

# Association Between Sleep Reactivity, Pre-Sleep Arousal State, and Neuroendocrine Hormones in Patients with Chronic Insomnia Disorder

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**Purpose:** The purpose of this study was to look into the relationship between pre-sleep arousal state, sleep reactivity, and serum levels of neuroendocrine hormones (cortisol, copeptin, and corticotropin-releasing hormone) in patients with chronic insomnia disorders (CID), and whether the effects of sleep reactivity and pre-sleep arousal on insomnia are related to the levels of these neuroendocrine hormones.

**Patients and Methods:** This study included 61 CID patients and 27 healthy controls (HC) whose base data were matched to those of the CID patients. The Pittsburgh Sleep Quality Index (PSQI), Pre-Sleep Arousal Scale (PSAS), and the Ford Insomnia Response to Stress Test (FIRST) were used to evaluate the participants' sleep, stress, and neuropsychological function. We measured the participants' serum concentration levels of cortisol, copeptin, and corticotropin-releasing hormone (CRH), using quantitative sandwich enzyme-linked immunosorbent assays.

**Results:** The CID group had significantly greater serum levels of copeptin, CRH, and cortisol, as well as higher FIRST and PSAS scores than the HC group. The partial correlation analysis revealed a substantial and positive association among cortisol, CRH, copeptin PSQI, PSAS, and FIRST after adjusting for sex, age, depression, and cognition. Principal component analysis showed that PSQI, FIRST, and PSAS, as well as cortisol, CRH, and copeptin, were all loaded on factor 1.

**Conclusion:** Patients with CID showed increased sleep reactivity and pre-sleep arousal, which correlated with serum levels of cortisol, copeptin, and CRH. Changes in neuroendocrine hormone levels may influence how pre-sleep arousal and sleep reactivity affect the development of insomnia.

**Keywords:** chronic insomnia disorder, pre-sleep arousal, sleep reactivity, hormones, neuroendocrine

## Introduction

Insomnia is the most prevalent sleep disorder. It refers to a subjective experience of unsatisfactory sleep duration and sleep quality. Cases that are of clinical significance (insomnia disorder) should meet a certain frequency ( $\geq 3$  times per week) with daytime dysfunction, despite the existence of appropriate sleep opportunities and sleep environments.<sup>1,2</sup> Overall, people with insomnia have objectively or/and subjectively reduced sleep duration, disrupted sleep continuity, and reduced sleep quality.<sup>3</sup> As a result, it is critical to investigate the mechanisms of insomnia to find appropriate treatment options and ultimately improve the life quality of these patients.

Studies on the mechanisms of insomnia have been ongoing, and over the decades, a variety of hypotheses have emerged. These models all emphasize the important role of cognition and hyperarousal in insomnia.<sup>4</sup> For example, Morin's model outlines a cycle of insomnia involving arousal, cognitive dysfunction, maladaptive habits, and consequences.<sup>5</sup> On the other hand, Ong et al's model suggests that metacognition of insomnia leads to cognitive arousal, exacerbating perceptions of insomnia.<sup>6</sup> Hyperarousal, encompassing cortical, physiological, and cognitive

aspects, plays a pivotal role in various insomnia mechanisms. Cognitive arousal, characterized by excessive worrying and rumination, particularly during pre-sleep phases, is a common complaint among insomnia patients. This hyperarousal, influenced by unhealthy sleep-related beliefs, is well-supported by research in insomnia's etiology and pathophysiology.<sup>7–10</sup>

Hyperarousal has been shown to be a form of stress response.<sup>11</sup> The onset of an acutely stressful event is often considered a trigger for insomnia.<sup>12</sup> Individuals often have different stress regulation abilities and behave differently in the face of stressful events.<sup>10</sup> Sleep reactivity is a generally steady feature that describes how much an individual experiences acute sleep problems in reaction to stress.<sup>13</sup> Pre-sleep arousal is defined as the state-dependent level of cognitive and somatic activation that occurs before bedtime, potentially hindering an individual's ability to begin and maintain sleep.<sup>9</sup> Furthermore, research has indicated that pre-sleep arousal appears to act as a mediator in the link between daily perceived stress and subjective sleep quality.<sup>14</sup> Researchers have suggested the existence of a bidirectional correlation between sleep reactivity and cognitive-emotional arousal, whereby after stressful events activate cognitive-emotional activities (eg, bedtime rumination), the hyperreactive sleep system's sensitivity is exploited, leading to nighttime insomnia and poor sleep.<sup>15</sup> When stressful events activate the sleep system, the resulting nocturnal arousal causes the brain to become hyperactive during this time, creating a vicious cycle.

Although the current understanding of the mechanisms of insomnia highlights the role of cognitive-emotional hyperarousal in the onset and development of the disorder, the role of neuroendocrine hormones in the pathophysiology of insomnia is also apparent.<sup>16</sup> To deepen our understanding of the mechanisms underlying insomnia, it is crucial to explore the intricate interplay between cognitive and physiological factors. Considering that common triggers of insomnia are stressful life events,<sup>17</sup> the ensuing physiological stress produces arousal in the individual, which can be both somatic and cognitive. Rumination may enhance acute reactions, postpone recovery, or reactivate responses at a later period in order to prolong physiological activation.<sup>18,19</sup> These effects are all consistent with the perseverative cognition hypothesis. In previous neurobiological studies on hyperarousal, the activity of the hypothalamic-pituitary-adrenal (HPA) axis has been the focus of research, and cortisol, as an end product of the HPA axis, has been verified in a large number of studies to be associated with CID.<sup>20</sup>

A previous study has established the presence of a connection between perseverative cognitive processes (such as worry and rumination) and extended cortisol activation.<sup>21</sup> This could suggest a complex interplay between cognitive factors and physiological responses in the context of CID. Cognitive processes may have potential impacts on the duration and intensity of physiological arousal. Reports indicate that when exogenous CRH is injected into patients with CID, the activity response of the HPA axis is significantly reduced and cortisol secretion is lower than normal.<sup>22</sup> Moreover, it has been suggested that cortisol has no strong pathophysiologic effects of its own and that its ability to affect the body is mainly through the regulation of other systems.<sup>23</sup> CRH, the releasing hormone that precedes cortisol in the axis, better reflects changes in HPA axis activity during stressful conditions.<sup>24</sup>

In addition to CRH, arginine vasopressin (AVP) is secreted by neurons in the paraventricular nucleus of the hypothalamus in response to events that cause an acute stress response.<sup>25</sup> Moreover, sympathetic hyperactivity is a component of hyperarousal, and neurons responsible for the synthesis and release of AVP are the effectors of this sympathetic activation.<sup>26</sup> Currently, AVP levels cannot be measured directly; however, as the C-terminal part of the vasopressin precursor, copeptin is highly stable and typically secreted in equal amounts with AVP.<sup>27</sup> Therefore, copeptin can be measured as a substitute to assess AVP secretion. Studies have shown that in healthy individuals, copeptin levels are closely related to the roles of cortisol and CRH in the HPA axis during periods of psychological stress or stress responses.<sup>24</sup> Therefore, copeptin can be used as an effective tool for assessing physiological changes associated with psychological stress.<sup>28</sup>

The relationship between neuroendocrine hormones and sleep reactivity, pre-sleep arousal state, and chronic insomnia (CID) needs further clarification. Previous research indicates that chronic insomnia can induce a state of chronic stress, leading to changes in neuroendocrine hormones that promote pre-sleep arousal. This arousal may perpetuate CID by maintaining stress and altering hormone levels. While it is suggested that pre-sleep arousal could be a factor in the chronicity of insomnia, the precise mechanisms are not yet understood.

To address this gap, we will measure serum cortisol, copeptin, and CRH levels to explore the interplay between cognitive processes, physiological responses, and CID. This multidimensional approach aims to enhance our understanding of the underlying mechanisms of CID and potentially uncover new therapeutic targets.

In this study, we hypothesize that: (1) there is a correlation between pre-sleep arousal, sleep reactivity, and physiological arousal in patients with CID; and (2) neuroendocrine hormone levels may mediate the impact of pre-sleep arousal and sleep reactivity on chronic insomnia. To our knowledge, this study is the first to investigate the interrelationships between the pre-sleep arousal state, sleep reactivity, and neuroendocrine hormones in patients with CID. Our goal is to provide new insights into the internal factors that may be linked to the mechanisms of chronic insomnia, contributing to a more comprehensive understanding of this complex condition.

## Materials and Methods

### Participants

In this study, 88 participants, including 61 patients with CID and 27 HCs, were included. The study data were gathered from the Sleep Disorders Clinic and Health Screening Centre, the Affiliated Chaohu Hospital of Anhui Medical University, located in Chaohu City, Hefei, Anhui Province, China. Our patients with CID were specifically recruited from among those who sought medical care at our clinic. A total of 61 patients (42 females and 19 males) met the criteria listed in the third edition of the International Classification of Sleep Disorders,<sup>29</sup> ie, (1) a report of difficulties initiating or maintaining sleep for a minimum of 3 months; (2) sufficient opportunity and conditions for sleeping; (3) functional implications of daytime; and (4) not attributable to other sleep or mental disorders. A senior sleep clinician and a sleep medicine specialist diagnosed the patients. Participants must also fulfill these conditions: (1) possess the capability to undertake assessment scales and memory tests unimpeded by hearing or visual impairments; (2) be free from any physical illnesses—including but not limited to immune system disorders, endocrine issues, cardiovascular diseases, neurological conditions, hepatic or renal impairments, and organic brain disorders; (3) have not ingested sedatives, antidepressants, antipsychotics, or any similar medications within the last four weeks; (4) be neither pregnant nor nursing.

The participants in the HC group were matched for age and sex with patients in the CID group, and had reported regular sleep patterns for at least 6 months prior to the study. Our professional physicians conducted a basic inquiry into their sleep patterns over the past six months through our self-developed sleep questionnaire, which was designed based on the Pittsburgh Sleep Quality Index (PSQI).<sup>30</sup> The inclusion criteria for the HC group were as follows: (1) no subjective complaints of sleeplessness or depression; and (2) scores below 7 on the PSQI,<sup>31</sup> below 7 on the 17-item Hamilton Depression Rating Scale (HAM-D-17)<sup>32</sup> and the score of greater than or equal to 26 on the Chinese-Beijing Version of Montreal Cognitive Assessment (MoCA-C).<sup>33</sup> The exclusion criteria for the HC group were as follows: (1) Clear neurological diseases (Parkinson's disease, dementia, multiple sclerosis, headache, etc.); (2) a diagnosis of psychiatric disorders, either recent or past, such as depressive disorder, bipolar disorder, or anxiety disorder; (3) patients with long-term medical conditions, such as diabetes, hypertension, and heart disease; (4) impaired vision, hearing, or movement; (5) administration of medications such as antidepressants, antipsychotics, or sedative-hypnotics within 2 weeks before the study; and (6) pregnant or nursing women. Prior to the start of the study, each participant signed a written informed consent form, and the same researcher individually evaluated all of them. The study was authorized by the Ethics Committee of the Affiliated Chaohu Hospital of Anhui Medical University (approval number 201805-KYXM-01).

### General Data Collection

Using a questionnaire created by our group, we collected data such as these participants' gender, age, and medical history. This data collection was conducted offline using paper questionnaires, which were personally distributed to participants and subsequently collected from them.

## Evaluation of Sleep Quality

The PSQI was used to measure subjective sleep quality. It comprises seven components: quality, latency, duration, the use of sleep medications, efficiency, disturbances, and daytime dysfunction. All seven components are scored on a 4-point scale from 0 (none) to 3 ( $\geq 3$  times per week). The total PSQI score ranges from 0 to 21, with higher scores indicating lower-quality sleep.<sup>30</sup> In China, separating people with inadequate sleep from healthy individuals can be done with a high degree of diagnostic sensitivity and specificity when the score is  $\geq 7$ .<sup>31</sup>

Polysomnography (PSG) was employed to assess objective sleep quality, recording sleep patterns and related physiological parameters. In our study, we used the Condi Graef sleep monitoring device, made in Australia, to collect sleep data from 34 patients with CID during their usual bedtime. The monitoring included a range of physiological parameters such as electroencephalogram, electrocardiogram lead II, electrooculogram, electromyogram, respiratory airflow, and chest and abdominal movements. The sleep parameters detected include total sleep time (TST), sleep efficiency (SE), rapid eye movement sleep latency (REMSL), and arousals, sleep onset latency (SOL), percentage of sleep stage 1 (N1%), stage 2 (N2%) and stage 3 (N3%), and percentage in rapid eye movement sleep (REM%). Following the completion of the monitoring, the same experienced sleep technologist analyzed the data the next day and scored adhering to the American Academy of Sleep Medicine (AASM) scoring manual (version 2.4).<sup>34</sup>

## Evaluation of Stress

To quantify sleep reactivity, we used the Ford Insomnia Response to Stress Test (FIRST), a validated measure of trait sleep reactivity, which is defined as the vulnerability to sleep disturbance in response to stressful events.<sup>35</sup> First, using a Likert scale (1 being extremely unlikely, 2 somewhat likely, 3 moderately likely, and 4 very likely), the respondents were asked if they were likely to have trouble falling asleep in nine different scenarios. These scenarios included: before a big meeting the following day; after feeling stressed during the day; after feeling stressed at night; after receiving bad news during the day; after watching a bad movie or TV show; after a bad day; after an argument; before having to speak in public; and before leaving for a vacation the following day. They were asked to assess their probability of having sleep-related difficulties, irrespective of whether they had not experienced them recently. The higher the FIRST score, the higher the sleep reactivity.<sup>35</sup>

## Assessment of Arousal Levels

State-dependent somatic and cognitive arousal that precedes sleep was assessed using the Pre-Sleep Arousal Scale (PSAS).<sup>36</sup> The PSAS consists of eight somatic and eight cognitive arousal symptoms, each rated on a 5-point Likert-like scale. The scale ranges from “1. Completely not felt” (indicating no experience of the symptom) to “5. Extremely strong felt” (indicating an extremely strong experience of the symptom). For each subscale, scores range from 8 to 40, with higher scores indicating greater arousal. Consequently, the total score of the PSAS ranges from 16 to 80 points. Higher PSAS scores suggest higher levels of activation before falling asleep.<sup>37</sup>

## Cognitive Assessment

The MoCA-C is a commonly used subjective cognitive screening instrument with high reliability and validity in Chinese populations. It has eight dimensions, which include visuospatial and executive processes, name, attention, language, abstraction, short-term memory, delayed recall, and orientation. A score of less than 26 indicates normal cognitive functioning; the maximum score is thirty.<sup>33</sup> In our analysis, the MoCA-C score will be used to control for cognitive variations.

## Assessment of Depression

The severity of depression was assessed using the 17-item HAM-D-17 rating scale, which is connected to depressed mood, guilt and suicidal thoughts, sleep, work and activity levels that are negatively affected by depressive symptoms.<sup>32</sup> The inclusion of this assessment in our study serves a dual purpose: firstly, to identify and quantify the presence of depressive symptoms among participants, and secondly, to control for the potential confounding effects of depression on cognitive performance. A healthy state is indicated by a score of less than 7, whereas mild, moderate, and severe depression are indicated by scores of 7–17, 18–24, and  $>24$ , respectively.<sup>32</sup> Our statistical analysis will include depression as a control variable to reduce the potential confounding effects of depression on our study.

## Storage and Measurement of Blood Samples

The participants' venous blood was drawn between 08:00 and 10:00 a.m. on the same day that the scales were administered. A 30-minute break was observed before blood was drawn to alleviate any bias in the data due to nervousness. Following centrifugal separation, serum was extracted and kept at  $-80^{\circ}\text{C}$  until the assay. The levels of cortisol, copeptin, and CRH were measured using quantitative sandwich ELISA according to the manufacturer's instructions (Wuhan USCN Business Co). All tests were performed by the same professional laboratory staff. The collection and experimental procedures of blood specimens strictly adhered to the "Collection and Processing of Blood Specimens for Clinical Chemistry" standard (WS/T 225—2002).<sup>38</sup>

## Statistical Analysis

Statistical analysis was performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). The mean  $\pm$  standard deviation (SD) was used to depict variables that were normally distributed. For non-parametric data, the 25th, 50th, and 75th percentiles [P50 (P25, P75)] were reported. Non-normally distributed variables were examined using the Mann–Whitney *U*-test, whereas normally distributed data were compared between groups using the Student's *t*-test. The participants' sleep quality, neuroendocrine hormone levels, PSAS scores, and FIRST scores were compared using partial correlation analysis. Principal component analysis was employed as a multivariate data reduction approach to examine the correlations between the measurements in patients with CID. A varimax rotation technique was used to rotate the original factor pattern, and factors with eigenvalues greater than one were set aside.

## Results

### Baseline Characteristics

Table 1 displays the background characteristics of participants in the CID and HC groups. There was no significant difference in age ( $t=-0.426$ ,  $P=0.671$ ) or gender ( $\chi^2=0.294$ ,  $P=0.588$ ) between the CID and HC groups. The CID group had significantly higher HAMD-17 ratings compared to the HC group ( $P<0.001$ ), but a lower MoCA-C score ( $P<0.001$ ).

**Table 1** Demographic and Clinical Data of Participants

Items	CID (n=61)	HC (n=27)	Statistics	P-values
Sex (male/female)	19/42	10/17	$\chi^2=0.294$	0.588
Age (year)	46.1 $\pm$ 8.2	46.9 $\pm$ 8.2	$t=-0.426$	0.671
HAMD (score)	9.0(5.0,13.0)	2.0(0,4.0)	$Z=-6.215$	<0.001
MoCA-C (score)	24.0 (23.0,25.0)	28.0(27.0,29.0)	$Z=-6.712$	<0.001
PSQI (score)	15.0(13.0,17.0)	4.0(3.0,5.0)	$Z=-7.470$	<0.001
PSAS (score)	32.0(28.0,39.0)	17.0(17.0,18.0)	$Z=-7.007$	<0.001
FIRST (score)	24.0 (18.5,28.0)	9.0(9.0,11.0)	$Z=-7.079$	<0.001
Cortisol (ng/mL)	434.3 $\pm$ 23.8	371.5 $\pm$ 13.9	$t=15.484$	<0.001
CRH (pg/mL)	338.7(287.0,380.2)	155.4(118.8,177.0)	$Z=-7.162$	<0.001
Copeptin (pg/mL)	390.2 $\pm$ 134.6	249.0 $\pm$ 73.4	$t=6.336$	<0.001
Polysomnogram (N=34)				
TST (min)	353.5(301.3,388.4)			
SOL (min)	19.0(11.0,33.1)			
SE (%)	68.5(62.5,76.6)			

(Continued)

**Table 1** (Continued).

Items	CID (n=61)	HC (n=27)	Statistics	P-values
N1 (%)	26.4±2.4			
N2 (%)	50.4±1.9			
N3 (%)	7.9(2.6,13.8)			
REM (%)	13.7±1.0			
REMSL (min)	177.7±17.0			
Arousals (times)	17.2±1.3			

**Notes:** Parametric tests were used for normally distributed data (mean ± SD), while non-normally distributed variables [P50 (P25, P75)] were evaluated non-parametrically.

**Abbreviations:** HAMD, Hamilton Depression Rating Scale; MoCA-C, Chinese-Beijing Version of Montreal Cognitive Assessment; PSAS, pre-sleep arousal scale; PSQI, Pittsburgh Sleep Quality Index; FIRST, Ford Insomnia Response to Stress Test; CRH, corticotropin-releasing hormone; CID, chronic insomnia disorder; HC, healthy control; SE, sleep efficiency; SOL, sleep onset latency; TST, total sleep time; N1%, percentage of the sleep stage 1; N2%, percentage of the sleep stage 2; N3%, percentage of the sleep stage 3; REM%, percentage of the rapid eye movement sleep time; REMSL, rapid eye movement sleep latency.

## Subjective and Objective Sleep Parameters

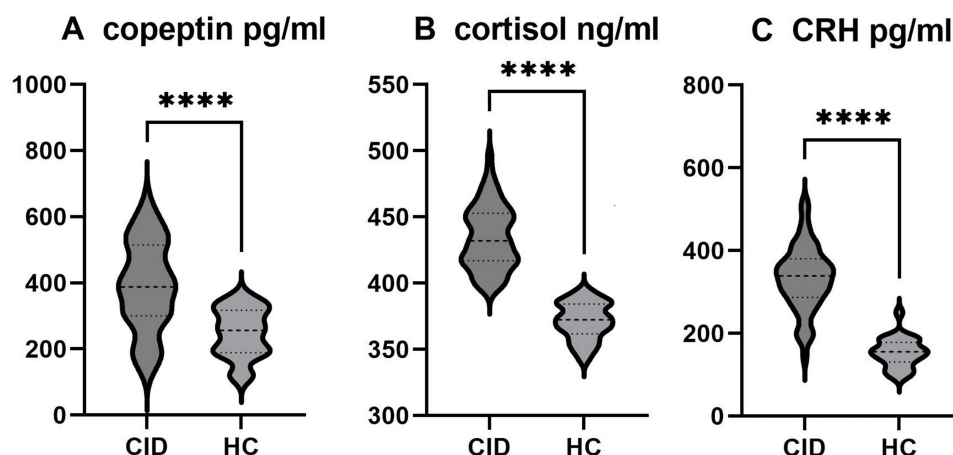
Table 1 shows the detail of subjective and objective sleep parameters. Participants with CID showed markedly higher PSQI scores than the HC group ( $P<0.001$ ), signifying more severe sleep disturbances. Their PSG findings feature reduced TST and SE, extended SOL, higher instances of arousals and N1% and N2%, and reduced N3% and REM%. These align with the criteria for diagnosing objective insomnia,<sup>39</sup> affirming the study's patient selection aligns with the condition's benchmarks.

## Scores of FIRST and PSAS

All scores of FIRST and PSAS in the CID group were higher than those in the HC group (all  $P_s<0.001$ , see Table 1).

## Levels of Neuroendocrine Hormones

Compared to the HC group, the CID group had significantly higher serum levels of cortisol, CRH, and copeptin (all  $P_s<0.001$ , see Table 1 and Figure 1).



**Figure 1** Comparison of serum neuroendocrine hormone levels between CID patients and HCs. (A) Serum copeptin levels in CID and HC groups. (B) Serum cortisol levels in CID and HC groups. (C) Serum CRH levels in CID and HC groups.

**Note:** \*\*\*\*  $P < 0.001$ .

**Abbreviations:** CID, chronic insomnia disorder; HC, healthy control; CRH, corticotropin-releasing hormone.



## Correlations of Serum Levels of Neuroendocrine Hormones, FIRST, PSAS, and Sleep Parameters

The connections between PSQI, PSAS, FIRST, and three biomarkers are shown in Table 2. In the partial correlations analysis (adjusted for sex, age, depression, and cognition), the serum level of cortisol was tightly and positively correlated with FIRST, PSAS, and PSQI scores ( $r=0.566$ ,  $P<0.001$ ;  $r=0.510$ ,  $P<0.001$ ;  $r=0.652$ ,  $P<0.001$ ). The serum level of CRH was also tightly and positively correlated with FIRST, PSAS, and PSQI scores ( $r=0.432$ ,  $P<0.001$ ;  $r=0.491$ ,  $P<0.001$ ;  $r=0.581$ ,  $P<0.001$ ). Furthermore, the serum level of copeptin was tightly and positively correlated with FIRST, PSAS, and PSQI scores ( $r=0.379$ ,  $P<0.001$ ;  $r=0.282$ ,  $P<0.001$ ;  $r=0.307$ ,  $P<0.001$ ). However, objective sleep parameters showed no significant correlation with FIRST, PSAS scores and serum levels of three biomarkers, as seen in Table 3.

## Principal Component Analysis

The dataset passed Bartlett's test of sphericity ( $P<0.001$ ) and the Kaiser-Meyer-Olkin ( $KMO=0.839$ ) criterion, indicating that principal component analysis could be performed. The use of principal component analysis to include FIRST, PSAS, and PSQI scores and the serum level of cortisol, copeptin, CRH is justified because there is significant correlation among them in the partial correlation analysis. The component loadings of the variables onto the rotational factors are illustrated

**Table 2** Correlation Coefficients Between the PSQI Score, PSAS Score, FIRST Score, Serum Cortisol, CRH, and Copeptin Levels in the Patients with CID

Items	PSQI	PSAS	FIRST	Cortisol	CRH	Copeptin
PSQI	I	0.539**	0.604**	0.652**	0.581**	0.307**
PSAS		I	0.504**	0.510**	0.491**	0.282**
FIRST			I	0.566**	0.432**	0.379**
Cortisol				I	0.683**	0.366**
CRH					I	0.371**
Copeptin						I

**Notes:** The partial correlation analysis was conducted after controlling for sex, age, depression, and cognition. \*\*  $P<0.001$ .

**Abbreviations:** PSAS, pre-sleep arousal scale; PSQI, Pittsburgh Sleep Quality Index; FIRST, Ford Insomnia Response to Stress Test; CRH, corticotropin-releasing hormone; CID, chronic insomnia disorder.

**Table 3** Correlation Coefficients Between Objective Sleep Parameters, PSAS Score, FIRST Score, Serum Cortisol, CRH, and Copeptin Levels in the Patients with CID

Items	R	N1%	N2%	N3%	SE	SOL	Ar	REMSL	TST
PSAS	0.167	-0.318	0.081	-0.008	0.195	-0.069	0.028	-0.220	-0.049
FIRST	-0.105	-0.215	0.148	0.199	0.172	0.337	-0.035	-0.185	0.174
Cortisol	-0.118	-0.220	0.255	-0.014	0.181	0.054	0.027	-0.008	0.068
CRH	-0.141	0.019	-0.045	-0.004	0.235	-0.063	0.023	0.343	0.303
Copeptin	0.007	0.278	-0.332	-0.150	0.091	-0.110	0.048	0.117	0.112

**Notes:** The partial correlation analysis was conducted after controlling for sex, age, depression, and cognition.

**Abbreviations:** PSAS, pre-sleep arousal scale; PSQI, Pittsburgh Sleep Quality Index; FIRST, Ford Insomnia Response to Stress Test; CRH, corticotropin-releasing hormone; CID, chronic insomnia disorder; SE, sleep efficiency; SOL, sleep onset latency; TST, total sleep time; N1%, percentage of the sleep stage 1; N2%, percentage of the sleep stage 2; N3%, percentage of the sleep stage 3; REM%, percentage of the rapid eye movement sleep time; REMSL, rapid eye movement sleep latency.

**Table 4** Principal Component Analysis for CID

Variables	Measures	Factor I
Subjective sleep quality	PSQI (score)	0.764
Pre-sleep arousal	PSAS (score)	0.781
Sleep reactivity	FIRST (score)	0.784
Neuroendocrine hormones	Cortisol (ng/mL)	0.720
	CRH (pg/mL)	0.727
	Copeptin (pg/mL)	0.631
Eigenvalues		3.252
Cumulative variance (%)		54.207

**Abbreviations:** PSAS, pre-sleep arousal scale; PSQI, Pittsburgh Sleep Quality Index; FIRST, Ford Insomnia Response to Stress Test; CRH, corticotropin-releasing hormone.

in Table 4; one significant factor was successfully generated. There was a strong loading of PSQI, FIRST, PSAS, cortisol, CRH, and copeptin on factor 1.

## Discussion

This study investigated the link between sleep reactivity, pre-sleep arousal, and neuroendocrine hormone levels in patients with CID, and explored how these factors contribute to insomnia. Our findings confirmed that patients with chronic insomnia had higher levels of neuroendocrine hormones (including cortisol, CRH, and copeptin), higher sleep reactivity, and a pre-sleep arousal state. Sleep reactivity and pre-sleep arousal had significantly positive correlations with CRH, cortisol, and copeptin. Principal component analysis also revealed that PSQI, FIRST, PSAS, cortisol, CRH, and copeptin were loaded highly on factor 1. These suggest that high sleep reactivity and pre-sleep arousal in patients with chronic insomnia are related to a high physical arousal level in patients with CID.

## Higher Serum Levels of Neuroendocrine Hormones in Patients with CID

Hyperarousal is one of the important components of the etiology of insomnia, which includes overactive neurobiological and psychological systems.<sup>16</sup> Previous neurobiological studies on hyperarousal focused on the activity of the HPA axis, and cortisol, as an end product of the HPA axis, has been confirmed by numerous studies to be associated with chronic insomnia. However, different studies have reported some inconsistent results.<sup>40,41</sup> In our sample of patients with chronic insomnia, serum levels of cortisol were found to be significantly higher than in healthy controls.

Cortisol does not fully reflect the stress response of the HPA axis; therefore, additional measurements of CRH levels were performed. Direct measurements of circulating CRH are relatively rare and can only be validly measured in blood.<sup>42</sup> Our results demonstrated that participants who suffered insomnia had significantly higher serum levels of CRH compared with the levels in healthy controls, which should be related to the role of CRH in promoting brain arousal and suppressing slow-wave sleep.<sup>43</sup>

Copeptin, as a glycopeptide comprising the C-terminal part of the AVP prohormone,<sup>27</sup> is a serum marker that consistently responds to sympathetic changes; moreover, our group previously verified that the serum levels of copeptin were associated with chronic insomnia.<sup>44</sup> This could be attributed to the inherent variability in the secretion patterns of these hormones. The results of our study reaffirmed this observation by showing that the serologic levels of copeptin were significantly elevated in the patients with chronic insomnia, which further validated the theory that implicates increased sympathetic excitability in the neurobiological mechanism of chronic insomnia. There was another study by our group that found both the CID patients and the HC group exhibited comparable levels of CRH, cortisol, and copeptin at the pre-sleep and morning waking-up time points.<sup>45</sup>



Moving forward, it is imperative to broaden our sample size and incorporate a more comprehensive set of temporal measurements to thoroughly investigate these hormonal variations.

## High Sleep Reactivity is Associated with Increased Serum Levels of CRH, Cortisol, and Copeptin in Patients with CID

The findings of previous studies suggest that sleep reactivity is a predisposing factor for insomnia disorder.<sup>13,46,47</sup> Most patients with chronic insomnia have high sleep reactivity, and even individuals with low sleep reactivity before having insomnia were found to have elevated sleep reactivity postdiagnosis.<sup>35</sup> Our findings also revealed that patients with chronic insomnia had significantly higher sleep reactivity than did healthy controls. The neurobiological causes of high sleep reactivity are poorly understood; however, disorders of the HPA axis, autonomic nervous system imbalance, and disruption of the cortical network may be potential causes.<sup>48,49</sup>

Cortisol, a neuroendocrine hormone produced by the HPA axis, is one of the most extensively studied. Studies have shown that there is a correlation between sleep reactivity and serum cortisol levels in young insomniac individuals and in adults without a history of insomnia.<sup>40,41</sup> In a previous study by our group, we found no significant difference in baseline cortisol levels between participants with high or low sleep reactivity prior to stress exposure. Additionally, under the same degree of stress, we observed that healthy young adults with high sleep reactivity developed a stronger salivary cortisol response compared to those with low sleep reactivity.<sup>50</sup> However, these findings are not consistent with the objectives of our study as patients with CID were not included in these. We can only speculate based on the results of studies on cortisol responses in patients with CID, those with post-stress acute insomnia, and people at high risk of insomnia. The effect of cortisol on insomnia is inconsistent and may be dose-dependent.<sup>51</sup> Therefore, in addition to studying serum levels of cortisol, we also included CRH, which has been shown to play a more pathophysiological role when individuals are exposed to stress; moreover, an increase in CRH levels immediately affects sleep.<sup>43</sup>

In addition to strengthening the HPA axis, insomnia also overactivates the sympathetic nervous system. Numerous studies have shown that patients with chronic insomnia have increased heart rates, increased metabolic rates, elevated cortisol and norepinephrine concentrations, and elevated body temperature.<sup>52</sup> Reffi et al explored the correlations between sleep reactivity and physiological arousal indices associated with the sympathetic nervous system, such as blood pressure, heart rate, and heart rate variability; however, there were no significant group differences.<sup>41</sup> The validity and consistency of the results of heart rate variability as a noninvasive tool for assessing sympathetic excitability have been questioned.<sup>53,54</sup> Previously, our research group used copeptin as a stable biomarker to evaluate sympathetic nerve activity, and blood copeptin levels in patients with CID were significantly greater than those in the HC group,<sup>44</sup> which is consistent with the findings of our study.

In the current study, partial correlation analysis demonstrated that the levels of serum cortisol ( $r=0.566$ ,  $P<0.001$ ), copeptin ( $r=0.379$ ,  $P<0.001$ ), and CRH ( $r=0.432$ ,  $P<0.001$ ) in the CID group were significantly positively linked with sleep reactivity. The results showed that physiological arousal is related to high sleep reactivity in patients with CID. Our results also suggest that enhanced hypothalamic activation and sympathetic nerve excitation may moderately predict high sleep reactivity in patients with CID.

## High Pre-Sleep Arousal is Associated with Increased Serum Levels of CRH, Cortisol, and Copeptin in Patients with CID

The mechanism of hyperarousal in insomnia can be roughly divided into cortical, cognitive, and physiological arousals, which do not function independently.<sup>11,16</sup> The correlation between cortical and physiological arousals has been demonstrated by several studies. Previous research has revealed that an increase in cortisol levels near bedtime was positively connected with increased EEG power spectral density across all frequency ranges in frontal areas, with the highest correlation identified in the beta1 frequency band in healthy adolescents.<sup>55</sup> Furthermore, some research suggests that the 24-hour urine levels of catecholamine metabolites and norepinephrine seem to correspond with the percentage of stage 1 sleep and wake time following sleep initiation, as well as the percentage of slow-wave sleep.<sup>56</sup> A recent study also

investigated the association between pre-sleep arousal and nocturnal cortical arousal, revealing that the PSAS score was substantially connected with changes in the gamma band during NREM and REM sleep in patients with insomnia.<sup>16</sup>

However, there are no clinical studies on the interaction between cognitive and physiological arousals in patients with CID. Our study focused on the pre-sleep arousal state of patients with chronic insomnia, which includes pre-sleep somatic arousal and pre-sleep cognitive arousal.<sup>57</sup> In our study, patients with chronic insomnia had significantly higher scores than healthy controls on the pre-sleep arousal scale. We hypothesized that pre-sleep cognitive arousal can promote the activation of physiological arousal networks, which may lead to changes in neuroendocrine hormone levels in patients with CID. It is worth noting that rumination, the main form of pre-sleep cognitive arousal, is thought to affect cortisol levels in healthy people after a stressful event.<sup>58,59</sup> According to our research, the serum levels of cortisol ( $r=0.510$ ,  $P<0.001$ ), CRH ( $r=0.491$ ,  $P<0.001$ ), and copeptin ( $r=0.282$ ,  $P<0.001$ ) in the CID group were significantly positively correlated with pre-sleep arousal in the partial correlation analysis. In other words, there is a correlation between physiological arousal characterized by elevated levels of neuroendocrine hormones and pre-sleep arousal in patients with CID. This may be due to pre-sleep arousal excessively focusing attention, thereby sustaining physiological arousal throughout sleep,<sup>60</sup> which may continue to prolong the duration of insomnia.

## The Effects of High Sleep Reactivity and High Pre-Sleep Arousal on Chronic Insomnia Disorder May Be Related to Neuroendocrine Hormones

The results described above may also help us better understand the relationship between sleep reactivity, pre-sleep arousal, and sleep quality in patients with CID. Regarding whether alterations in neuroendocrine hormones were one of the mediating pathways, our principal component analysis showed that PSQI, FIRST, PSAS, cortisol, CRH, and copeptin all loaded highly on one factor. These components were significantly positively correlated with each other, and they collectively explained approximately 54.207% of the variance in the dataset. These results suggest that poor sleep quality in patients with CID is closely associated with high sleep reactivity, high pre-sleep arousal, and increased serum levels of neuroendocrine hormones, including cortisol, CRH, and copeptin. The notable correlations among these factors underscore their collective role in the etiology of chronic insomnia, which can be used to simplify the diagnosis and treatment process of chronic insomnia without compromising diagnostic accuracy, especially in outpatient settings. However, the causal relationship between these indicators and the extent to which they contribute to CID cannot be determined. Moreover, when exploring the correlations between objective sleep data and other variables, we did not find the expected significant associations. We believe this is mainly because the sample size limited our ability to detect weaker relationships. Therefore, future studies should consider using larger and more diverse samples, as well as more refined measurement tools, to enhance the statistical power and external validity of the findings. Despite this limitation, our study still reveals the correlation between sleep reactivity and pre-sleep arousal with neuroendocrine hormones in patients with chronic insomnia, which is significant for understanding the influencing factors of chronic insomnia. These findings provide valuable insights for further exploring the potential mechanisms of chronic insomnia and offer new directions for future research.

## Limitation

This study has some limitations. First, a larger sample size is required to validate our results, as the current sample size is insufficient to adequately examine the distinct contributions of each biomarker to the connection. Furthermore, our samples were obtained from outpatient clinics and physical examination centers; therefore, the collection of serum specimens was limited in time, and specimens with obvious secretion rhythms could not be collected at multiple time points. This should be considered for further improvement in future studies. Although we attempted to identify some directionality in these indicators through mediating analysis or moderating analysis, the model we established did not pass the fitting test. This outcome is attributed to the inherent limitations of our cross-sectional study design, which does not allow for the establishment of causality or the direction of effects among variables. Cross-sectional data, by its very nature, provides a static view of the research subjects at a single point in time, thereby precluding the ability to infer temporal precedence—a critical component in mediation analysis. In light of these limitations, we have chosen not to

overinterpret the cross-sectional associations and have opted for a more descriptive approach to analyze the relationships among our variables. Given the limitations of our current cross-sectional study, which does not allow us to determine the causal relationships or the directionality of effects among variables, we plan to design a longitudinal study in the future. This future study will build upon the results presented in this paper and delve deeper into the directional issues of sleep reactivity, pre-sleep arousal, and neuroendocrine hormone levels in chronic insomnia patients as their insomnia progresses.

## Conclusions

Pre-sleep arousal and sleep reactivity are associated with elevated serum cortisol, CRH, and copeptin levels in patients with CID. Our results suggest the need to explore the role that changes in the levels of neuroendocrine hormones play in the effects of pre-sleep arousal and sleep reactivity on sleep quality.

## Ethics Approval and Consent to Participate

The procedures adhered to the principles outlined in the Declaration of Helsinki and applicable regulations. Approval for this study was obtained from the Ethics Committee of the Affiliated Chaohu Hospital of Anhui Medical University under the reference number 201805kyxm-01. Prior to participating in the survey, patients gave their informed consent in writing.

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## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

The authors declare that they have no competing interests.

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