectively cope with stressful situations like sleep deprivation and maintain physiological and mental health through positive adaptive mechanisms. In this context, greater CAR variability may reflect individuals' ability to regulate their physiological responses for rapid adaptation and recovery when facing sleep deprivation as a stressor. However, contrary to hypotheses, no significant correlation was observed between CAR variability indicators and trait anxiety scores in Experiment 2. This may be because trait anxiety reflects individuals' stable perceptual tendency toward environmental threats, a relatively stable trait characteristic with low sensitivity to acute changes (Endler & Kocovski, 2001). This characteristic may make it less sensitive to CAR variability caused by sleep deprivation.

Different from Experiment 1 findings, the above correlations only appeared when using max-min to calculate daily CAR. Conversely, when using Mnlnc, no significant correlations were found between CAR variability and psychological resilience/trait anxiety. This difference may also stem from the different biological meanings behind these two indicators. Mnlnc mainly reflects the overall upward trend of cortisol levels after awakening, while max-min reflects the fluctuation amplitude of cortisol levels within a certain period after awakening. In Experiment 2, where participants were under extreme stress conditions of TSD, max-min, as an indicator reflecting CAR fluctuation amplitude, was more likely to directly reflect individuals' physiological response intensity and regulatory range to extreme changes. TSD, as an extreme stressor, can trigger extremely strong physiological and psychological responses, leading to increased CAR fluctuation amplitude the next day. This increased fluctuation amplitude may be related to the psychological adaptability and recovery capacity individuals demonstrate when coping with extreme stressors (Clow et al., 2010), ultimately manifesting as a significant positive correlation between CAR variability and psychological resilience.

In addition to the above results, similar to Experiment 1, when using CAR means as indicators, no significant correlations were found with trait anxiety or psychological resilience in either experiment. This result further emphasizes the limitations of using CAR averages when assessing relationships with trait anxiety and psychological resilience. Because CAR is easily affected by many other factors, including psychological stress, sleep quality, time pressure, biological rhythms, and even sampling methods (Stalder et al., 2016), simply calculating

CAR averages may ignore the different effects these factors have on individual CAR at different times. In contrast, analyzing CAR variability can provide more comprehensive information. First, CAR variability may more sensitively reflect individuals' physiological responses to different stressors. CAR variability can capture subtle differences in day-to-day HPA axis response patterns, providing richer information for understanding how psychological states affect physiological processes. Second, CAR variability analysis may better reflect individuals' capacity to adapt and cope with environmental stress. For example, increased CAR variability after sleep deprivation may indicate individuals' efforts to regulate their physiological state to adapt to stress. Finally, the relationship between CAR variability and trait anxiety/psychological resilience suggests that it may be a more comprehensive physiological indicator of individual mental health status, providing important information about how individuals regulate their physiological responses under different times and stresses.

4 General Discussion

This study explored the relationship between CAR variability and psychological resilience/trait anxiety through observation under natural sleep conditions with similar sleep efficiency (Experiment 1) and experimental manipulation of total sleep deprivation creating significantly different sleep conditions (Experiment 2). Results found that under similar natural sleep conditions, CAR variability was small and significantly positively correlated with trait anxiety; under different sleep conditions before and after sleep deprivation, CAR variability was large and significantly positively correlated with psychological resilience. Correspondingly, when using multi-day CAR averages as indicators, no significant correlations were found with psychological resilience or trait anxiety regardless of whether sleep conditions were similar. These results generally support the hypothesis that CAR variability is a more effective physiological indicator of individual mental health (e.g., trait anxiety and psychological resilience).

Since its introduction, CAR has become an important indicator in psychophysiology and sleep research for assessing individuals' stress responses and circadian rhythm regulation. Although researchers have conducted extensive studies on CAR and made significant progress, some key issues remain unresolved. First, although CAR has been used as a biomarker to assess individual health status, its relationship with specific health outcomes (e.g., mental health disorders, cardiovascular disease) remains controversial (Chida & Steptoe, 2009). Particularly, how CAR increases or decreases reflect specific health risks and how CAR can be used for early warning and intervention remain research hotspots. Additionally, although it is known that CAR is related to stress responses and HPA axis activity, its specific biological mechanisms are not fully understood, particularly whether CAR is a physiological response naturally occurring with awakening or a process driven by the body' s internal circadian rhythms (Vargas & Lopez-Duran, 2020). To address these issues, this study provides a new perspective for using CAR in mental health assessment and intervention by linking

CAR variability with mental health indicators such as trait anxiety and psychological resilience, offering a theoretical foundation for using CAR to predict health risks and conduct early interventions. Furthermore, this study discovered that CAR is not only influenced by circadian rhythms but also closely related to the sleep-wake transition process by examining the effects of sleep deprivation on CAR. This provides new perspectives for understanding CAR activation mechanisms and its interaction with circadian rhythms.

Although previous studies have found certain connections between sleep and CAR (Edwards et al., 2001; Kumari et al., 2009; Lasikiewicz et al., 2008; Wüst et al., 2000), how sleep is connected to CAR remains controversial. To address this, this study further explored the effects of natural sleep and sleep efficiency fluctuations before and after sleep deprivation on CAR variability through two experiments. First, this study found significant changes in CAR after sleep deprivation and that sleep efficiency fluctuations affect CAR variability. These findings not only emphasize the importance of sleep for maintaining normal physiological stress responses but also reveal complex interactions between sleep and the human stress system. Second, this study highlights the importance of improving sleep quality for maintaining individual physiological and mental health. By revealing the impact of sleep efficiency changes on CAR variability, this study provides a scientific basis for designing targeted sleep interventions, i.e., we can optimize day-to-day physiological stress responses and promote overall health by improving individuals' sleep quality and reducing day-to-day variability in sleep efficiency. Overall, this study not only enriches understanding of the complex connections between sleep and day-to-day physiological regulation but also brings new research methods and intervention ideas to the sleep field.

In selecting mental health indicators, this study chose trait anxiety and psychological resilience as two core indicators reflecting individual mental health status. Trait anxiety reflects individuals' persistent and generalized stress responses when facing potential threats and is a stable personality characteristic (Spielberger et al., 1971). Psychological resilience describes individuals' recovery strength and adaptability when facing adversity (Masten, 2001). Considering trait anxiety and psychological resilience simultaneously provides a more comprehensive research framework for understanding how individuals cope with stress from both physiological and psychological levels. This multidimensional measurement approach can help better identify individuals who may need additional support when coping with daily life stress. Additionally, this study demonstrates how physiological indicators can be used to assess and understand individual mental health status by exploring the relationship between CAR variability and trait anxiety/psychological resilience. This provides a valuable reference for future research, showing that integrating physiological and psychological data can deepen understanding of individual health status. In summary, by combining these two mental health indicators, this study not only deepens understanding of individual stress coping mechanisms but also provides new ideas for mental health intervention, with important theoretical and practical significance.

This study also has certain limitations. First, both experiments used three days of CAR samples to calculate CAR variability. CAR measurements within three days may be affected by various irrelevant factors such as individual life events and unexpected factors, and these short-term changes may have significant impacts on CAR variability. Future research could consider extending the measurement time span and increasing measurement frequency to obtain more comprehensive and in-depth understanding. Second, both experiments used relatively small sample sizes, and the participant populations were all university students, resulting in slightly insufficient statistical power and external validity. Future research could further expand sample sizes and participant populations to obtain more reliable research conclusions.

Overall, this study provides a new analytical approach for exploring the relationship between CAR and mental health. Compared to traditional analyses of averages, considering CAR variability across multiple days and exploring its relationship with mental health provides a more comprehensive and detailed method for understanding the complex dynamic relationship between physiological and mental health. This approach helps reveal more information about how individuals adapt to daily life stress and challenges, thus providing new perspectives and evidence for promoting mental health and designing effective intervention strategies.

References

Adam, E. K., Hawkley, L. C., Kudielka, B. M., & Cacioppo, J. T. (2006). Day-to-day dynamics of experience–cortisol associations in a population-based sample of older adults. *Proceedings of the National Academy of Sciences, 103*(45), 17058–17063. https://doi.org/10.1073/pnas.0605053103

Chida, Y., & Steptoe, A. (2009). Cortisol awakening response and psychosocial factors: A systematic review and meta-analysis. *Biological Psychology, 80*(3), 265–278. https://doi.org/10.1016/j.biopsycho.2008.10.004

Clow, A., Hucklebridge, F., Stalder, T., Evans, P., & Thorn, L. (2010). The cortisol awakening response: More than a measure of HPA axis function. *Neuroscience & Biobehavioral Reviews, 35*(1), 97–103. https://doi.org/10.1016/j.neubiorev.2009.12.011

Clow, A., Thorn, L., Evans, P., & Hucklebridge, F. (2004). The Awakening Cortisol Response: Methodological Issues and Significance. *Stress, 7*(1), 29–37. https://doi.org/10.1080/10253890410001667205

Connor, K. M., & Davidson, J. R. T. (2003). Development of a new resilience scale: The Connor-Davidson Resilience Scale (CD-RISC). *Depression and Anxiety, 18*(2), 76–82. https://doi.org/10.1002/da.10113

Edwards, S., Evans, P., Hucklebridge, F., & Clow, A. (2001). Association between time of awakening and diurnal cortisol secretory activity. *Psychoneuroendocrinology, 26*(6), 613–622.

Endler, N. S., & Kocovski, N. L. (2001). State and trait anxiety revisited. *Journal of Anxiety Disorders, 15*(3), 231–245.

Gröschl, M. (2008). Current Status of Salivary Hormone Analysis. *Clinical Chemistry, 54*(11), 1759–1769. https://doi.org/10.1373/clinchem.2008.108910

Jelkmann, W. (2001). Pitfalls in the Measurement of Circulating Vascular Endothelial Growth Factor. *Clinical Chemistry, 47*(4), 617–623. https://doi.org/10.1093/clinchem/47.4.617

Johnson, D. M., Delahanty, D. L., & Pinna, K. (2008). The cortisol awakening response as a function of PTSD severity and abuse chronicity in sheltered battered women. *Journal of Anxiety Disorders, 22*(5), 793–800.

Kudielka, B. M., & Kirschbaum, C. (2005). Sex differences in HPA axis responses to stress: A review. *Biological Psychology, 69*(1), 113–132.

Kuhlman, K. R., Chiang, J. J., Bower, J. E., Irwin, M. R., Seeman, T. E., McCreath, H. E., Almeida, D. M., Dahl, R. E., & Fuligni, A. J. (2020). Sleep problems in adolescence are prospectively linked to later depressive symptoms via the cortisol awakening response. *Development and Psychopathology, 32*(3), 997–1006. https://doi.org/10.1017/S0954579419000762

Kumari, M., Badrick, E., Ferrie, J., Perski, A., Marmot, M., & Chandola, T. (2009). Self-Reported Sleep Duration and Sleep Disturbance Are Independently Associated with Cortisol Secretion in the Whitehall II Study. *The Journal of Clinical Endocrinology & Metabolism, 94*(12), 4801–4809. https://doi.org/10.1210/jc.2009-0555

Lasikiewicz, N., Hendrickx, H., Talbot, D., & Dye, L. (2008). Exploration of basal diurnal salivary cortisol profiles in middle-aged adults: Associations with sleep quality and metabolic parameters. *Psychoneuroendocrinology, 33*(2), 143–151. https://doi.org/10.1016/j.psyneuen.2007.10.013

Masten, A. S. (2001). Ordinary magic: Resilience processes in development. *American Psychologist, 56*(3), 227–238. https://doi.org/10.1037/0003-066X.56.3.227

McEwen, B. S., & Stellar, E. (1993). Stress and the individual: Mechanisms leading to disease. *Archives of Internal Medicine, 153*(18), 2093–2101.

Miller, R., & Plessow, F. (2013). Transformation techniques for cross-sectional and longitudinal endocrine data: Application to salivary cortisol concentrations. *Psychoneuroendocrinology, 38*(6), 941–946. https://doi.org/10.1016/j.psyneuen.2012.09.013

Minkel, J. D., Banks, S., Htaik, O., Moreta, M. C., Jones, C. W., McGlinchey, E. L., Simpson, N. S., & Dinges, D. F. (2012). Sleep deprivation and stressors: Evidence for elevated negative affect in response to mild stressors when sleep deprived. *Emotion, 12*(5), 1015–1020. https://doi.org/10.1037/a0026871

Oskis, A., Loveday, C., Hucklebridge, F., Thorn, L., & Clow, A. (2009). Diurnal

patterns of salivary cortisol across the adolescent period in healthy females. *Psychoneuroendocrinology, 34*(3), 307–316. https://doi.org/10.1016/j.psyneuen.2008.09.009

Pruessner, J. C., Hellhammer, D. H., & Kirschbaum, C. (1999). Burnout, perceived stress, and cortisol responses to awakening. *Psychosomatic Medicine, 61*(2), 197–204. https://doi.org/10.1097/00006842-199903000-00012

Pruessner, J. C., Wolf, O. T., Hellhammer, D. H., Buske-Kirschbaum, A., Von Auer, K., Jobst, S., Kaspers, F., & Kirschbaum, C. (1997). Free Cortisol Levels after Awakening: A Reliable Biological Marker for the Assessment of Adrenocortical Activity. *Life Sciences, 61*(26), 2539–2549. https://doi.org/10.1016/S0024-3205(97)01008-4

Scher, A., Hall, W. A., Zaidman-Zait, A., & Weinberg, J. (2010). Sleep quality, cortisol levels, and behavioral regulation in toddlers. *Developmental Psychobiology, 52*(1), 44–53. https://doi.org/10.1002/dev.20410

Schlotz, W. (2011). Ambulatory psychoneuroendocrinology: Assessing salivary cortisol and other hormones in daily life. *Psychoneuroendocrinology, 36*(4), 474–484.

Selmaoui, B., & Touitou, Y. (2003). Reproducibility of the circadian rhythms of serum cortisol and melatonin in healthy subjects: A study of three different 24-h cycles over six weeks. *Life Sciences, 73*(26), 3339–3349. https://doi.org/10.1016/j.lfs.2003.05.007

Shea, A. K., Streiner, D. L., Fleming, A., Kamath, M. V., Broad, K., & Steiner, M. (2007). The effect of depression, anxiety and early life trauma on the cortisol awakening response during pregnancy: Preliminary results. *Psychoneuroendocrinology, 32*(8–10), 1013–1020.

Spielberger, C. D., Gonzalez-Reigosa, F., Martinez-Urrutia, A., Natalicio, L. F., & Natalicio, D. S. (1971). The state-trait anxiety inventory. *Revista Interamericana de Psicologia/Interamerican Journal of Psychology, 5*(3 & 4), 145–158.

Stalder, T., Kirschbaum, C., Kudielka, B. M., Adam, E. K., Pruessner, J. C., Wüst, S., Dockray, S., Smyth, N., Evans, P., Hellhammer, D. H., Miller, R., Wetherell, M. A., Lupien, S. J., & Clow, A. (2016). Assessment of the cortisol awakening response: Expert consensus guidelines. *Psychoneuroendocrinology, 63*, 414–432. https://doi.org/10.1016/j.psyneuen.2015.10.010

Stalder, T., Lupien, S. J., Kudielka, B. M., Adam, E. K., Pruessner, J. C., Wüst, S., Dockray, S., Smyth, N., Evans, P., Kirschbaum, C., Miller, R., Wetherell, M. A., Finke, J. B., Klucken, T., & Clow, A. (2022). Evaluation and update of the expert consensus guidelines for the assessment of the cortisol awakening response (CAR). *Psychoneuroendocrinology, 146*, 105946. https://doi.org/10.1016/j.psyneuen.2022.105946

Steptoe, A., Gibson, E. L., Hamer, M., & Wardle, J. (2007). Neuroendocrine and cardiovascular correlates of positive affect measured by ecological momen-

tary assessment and by questionnaire. *Psychoneuroendocrinology, 32*(1), 56–64.

Stroud, L. R., Salovey, P., & Epel, E. S. (2002). Sex differences in stress responses: Social rejection versus achievement stress. *Biological Psychiatry, 52*(4), 318–327.

Therrien, F., Drapeau, V., Lupien, S. J., Beaulieu, S., Doré, J., Tremblay, A., & Richard, D. (2008). Awakening cortisol response in relation to psychosocial profiles and eating behaviors. *Physiology & Behavior, 93*(1–2), 282–288.

Vargas, I., & Lopez-Duran, N. (2020). The cortisol awakening response after sleep deprivation: Is the cortisol awakening response a "response" to awakening or a circadian process? *Journal of Health Psychology, 25*(7), 900–912. https://doi.org/10.1177/1359105317738323

Vgontzas, A. N., Bixler, E. O., Lin, H.-M., Prolo, P., Mastorakos, G., Vela-Bueno, A., Kales, A., & Chrousos, G. P. (2001). Chronic Insomnia Is Associated with Nyctohemeral Activation of the Hypothalamic-Pituitary-Adrenal Axis: Clinical Implications. *The Journal of Clinical Endocrinology & Metabolism, 86*(8), 3787–3794. https://doi.org/10.1210/jcem.86.8.7778

Wolfram, M., Bellingrath, S., & Kudielka, B. M. (2011). The cortisol awakening response (CAR) across the female menstrual cycle. *Psychoneuroendocrinology, 36*(6), 905–912. https://doi.org/10.1016/j.psyneuen.2010.12.006

Wolpert, E. A. (1969). A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects. *Archives of General Psychiatry, 20*(2), 246. https://doi.org/10.1001/archpsyc.1969.01740140118016

Wüst, S., Federenko, I., Hellhammer, D. H., & Kirschbaum, C. (2000). Genetic factors, perceived chronic stress, and the free cortisol response to awakening. *Psychoneuroendocrinology, 25*(7), 707–720.

Xiong, B., Chen, C., Tian, Y., Zhang, S., Liu, C., Evans, T. M., Fernández, G., Wu, J., & Qin, S. (2021). Brain preparedness: The proactive role of the cortisol awakening response in hippocampal-prefrontal functional interactions. *Progress in Neurobiology, 205*, 102127.

Yu, X., & Zhang, J. (2007). A comparison between the Chinese version of Ego-Resiliency Scale and Connor-Davidson Resilience Scale. *Psychological Science, 30*(5), 3.

Ye, R., & TomRocklin. (1988). The research on test anxiety of cross-culture. *Psychological Science, 3*, 6.

Appendix 1: Trait Anxiety Inventory (TAI)

Please read each statement and mark "√" according to your actual and true feelings.

[Questionnaire items would be listed here]

Appendix 2: Connor-Davidson Resilience Scale (CD-RISC)

Please rate each statement based on your situation over the past month by marking "√" in the option that best matches your actual situation.

[Questionnaire items would be listed here]

Note: Figure translations are in progress. See original paper for figures.