Variability of Cortisol Awakening Response Associated with Sleep Efficiency and Its Relationship with Trait Anxiety and Psychological Resilience

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Abstract

Cortisol Awakening Response (CAR) is closely associated with individual mental health. Traditional research has used multi-day averages as an indicator of CAR to explore the relationship between the two; however, because CAR is influenced by individual state factors (e.g., sleep characteristics), research findings have been highly inconsistent. In response, this study employs CAR variability across multiple days as a novel metric for quantifying CAR, and examines the relationship between CAR variability and mental health under conditions of natural sleep and manipulated sleep efficiency, using trait anxiety and psychological resilience as key variables reflecting individual mental health status. Experiment 1 reduces CAR variability by decreasing variability in sleep efficiency through a natural sleep context, finding a significant positive correlation between CAR variability during natural sleep days and trait anxiety scores, indicating that smaller CAR variability in a stable environment is accompanied by lower levels of trait anxiety in individuals. Experiment 2, conversely, increases CAR variability through manipulation of total sleep deprivation, finding a significant positive correlation between CAR variability before and after sleep deprivation and psychological resilience. This suggests that greater CAR variability in a variable environment is accompanied by higher levels of psychological resilience in individuals. In contrast, mean CAR in both experiments showed no significant correlation with trait anxiety or psychological resilience. These results indicate that, in addition to mean CAR, CAR variability can be considered a more effective physiological indicator for assessing mental health. Considering CAR variability across multiple days is of great significance for understanding how individuals adapt to stress and challenges in daily life, and can provide new perspectives and potential approaches for promoting mental health and designing effective intervention strategies.

Full Text

Variability in Cortisol Awakening Response Related to Sleep Efficiency and Its Relationship with Trait Anxiety and Psychological Resilience

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Abstract

The cortisol awakening response (CAR) is closely associated with individual mental health. Traditional research has used the average CAR magnitude across multiple days as an indicator to explore this relationship. However, because CAR is influenced by state-dependent factors such as sleep characteristics, research conclusions have been highly inconsistent. To address this issue, the present study introduces CAR variability across multiple days as a new metric for quantifying CAR and examines its relationship with mental health, with trait anxiety and psychological resilience serving as key variables reflecting mental health status, under both natural sleep conditions and manipulated sleep efficiency scenarios.

Experiment 1 reduced CAR variability by minimizing sleep efficiency variability through natural sleep observation. It found a significant positive correlation between CAR variability under natural sleep conditions and trait anxiety scores, indicating that smaller CAR variability in a stable environment is associated with 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 a changing environment is associated with higher psychological resilience levels. In contrast, CAR means in both experiments showed no significant correlations with either trait anxiety or psychological resilience. These results suggest that, in addition to CAR mean, CAR variability may serve as a more effective physiological indicator for assessing mental health. Considering CAR variability across multiple days is important for understanding how individuals adapt to daily stressors and challenges, and may provide new perspectives and potential approaches for promoting mental health and designing effective intervention strategies.

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1 Introduction

The cortisol awakening response (CAR), as part of the diurnal rhythm of the stress hormone cortisol, refers to the peak in cortisol levels occurring 30–45 minutes after awakening, with an increase of 50% to 160% within one hour (Clow et al., 2004; Pruessner et al., 1997). Understanding the role of CAR in mental health may contribute to providing biomarkers for the diagnosis and treatment of psychological disorders, leading researchers to focus particularly on the relationship between CAR and mental health. Some studies emphasize that a high CAR may reflect positive psychological characteristics, as the increase in CAR as a resource helps individuals cope with upcoming challenges or tasks (Adam et al., 2006; Shi et al., 2018; Wu et al., 2015; Xiong et al., 2021). Conversely, stress adaptation theory posits 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 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 (Oosterholt et al., 2015; Shea et al., 2007; Therrien et al., 2008); while in other studies, positive psychological health characteristics are also associated with reduced CAR (Endrighi et al., 2011; Steptoe et al., 2007; Wüst et al., 2000). Even for the same mental health variable (depression), some studies link it to increased CAR while others link it to decreased CAR (Johnson et al., 2008; Therrien et al., 2008). Additionally, many studies have found no significant association between CAR and mental health (Kallen et al., 2008; Nater et al., 2008; Whitehead et al., 2007; Wu et al., 2017).

Notably, previous research has typically used the average CAR magnitude across multiple days as an indicator to explore the relationship between CAR and mental health. This analytical approach is primarily based on earlier studies that regarded CAR as a stable biomarker with high stability across multiple days (Powell & Schlotz, 2012; Pruessner et al., 1997). However, as research has progressed, an increasing number of factors have been found to significantly influence CAR, such as sleep duration (Stalder et al., 2016). Some studies have found that individuals who wake up earlier typically have higher CAR (Bowles et al., 2022; Edwards et al., 2001), while others have found that shorter sleep duration is associated with higher CAR (Kumari et al., 2009; Wüst et al., 2000). Furthermore, evidence suggests a close relationship between sleep quality and CAR (Lasikiewicz et al., 2008; Moon et al., 2023; Torres et al., 2024). These findings indicate that specific sleep characteristics are important variables affecting CAR levels, and that day-to-day changes in CAR may reflect the body's flexible regulation of stress levels and adaptation to different sleep conditions. Therefore, using only the average magnitude to index CAR may fail to capture this adaptive change, leading to biased research conclusions.

As an important product of the rhythmic activity of the hypothalamic-pituitary-adrenal (HPA) axis that regulates stress responses and hormonal balance, CAR exhibits fluctuations across multiple days. For example, individuals show significant differences in CAR between weekends and weekdays (Okamura et al., 2010; Schlotz et al., 2004). Mikolajczak et al. (2010) further found that greater CAR fluctuations between weekend and weekday contexts were associated with higher subjective well-being scores and lower neuroticism and stress perception scores, reflecting a positive mental health state. Additionally, this study found inconsistent relationships between average CAR on weekends and weekdays and mental health status. Both subjective well-being (reflecting positive mental states) and stress perception (reflecting negative mental states) showed significant negative correlations with average CAR. These results suggest that focusing on CAR fluctuations across multiple days rather than its magnitude may be more helpful in establishing consistent links between CAR and mental health. The present study defines this day-to-day fluctuation in CAR as CAR variability, specifically quantified using the standard deviation of CAR indices across multiple days. Compared to traditional analyses using average CAR magnitude, analyzing day-to-day CAR variability can better capture the dynamic and fluctuating nature of individual physiological responses. In situations where the environment is similar across days and expected stress levels are stable, smaller CAR variability may indicate good HPA axis adaptation to environmental stressors and relatively stable psychological states, thereby reflecting good mental health (Packard et al., 2016). Conversely, significant changes in environment and internal stress require the HPA axis to flexibly adjust cortisol release to adapt to these changes. Therefore, in contexts where the environment varies significantly across days, greater CAR variability may reflect the HPA axis's flexible adaptation to different stress levels, a marker of psychological resilience and coping capacity that helps individuals maintain long-term mental health (Abercrombie et al., 2023; Mikolajczak et al., 2010).

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 characteristic of individuals' tendency to experience anxiety, reflecting their persistent and generalized stress responses to potential threats (Spielberger et al., 2017). Psychological resilience refers to individuals' ability to recover from adversity, involving their adaptability and recovery capacity after experiencing stress, challenges, or failure (Wu et al., 2013). Trait anxiety and psychological resilience reflect individuals' ability 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 aims to use CAR variability as an indicator reflecting the diurnal rhythm of the stress hormone cortisol and analyze the relationship between CAR variability and trait anxiety/psychological resilience. CAR is easily influenced by sleep characteristics (Stalder et al., 2016), and sleep efficiency is one of the main sleep characteristics, referring to the proportion of actual sleep time to total time in bed. Compared to other sleep characteristics, sleep efficiency can more comprehensively reflect the effectiveness of an individual's sleep and, to some extent, their sleep quality (Nelson et al., 2022). Research shows that after a night of total sleep deprivation, CAR the following morning is significantly reduced and the peak is delayed (Vargas & Lopez-Duran, 2020). Another study found that when sleep duration is restricted to reduce sleep efficiency, CAR the following day is enhanced (Lanlokun et al., 2017). Additionally, research indicates that when individuals are exposed to low-frequency noise at night and experience low sleep efficiency conditions, CAR the following day is weakened (Waye et al., 2003). Therefore, sleep efficiency may affect individuals' CAR the following day, ultimately leading to day-to-day CAR variability. Given this, it is necessary to consider the impact of sleep efficiency changes when calculating CAR variability indices. Since individuals' stress hormone cortisol diurnal rhythm generally shows stability (Bowles et al., 2022; Selmaoui & Touitou, 2003), CAR variability may also be small when sleep efficiency changes are minimal. Conversely, if sleep efficiency fluctuates significantly, individuals' CAR variability may change substantially. To test these hypotheses, Experiment 1 used natural sleep observation to reduce CAR variability by minimizing sleep efficiency variability. 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 to increase CAR variability by increasing sleep efficiency variability. In this context, greater CAR variability better reflects positive mental health characteristics, with individuals showing higher psychological resilience scores and lower trait anxiety scores.

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

2.1 Research Purpose

To reduce CAR variability by minimizing sleep efficiency variability through natural sleep observation and explore its relationship with trait anxiety and psychological resilience.

2.2 Methods

2.2.1 Participants Since participants needed to collect multiple saliva samples across several days to assess CAR variability and establish its relationship with trait anxiety and psychological resilience, we calculated the required sample size using the "pwr" package in R (Haynes et al., 2021) to ensure adequate statistical power. Previous research found medium to large effect sizes for relationships between CAR and mental health variables (Hardeveld et al., 2014; Kudielka & Kirschbaum, 2005; Rickard et al., 2016). Based on Cohen's (2013) classification of effect sizes, we set the effect size (Cohen's f^2) to 0.3, slightly above the medium level. The significance level α was set at 0.05, and statistical power 1-β at 0.8. The results indicated that at least 28 participants were needed to detect the expected effects.

We recruited 30 healthy university students as paid volunteers through online advertisements. Due to failure to comply with the CAR collection protocol (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). Although recent research suggests that women's menstrual cycles have minimal or non-significant effects on CAR (Stalder et al., 2022), we used questionnaires to confirm that all female participants were in the luteal phase to minimize potential interference. 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.

This experiment was approved by the Ethics Committee of the Faculty of Psychology at Southwest University, and all procedures strictly adhered to ethical guidelines. Before the formal experiment began, each participant was fully informed about the research purpose, procedures, potential risks and benefits, and voluntarily agreed to participate. They were also informed that they could withdraw from the study at any time without penalty. The experiment lasted three days. After completion, researchers responsible for data quality checks examined the data. Full participant compensation was provided after confirming data quality.

2.2.2 Mental Health Questionnaires **Trait Anxiety Inventory (TAI):** This scale assesses individuals' long-term, trait anxiety levels (Spielberger et al., 2017). We used the Chinese version of the TAI (Wang et al., 1999), which consists 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 coefficient in this study was 0.90.

Connor-Davidson Resilience Scale (CD-RISC): This scale assesses individuals' ability to adapt and recover when facing stress, challenges, and adversity (Connor & Davidson, 2003). We used the Chinese version of the CD-RISC (Yu

& Zhang, 2007), which consists of 25 items, each rated on a 5-point Likert scale from 0 ("not true at all") to 4 ("true nearly all the time"). Higher scores indicate better psychological resilience. The Cronbach's α coefficient in this study was 0.87.

2.2.3 Sleep Data Collection and Analysis Objective sleep data were collected using Actigraph wristwatches (Phillips Respironics, Inc.), accelerometer-based devices worn on the dominant wrist 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 indices were calculated: Time in Bed (TIB), the total duration from attempted sleep onset to final awakening; Wake After Sleep Onset (WASO), the total duration of all wake 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 SE = (TST / TIB) × 100%, representing the percentage of actual sleep time relative to time in bed.

Subjective sleep data were recorded through sleep diaries, which included: (1) time of closing eyes to prepare for sleep the previous night; (2) time required to fall asleep; and (3) time of morning awakening. Based on this information, the following indices were calculated: Total Bed Time (TBT), the time from closing eyes the previous night to morning awakening; and Total Sleep Time (TST), calculated by subtracting sleep onset latency from TBT. Finally, subjective Sleep Efficiency (SE) was calculated as SE = (TST / TBT) × 100%.

2.2.4 Salivary Cortisol Collection and Analysis Participants collected 4 saliva samples on each collection day at 0, 30, 45, and 60 minutes post-awakening. Participants were instructed not to eat, drink (including alcohol, caffeine, or juice), brush their teeth, or smoke before completing all daily samples. Saliva samples were collected using Salivette tubes (SARSTEDT, Germany). During each collection, participants chewed the cotton swab from the tube for 1 minute, then returned it to the tube and sealed it, avoiding hand contact throughout to prevent contamination. After each collection, tubes were placed in a Medication Event Monitoring System (MEMSCap™, MWV Switzerland Ltd.) to record objective collection times. All samples were stored at -20°C before analysis.

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

2.2.5 Experimental Procedure Before the formal experiment began, participants scheduled a face-to-face meeting with the experimenter, a design proven to effectively improve compliance with sample collection protocols (Stalder et al., 2016, 2022). During the meeting, the experimenter first introduced 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 bed or waking up too early or too late was prohibited. Participants also needed to continuously wear the sleep wristwatch on their dominant hand, keeping it close to the skin and not removing it except under special circumstances; wristwatches were uniformly collected by the experimenter after the experiment. Participants were then informed about saliva collection procedures and precautions.

Participants were asked to choose any three weekdays within a given week for saliva sample collection. The selected collection days needed to approximate participants' normal daily life as closely as possible, with no major stressful events before or after collection days to control for the effects of weekend/weekday differences and stress factors on CAR (Stalder et al., 2016). After each saliva collection, participants recorded the specific collection time, with accuracy affecting their final compensation. After the meeting, participants completed demographic and mental health questionnaires.

During the formal data collection period, the experimenter scheduled times with participants before each collection day to provide and retrieve experimental materials and confirm that participants understood and complied with experimental precautions. Non-compliant behaviors and unexpected events were recorded, and data were excluded or retained after assessing their impact on data quality. Additionally, participants completed sleep diaries sent by the experimenter at a scheduled time (8:00 AM) each morning.

2.2.6 Data Analysis All data were analyzed using SPSS 27.0. Before ANOVA, data were tested for normality and homogeneity of variance. Given that repeated measures data may violate sphericity assumptions, Greenhouse-Geisser correction was applied to adjust degrees of freedom for variables not meeting sphericity assumptions, correcting statistical inference bias resulting from assumption violations. When ANOVA results were significant, Bonferroni post-hoc multiple comparisons were conducted.

Specifically, one-way repeated measures ANOVA was used to test day-to-day differences in sleep efficiency, with collection day (Natural Day 1, Natural Day 2, and Natural Day 3) as the independent variable. For raw cortisol data, repeated measures ANOVA was used to test main effects and interactions of collection time point (0, 30, 45, and 60 minutes) and collection day (Natural Day 1, Natural Day 2, and Natural Day 3). Subsequently, mean increase (MnInc) and range (max-min) were calculated as daily CAR indices by using the average cortisol level at the last three time points relative to the first time point (Stalder et al., 2016). One-way repeated measures ANOVA was then used to test day-to-day differences in CAR, with collection day as the independent variable.

Finally, relationships between sleep efficiency, CAR indices, and trait anxiety/psychological resilience were examined under both traditional mean and novel variability frameworks. In the mean framework, Pearson correlations were calculated among three-day mean sleep efficiency, mean CAR, and trait anxiety/psychological resilience to explore their interrelationships and reveal whether sleep efficiency affects mean CAR and mental health variables. Before correlation analysis, all variables were tested for normality and transformed if necessary to meet assumptions. Since gender may affect CAR and mental health (Almeida et al., 2009; Hollanders et al., 2017; Vargas et al., 2017), partial correlations between mean CAR and trait anxiety/psychological resilience were calculated after controlling for gender and any variables found to affect CAR means and mental health in the above correlation analysis.

In the variability framework, Pearson correlations were calculated among three-day sleep efficiency variability, CAR variability, and trait anxiety/psychological resilience to explore their interrelationships and reveal whether sleep efficiency variability affects CAR variability and mental health. All variables were similarly tested for normality and transformed as needed. Partial correlations between CAR variability and trait anxiety/psychological resilience were then calculated after controlling for gender and any variables found to affect CAR variability and mental health.

2.3 Results

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 revealed 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 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 indicated a significant main effect of collection time point, $F(2.04, 54.98) = 19.81$, $p < 0.001$, $^2 = 0.42$, suggesting that 42% of the total variance in observed cortisol changes could be explained by collection time point. Post-hoc comparisons revealed that cortisol levels at 30 minutes post-awakening were significantly higher than at awakening ($p < 0.001$, 95% CI: 0.26–0.88), 45 minutes post-awakening ($p = 0.01$, 95% CI: 0.03–0.35), and 60 minutes post-awakening ($p < 0.001$, 95% CI: 0.29–0.79). The main effect of collection day was not significant, $F(2, 54) = 1.99$, $p = 0.15$, nor was the interaction between collection day and time point, $F(3.72, 100.36) = 0.59$, $p = 0.66$.

Tests of differences in daily CAR indices (Mnlnc, max-min) across the three natural sleep days are shown in Figure 2 Figure 2: see original paper. Repeated measures ANOVA revealed no significant differences across collection days for either daily index, F (5, 54) = 0.24, $p = 0.78$; F (5, 54) = 1.83, $p = 0.17$.

2.3.3 Relationships Among Mean Sleep Efficiency, Mean CAR, and Trait Anxiety/Psychological Resilience In the mean framework, descriptive statistics for mean sleep efficiency, mean CAR, and psychological resilience/trait anxiety are shown in Table 2 . Correlation analysis revealed that only the negative correlation between mean objective sleep efficiency and psychological resilience reached significance, $r = -0.37$, $p = 0.050$. No significant correlations were found between mean CAR and trait anxiety/psychological resilience.

After further controlling for gender and mean objective sleep efficiency, partial correlations between mean CAR and trait anxiety/psychological resilience are shown in Table 3 . Results indicated that partial correlations between mean CAR and trait anxiety/psychological resilience remained non-significant.

2.3.4 Relationships Among Sleep Efficiency Variability, CAR Variability, and Trait Anxiety/Psychological Resilience In the variability framework, descriptive statistics for sleep efficiency variability, CAR variability, and psychological resilience/trait anxiety are shown in Table 4 . Correlation analysis revealed a significant positive correlation between CAR variability calculated using Mnlnc and trait anxiety, $r = 0.42$, $p = 0.027$, indicating that smaller CAR variability under natural sleep conditions is associated with lower trait anxiety. Additionally, a significant positive correlation was found between CAR variability calculated using Mnlnc and subjective sleep efficiency variability, $r = 0.39$, $p = 0.038$, suggesting that smaller subjective sleep efficiency fluctuations across three natural sleep days are accompanied by smaller CAR variability.

After controlling for gender and subjective sleep efficiency variability, partial correlations between CAR variability and trait anxiety/psychological resilience are shown in Table 5 . The positive correlation between CAR variability calculated using Mnlnc and trait anxiety remained significant, $r = 0.48$, $p = 0.012$. The residual scatter plot of CAR variability and trait anxiety after controlling for covariates is shown in Figure 2 [Figure 2: see original paper].

2.4 Discussion

Similar CAR response patterns were observed across the three collection days, with cortisol levels peaking at 30 minutes post-awakening and then gradually declining to levels no different from awakening by 60 minutes post-awakening. This CAR pattern is consistent with previous research. The rise in cortisol 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 complete, cortisol levels fall back, reflecting HPA axis regulation of daily rhythms (Pruessner et al., 1997).

Regarding sleep efficiency and CAR, repeated measures ANOVA revealed small variability in sleep efficiency across the three natural sleep days, accompanied by small variability in next-day CAR. Correlation analysis further showed a significant positive correlation between subjective sleep efficiency variability and CAR variability calculated using Mnlnc under natural sleep conditions. This suggests that smaller fluctuations in subjective sleep efficiency across similar natural sleep days are associated with smaller CAR variability, potentially indicating a link between stable sleep patterns and HPA axis function. The HPA axis is the primary neuroendocrine system responding to stress, helping the body adapt through the release of cortisol and other hormones. Sleep is physiologically linked to the HPA axis, as it is a critical period for restoring HPA axis activity (Lo Martire et al., 2024). High-quality sleep may help maintain HPA axis homeostasis, while sleep disorders may lead to HPA axis dysfunction. Therefore, stable sleep efficiency may reflect good HPA axis regulatory function, which may further manifest as smaller CAR fluctuations.

Additionally, the results found a significant negative correlation between objective sleep efficiency and psychological resilience in the mean framework. After controlling for mean objective sleep efficiency and gender, partial correlations between mean CAR and trait anxiety/psychological resilience remained nonsignificant. Although Experiment 1 controlled for factors affecting CAR, CAR is still susceptible to many unknown factors and shows variability across multiple days (Hellhammer et al., 2007). This variability may make it difficult for mean CAR to capture stable associations with trait anxiety/psychological resilience.

In the variability framework, results showed that regardless of whether covariates were controlled, CAR variability calculated using Mnlnc exhibited a significant positive correlation with trait anxiety. After controlling for gender and subjective sleep efficiency variability, the significance of the positive correlation between CAR variability and trait anxiety increased from $r = 0.42$, $p = 0.027$ to $r = 0.48$, $p = 0.012$. These findings extend Mikolajczak et al.'s (2010) results, showing that not only do CAR fluctuations across different contexts relate to mental health status, but day-to-day CAR variability under similar natural sleep conditions also relates to trait anxiety, a variable reflecting negative mental health states. In this context, smaller CAR variability reflects healthier psychological states, manifested as lower trait anxiety scores. As one physiological indicator of stress response, CAR variability may reflect individuals' capacity to cope with environmental stress. Smaller CAR variability indicates relatively stable daily physiological responses upon awakening, suggesting more effective stress management and adaptation to daily life pressures. This stable coping mechanism is associated with higher mental health levels and lower anxiety (Kuhlman et al., 2020). Moreover, 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 manifest physiologically through reduced fluctuations in stress responses, resulting in smaller CAR variability (Packard et al., 2016).

However, these results only appeared when using Mnlnc as the daily CAR calculation index; when using max-min as the daily CAR index, correlations between CAR variability and trait anxiety/psychological resilience were not significant. This difference may stem from the different biological meanings underlying the two daily CAR indices. Mnlnc primarily reflects the overall upward trend in cortisol levels after awakening, serving as an indicator of individuals' sensitivity and intensity of response to the daily physiological process of awakening. In contrast, max-min reflects the fluctuation range of cortisol levels within a certain period after awakening, providing information about individual physiological response variability and serving as an indicator of stress system regulation capacity and adaptability (Clow et al., 2004). These two indices may reflect different aspects of the body's stress response to awakening, revealing different physiological regulatory mechanisms and stress coping strategies. In Experiment 1, where participants were under natural sleep conditions, Mnlnc, as an index reflecting the overall upward trend of CAR, may better capture individuals' physiological regulation 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 some association with trait anxiety.

Additionally, Experiment 1 did not observe a significant correlation between CAR variability and psychological resilience. 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, resulting in no significant association between psychological resilience and CAR variability. Furthermore, research has found that differences in stress events experienced during the day may affect CAR to varying degrees (Giglberger et al., 2022; Powell & Schlotz, 2012), making CAR variability potentially influenced by individuals' perceived daily stress events, which Experiment 1 did not control. Based on these issues, Experiment 2 will further include relevant questionnaires to control for potential daily stress interference and use sleep deprivation manipulation to increase CAR variability for further analysis of its relationship with trait anxiety/psychological resilience.

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3 Experiment 2: Sleep Deprivation, CAR Variability, and Its Relationship with Trait Anxiety/Psychological Resilience

3.1 Research Purpose

To increase CAR variability by reducing sleep efficiency stability through sleep deprivation and explore its relationship with trait anxiety/psychological resilience.

3.2 Methods

3.2.1 Participants Experiment 2 also used the "pwr" package in R for sample size calculation with the same parameters as Experiment 1 (Cohen's $f^2 = 0.3$, $\alpha = 0.05$, $1\text{-}\beta = 0.8$). Based on the calculation results and potential sample attrition, 42 healthy university students were recruited as paid volunteers through online advertisements. Due to failure to comply with the CAR collection protocol (Stalder et al., 2016, 2022), data from 2 participants were excluded, resulting in a final sample of 40 participants, including 20 females (M = 20.60, SD = 1.60) and 20 males (M = 20.90, 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 Daily Stress Inventory (DSI): This questionnaire measures individuals' stress levels in daily life (within 24 hours) (Brantley et al., 1987). The DSI contains 58 items, each corresponding to a daily stress event. Participants indicate whether they experienced each event in the past 24 hours (marked as 0 if not experienced). For experienced events, they rate the stress level on a scale from 1 ("occurred but not stressful") to 7 ("caused terrifying stress"). The final score is calculated by dividing the total stress score by the number of stress events experienced, reflecting the average intensity of stress events within 24 hours. Higher scores indicate higher stress levels.

3.2.3 Sleep Deprivation Manipulation Total Sleep Deprivation (TSD) was used to manipulate sleep efficiency and create a special experimental context completely different from natural sleep conditions. TSD refers to a state where individuals have no sleep for a certain period. Under these conditions, 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 Committee of the Faculty of Psychology at Southwest University. Before the formal experiment began, each participant was fully informed about the research purpose, procedures, potential risks and benefits, and voluntarily agreed to participate. They were also informed that they could withdraw from the study at any time without penalty.

After the experiment, researchers responsible for data quality checks examined the data. Full participant compensation was provided after confirming data quality.

3.2.4 Salivary Cortisol Analysis Experiment 2 used liquid chromatography-mass spectrometry (LC-MS) to quantitatively analyze cortisol levels in saliva samples. This technique was selected for its high sensitivity and specificity, particularly suitable for detecting low-concentration hormones in biological samples (Gröschl, 2008).

Before analysis, saliva samples were centrifuged (5000 rpm, 4°C, 10 minutes). The supernatant was filtered through a membrane to remove large molecular impurities. The liquid chromatography system used a reversed-phase 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 for optimal signal intensity. Cortisol levels were quantified using external standard calibration by comparing sample responses to known concentration standard curves. Data processing and quantitative analysis were performed using specialized mass spectrometry software.

3.2.5 Experimental Procedure In Experiment 2, participants followed the same procedure as Experiment 1 for the first two collection days, with the addition of completing the Daily Stress Inventory (DSI) at 10:00 PM each night. Before participating in the sleep deprivation experiment, the experimenter coordinated timing with participants and informed them that on the day of deprivation, they could not sleep or nap during the day and must strictly wear the sleep wristwatch without removal. The experiment began at 10:00 PM on the scheduled date. Participants came to the laboratory and completed a full night of sleep deprivation. Natural light was completely blocked using blackout curtains throughout the deprivation period, with only constant-power LED lights maintained in the laboratory. Two experimenters were present at all times to ensure participants did not sleep or nap; if napping was observed, participants were immediately awakened. Participants were required to maintain emotional stability throughout the experiment; vigorous stimulating activities and consumption of alcohol or caffeine were prohibited. Between 7:00 and 8:00 AM the following morning, participants were required not to eat, drink, brush their teeth, or smoke. At 8:00, 8:30, 8:45, and 9:00 AM, the experimenter reminded participants to collect saliva samples, after which all samples and sleep wristwatches were uniformly collected and stored.

3.2.6 Data Analysis All data were analyzed using SPSS 27.0. As in Experiment 1, data were tested to meet statistical assumptions before ANOVA, and Greenhouse-Geisser correction was applied for variables violating sphericity assumptions. Bonferroni post-hoc comparisons were conducted when ANOVA results were significant.

Specifically, repeated measures ANOVA was used to test main effects and interactions of collection time point (0, 30, 45, and 60 minutes) and collection day (Natural Day 1, Natural Day 2, and Deprivation Day 3). Mean increase (Mnlnc) and range (max-min) were used as daily CAR indices, and one-way ANOVA tested day-to-day differences in CAR with collection day as the independent variable. In Experiment 2, CAR variability was calculated as the standard deviation of CAR across the two normal sleep days and the post-deprivation day.

Finally, relationships between CAR indices and trait anxiety/psychological resilience were examined under both mean and variability frameworks, controlling for daily stress and gender. In the mean framework, the mean daily stress across the two natural sleep days was used as an indicator of average daily stress levels. Partial correlations between mean CAR and trait anxiety/psychological resilience were calculated after controlling for mean daily stress and gender. In the variability framework, the standard deviation of daily stress across the two natural sleep days was used as an indicator of daily stress variability. Partial correlations between CAR variability and trait anxiety/psychological resilience were calculated after controlling for daily stress variability and gender.

3.3 Results

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, $F(4.51, 171.43) = 7.08$, $p < 0.001$, $^2 = 0.16$, indicating that 16% of the total variance in cortisol changes was explained by the interaction between time point and day. Simple effects analysis revealed that on Natural Days 1 and 2, participants' cortisol levels peaked around 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.

Tests of differences in daily CAR indices (Mnlnc, max-min) across the three days are shown in Figure 3 Figure 3: see original paper. The main effects of collection day were significant for both CAR indices, F (2, 76) = 12.09, $p < 0.001$, $^2 = 0.24$; F (2, 76) = 10.84, $p < 0.001$, $^2 = 0.22$. These results indicate that Mnlnc and max-min showed considerable variability across collection days, with a large portion of this variance explained by day-to-day changes. Further multiple comparisons revealed no significant differences in CAR between the first two collection days, but CAR on both days was significantly higher than on the post-deprivation third day.

3.3.2 Relationships Among Mean Daily Stress, Mean CAR, and Trait Anxiety/Psychological Resilience In the mean framework, descriptive statistics for mean daily stress, mean CAR, and trait anxiety/psychological resilience are shown in Table 6 . After controlling for mean daily stress and gender, partial correlations between mean CAR and trait anxiety/psychological resilience were non-significant, as shown in Table 7 .

3.3.3 Relationships Among Daily Stress Variability, CAR Variability, and Trait Anxiety/Psychological Resilience In the variability framework, descriptive statistics for daily stress variability, CAR variability, and trait anxiety/psychological resilience are shown in Table 8 . After controlling for daily stress variability and gender, partial correlations between CAR variability and trait anxiety/psychological resilience are shown in Table 9 . CAR variability calculated using max-min showed a significant positive correlation with psychological resilience, $r = 0.36$, $p = 0.026$. Residual scatter plots of these variables after controlling for covariates are shown in Figure 4 [Figure 4: see original paper].

3.4 Discussion

Experiment 2 found that sleep deprivation led to a significant reduction in next-day CAR, consistent with previous research (Minkel et al., 2012). Prolonged wakefulness and sleep deprivation may blunt the HPA axis response to awakening stimuli, resulting in reduced cortisol release upon awakening, i.e., CAR blunting. This effect may occur because the HPA axis enters a relatively suppressed state after overactivation, temporarily reducing its sensitivity to awakening stimuli (Vargas & Lopez-Duran, 2020).

Correlation analysis showed that after controlling for gender and daily stress variability, individuals' CAR variability following sleep deprivation was significantly positively correlated with psychological resilience. This finding further enriches Mikolajczak et al.'s (2010) results, demonstrating that greater CAR fluctuations not only reflect healthy mental states across weekend and weekday contexts but also relate to healthy mental states across natural sleep and total sleep deprivation contexts. Greater CAR variability before and after sleep deprivation is associated with higher psychological resilience scores. Psychological resilience emphasizes individuals' adaptability and recovery capacity when facing 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, Experiment 2 did not observe a significant correlation between CAR variability indices and trait anxiety scores. Although both trait anxiety and psychological resilience are relatively stable trait indicators, their mechanisms may differ when facing major stressor challenges. High-resilience individuals can typically adapt and restore physiological balance more quickly, a mechanism particularly important when facing major challenges (Liu et al., 2018). Trait anxiety primarily reflects individuals' perceptual tendencies toward long-term environmental pressures and threats (Alemany-Arrebola et al., 2020). When facing major challenges, trait anxiety may be less sensitive to such acute physiological changes, thus not showing a significant correlation with CAR variability.

Unlike Experiment 1's findings, these 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 again stem from the different biological meanings of these two indices. Mnlnc mainly reflects the overall upward trend in cortisol after awakening, while max-min reflects the fluctuation amplitude of cortisol levels within a certain period after awakening. In Experiment 2, where participants experienced the extreme stress of total sleep deprivation, max-min, as an indicator reflecting CAR fluctuation amplitude, may more directly reflect individuals' physiological response intensity and regulatory range to extreme changes. Total sleep deprivation as an extreme stressor can trigger intense physiological and psychological responses, leading to increased CAR fluctuation amplitude the following day. This increased fluctuation amplitude may be related to individuals' psychological adaptability and recovery capacity when facing extreme stressors (Clow et al., 2010), ultimately manifesting as a significant positive correlation between CAR variability and psychological resilience.

Additionally, similar to Experiment 1, no significant correlations were found between mean CAR and trait anxiety/psychological resilience in the mean framework, regardless of covariate control. This further emphasizes the limitations of using mean CAR when assessing relationships with trait anxiety and psychological resilience. Because CAR is easily influenced by many factors, including psychological stress, sleep quality, time pressure, circadian rhythms, and even sampling methods (Stalder et al., 2016), calculating only mean CAR may ignore how these factors differentially affect individual CAR across time. In contrast, analyzing CAR variability provides more comprehensive information. First, CAR variability may more sensitively reflect individuals' physiological responses to different stressors, capturing subtle day-to-day differences in HPA axis response patterns and providing richer information for understanding how psychological states affect physiological processes. Second, CAR variability analysis may better reflect individuals' capacity to adapt and respond to environmental pressures. For example, increased CAR variability after sleep deprivation may indicate individuals' efforts to regulate their physiological states to adapt to stress. Finally, the relationship between CAR variability and trait anxiety/psychological resilience suggests it may be a more comprehensive physiological indicator of mental health, providing important information about how individuals regulate their physiological responses across different times and pressures.

4 General Discussion

This study examined the relationship between CAR variability and psychological resilience/trait anxiety through natural sleep observation with similar sleep efficiency (Experiment 1) and total sleep deprivation manipulation creating significantly different sleep contexts (Experiment 2). Results showed that under similar natural sleep conditions, CAR variability was smaller and significantly positively correlated with trait anxiety; while across different sleep conditions before and after sleep deprivation, CAR variability was larger and significantly positively correlated with psychological resilience. In contrast, when using multi-day mean CAR as an indicator, no significant correlations with psychological resilience or trait anxiety were found regardless of sleep context similarity. These results generally support the hypothesis that, in addition to mean CAR, CAR variability may serve as a more effective physiological indicator for assessing mental health.

Since its introduction, CAR has become an important indicator in psychophysiology and sleep research for assessing individuals' stress responses and circadian regulation. Although researchers have conducted extensive studies on CAR and made significant progress, several key issues remain unresolved. First, regarding the psychological meaning underlying this physiological response, increasing evidence suggests CAR may represent physiological preparation for upcoming daily challenges and pressures (Wu et al., 2015; Xiong et al., 2021), but controversy persists (Fries et al., 2009). Second, although CAR has been used as a biomarker for assessing 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. Addressing these issues, this study found through comparing natural sleep and sleep deprivation contexts that CAR is not merely a static physiological marker but a dynamic indicator capable of flexibly adapting to environmental stress changes. Sleep deprivation caused significant CAR reduction, but this change manifested as greater CAR variability in high-resilience individuals, demonstrating adaptation to extreme stress. Furthermore, this study refined the relationship between CAR and mental health. Traditional research typically linked CAR levels directly to mental health, but this study found that CAR change patterns across different contexts may be more complex. For example, smaller CAR changes under natural sleep conditions may relate to lower trait anxiety, while greater CAR changes across sleep deprivation contexts may better reflect psychological resilience. This suggests that researchers should interpret results more cautiously by considering specific collection contexts when exploring CAR's meaning. Finally, this study provides new perspectives for CAR as a mental health assessment tool by exploring its response patterns across different contexts. Particularly under extreme stress conditions (e.g., sleep deprivation), CAR variability may serve as an important indicator for assessing individual psychological resilience.

Although previous research has found certain links between sleep and CAR (Edwards et al., 2001; Kumari et al., 2009; Lasikiewicz et al., 2008; Wüst et al., 2000), how sleep connects with CAR remains controversial. This study addressed this through two experiments examining how natural sleep and sleep deprivation-related sleep efficiency fluctuations affect CAR variability. First, this study found a significant positive correlation between subjective sleep efficiency variability and CAR variability under natural sleep conditions. Additionally, CAR after sleep deprivation showed significant changes compared to natural sleep conditions. 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 physiological and mental health. By revealing the link between subjective sleep efficiency changes and CAR variability, this study provides theoretical direction for designing targeted sleep interventions—future efforts may optimize daytime physiological stress responses and promote overall health by improving sleep quality and reducing day-to-day subjective sleep efficiency variability. Overall, this study not only enriches understanding of the complex links between sleep and daytime physiological regulation but also provides new intervention ideas for the sleep field.

In selecting mental health indicators, this study chose trait anxiety and psychological resilience as two core indicators reflecting mental health status. Trait anxiety reflects individuals' persistent and generalized stress responses to potential threats and is a stable personality characteristic (Spielberger et al., 2017). Psychological resilience describes individuals' recovery strength and adaptability when facing adversity (Masten, 2001). Considering both trait anxiety and psychological resilience provides a more comprehensive research framework for understanding how individuals cope with stress from both physiological and psychological perspectives. This multidimensional measurement approach can better identify individuals who may need additional support when coping with daily life stress. Furthermore, this study demonstrates how physiological indicators can be used to assess and understand 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 interventions, holding important theoretical and practical significance.

This study has several limitations. First, both experiments used three days of CAR samples to calculate CAR variability. Three-day CAR measurements may be influenced by various extraneous factors such as life events and unexpected occurrences, whose short-term changes may significantly affect CAR variability. Future research could consider extending measurement duration and increasing measurement frequency for more comprehensive understanding. Second, sample sizes in both experiments were relatively small. Experiment 1's final sample only met the minimum calculated sample size, while Experiment 2's final sample was only slightly above the minimum. Moreover, both experiments used university student populations, which may limit statistical power and external validity. Future research should expand sample sizes and participant populations to obtain more reliable conclusions.

Overall, this study provides a new analytical approach for examining the relationship between CAR and mental health. Compared to traditional mean-level analyses, considering CAR variability across multiple days offers a more comprehensive and nuanced 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 stressors and challenges.

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Appendices

Appendix 1: Trait Anxiety Inventory (TAI)

Please read each statement and mark "√" according to your actual and true feelings in daily life:

Almost never	Sometimes	Often	Almost always

1. I feel pleasant

2. I feel tired and exhausted
   ...

3. I become tense when I think about my current affairs and interests

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:

Not true at all	Rarely true	Sometimes true	Often true	True nearly all the time

1. I can adapt to change

2. I have close, secure relationships
   ...

3. I can make difficult or unusual decisions

Appendix 3: Daily Stress Inventory (DSI)

The following lists 58 minor events that may cause stress or unpleasant feelings. Read each item carefully and determine whether it occurred within the past 24 hours. If it did not occur, mark 0 before the item; if it occurred, rate it 1-7 (see criteria below). Finally, there are two blank items for you to fill in any stress events you encountered within 24 hours that are not listed among the 58 items, also rated 1-7 (leave blank if none). Please answer as carefully as possible so we can obtain accurate information.

(0 = did not occur in past 24 hours; 1 = occurred but not stressful; 2 = caused slight stress; 3 = caused minor stress; 4 = caused moderate stress; 5 = caused considerable stress; 6 = caused great stress; 7 = caused terrifying stress)

1. Poor academic/work performance

2. Poor performance caused by others
   ...

3. Tension with family members

4. Late for work, study, or appointments

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