Physiologic Indexes in Chronic Insomnia During a Constant Routine: Evidence for General Hyperarousal?

Michael Varkevisser, PhD1; Hans P.A. Van Dongen, PhD2; Gerard A. Kerkhof, PhD3,4

1Department of Psychology, University of Amsterdam, Amsterdam, The Netherlands; 2Cognitive Psychology Unit, Department of Psychology, Leiden University, Leiden, The Netherlands; 3Unit for Experimental Psychiatry, Division of Sleep and Chronobiology, Department of Psychiatry, and Center for Sleep and Respiratory Neurobiology, University of Pennsylvania School of Medicine, Philadelphia, PA; 4Centre for Sleep and Wake Disorders, MCH Westeinde, The Hague, The Netherlands

Study Objectives: It has been hypothesized that general hyperarousal, present during both sleep and wakefulness, may underlie chronic insomnia. The present study explored, under strictly controlled conditions, whether chronic insomnia is associated with altered physiologic markers of arousal, both in absolute levels and in terms of circadian rhythmicity, relative to controls.

Design: A 24-hour constant-routine protocol was implemented to assess physiologic measures.

Setting: The study was conducted in an isolated, temperature- and light-controlled, sound-attenuated sleep laboratory.

Participants: Eleven subjects with clinically diagnosed chronic insomnia were compared with 13 healthy matched controls.

Interventions: The subjects underwent physiologic parameter recordings and cognitive performance testing during 24 hours of total sleep deprivation under strictly controlled circumstances.

Measurements and Results: Cardiovascular parameters, free cortisol, and body temperature were subjected to mixed-model analysis of variance and mixed-model harmonic regression. Overall, no differences were found in either the absolute level or the circadian parameters (amplitude, phase) of these variables between the insomniacs and the control subjects.

Conclusions: Although physiologic indexes of arousal were slightly elevated in the insomnia group relative to the controls, the differences between the groups were not statistically significant. This could have been due to a lack of statistical power or could reflect the actual absence of arousal in our sample of chronic insomniacs. Systematic interindividual level differences overwhelmed any differences between the 2 groups, making it unlikely that general hyperarousal was a critical underlying factor in our sample. Earlier findings of hyperarousal in insomnia during studies that allowed sleep may have been specifically related to the sleep state.

Keywords: Chronic insomnia, general hyperarousal, constant routine, sleep deprivation, sympathovagal activity, free cortisol, core body temperature, interindividual differences

Disclosure Statement
This was not an industry supported study. Dr. Van Dongen has received research support from Cephalon, Inc. Drs. Varkevisser and Kerkhof have indicated no financial conflicts of interest.

Submitted for publication August 2004
Accepted for publication August 2005
Address correspondence to: Michael Varkevisser, Department of Psychology, University of Amsterdam, Roetersstraat 15, 1018 WB Amsterdam, The Netherlands; Telephone: 31 15 2784640; Fax: 31 15 2787316; E-mail: M.Varkevisser@uva.nl

SLEEP, Vol. 28, No. 12, 2005

INTRODUCTION

SEVERAL STUDIES HAVE SUGGESTED A CHRONIC STATE OF HYPERAROUSAL AS THE UNDERLYING MECHANISM OF CHRONIC INSOMNIA.1-3 IN A SIMILAR VEIN, insomniacs’ complaints of elevated daytime levels of anxiety and fatigue have been hypothesized to be attributable to chronic hyperarousal, rather than being a consequence of the previous night’s poor sleep.4,5 Such hyperarousal could result from a combination of internalization of significant stressful events (e.g., divorce, poor job satisfaction) and certain predisposing factors.5,6 In the context of insomnia, hyperarousal is often conceptually differentiated in cognitive, emotional, and physiologic hyperarousal. In experimental studies of insomnia, it has been difficult to discriminate these concepts,4 suggesting that they may have the same etiology.

Bonnet and Arand6 assessed cardiac autonomic activity in insomniacs and good sleepers as a marker of physiologic arousal. They reported that insomniacs showed increased sympathetic and decreased parasympathetic nervous system activity during sleep, which the authors attributed to the influence of hyperarousal. Domitrovich et al7 presented preliminary data of decreased parasympathetic activity specifically in non-rapid eye movement (NREM) sleep in a group of insomniacs. The increase in physiologic arousal that would accompany this change in sympathovagal influence might be a reason why insomniacs do not sleep well.8

Other indexes have been employed to assess the presence of physiologic arousal as well. For instance, in a study by Vgontzas et al,9 an increase in the insomniacs’ cortisol level during the nocturnal period was found, particularly around the sleep-onset period. The authors related the increase in nighttime cortisol secretion to hyperarousal rather than to the previous night’s poor sleep, based on the finding that sleep loss does not disrupt the usual pattern of cortisol levels in healthy young adults.10 Similar findings were shown in other studies measuring hypothalamic-pituitary-adrenal axis activity.11,12 Further studies have shown an increase in alpha and beta frequencies in the electroencephalograms of insomniacs during sleep,13,14 an increase in 24-hour whole-body metabolism15 and brain metabolism,16 an elevation in body temperature.17,18,19 These effects have been interpreted as converging evidence of a relationship between insomnia and hyperarousal.

Despite these consistent findings, there is still some doubt
whether physiologic hyperarousal is a primary cause of chronic insomnia. A study by Riemann and colleagues measuring nocturnal plasma cortisol levels did not replicate the findings of Vgontzas et al. Furthermore, in a study of Stepanski et al., no evidence of hyperarousal was found when insomniacs were sleep deprived. In the latter study, the influence of hyperarousal was expected to moderate sleepiness as induced by sleep loss. However, the level of physiologic sleepiness after a night of sleep deprivation was comparable in the insomnia group and in a healthy control group. Finally, in a laboratory study by Lichstein et al., daytime pupillometry was applied as an index for arousal in the sympathetic nervous system. The results showed no differences in pupil diameter between insomniacs and healthy controls, indicating no daytime differences in sympathetic nervous system activity. While the majority of published studies offer support for some type of hyperarousal, these contradictory findings suggest that the role of arousal as a major contributor to poor sleep and daytime symptoms (i.e., sleepiness) in insomnia should be considered with some caution.

Most studies to date have not controlled for exogenous or physiologic factors that could influence the level of arousal, such as posture, light exposure, etc. It is noteworthy that laboratory studies in which these so-called masking factors were not controlled well failed to find any distinct differences in cognitive performance between insomniacs and controls. Under the strictly controlled conditions of a constant-routine study, however, we have found that cognitive performance is consistently impaired in chronic insomniacs compared with healthy controls. Precise experimental control over masking factors may be equally crucial for measuring hyperarousal in insomnia.

The aim of the present study was to assess, in a constant-routine protocol employed to neutralize masking factors, whether chronic insomnia is associated with changes in sympathovagal activity and changes in cortisol and temperature levels across the 24-hour day. Our research hypothesis was that, throughout the 24 hours, chronic insomniacs would display an increased level of arousal as measured by these physiologic indexes, relative to age- and sex-matched healthy controls. We expected to observe 1 of 3 possible outcomes for the insomniacs: (a) a systematically elevated level of arousal (i.e., general hyperarousal across the 24-hour day), (b) a specifically nocturnal increase in arousal (around the normal time for sleep), or (c) no differences in the level of arousal relative to healthy controls.

METHODS

Subjects

The patient group of this study consisted of 13 individuals diagnosed with chronic psychophysio逻辑 insomnia (7 men, 6 women; mean age ± SD: 43.8 ± 8.9 years; range 31-54). Patients had been referred to the Centre for Sleep and Wake Disorders at the Westeinde Hospital (The Hague) by their physician. All patients underwent 48-hour ambulatory polysomnography according to the standard protocol of the Centre for Sleep and Wake Disorders. Inclusion criteria were taken from the International Classification of Sleep Disorders, according to which the insomnia complaint should (a) have a duration of more than 6 months, (b) occur 3 nights or more per week, and (c) be confirmed by polysomnography (sleep efficiency less than 85%; sleep latency more than 30 minutes and/or wake time during sleep more than 45 minutes). Since circadian rhythm problems are not part of the psychophysio逻辑 insomnia disorder, shift workers were not included in the study.

None of the insomniacs had evidence of psychiatric disease, as indicated by the Symptom Checklist-90. To further ensure that the participants were properly classified as having psychophysio逻辑 insomnia, 2 subjects (1 man, one woman) of the original 13 subjects were excluded from data analysis due to daily intake of an antidepressant medication. Three subjects of the remaining 11 patients occasionally took central nervous system-active medication, as prescribed by their physician: a benzodiazepine (1 subject, on average once a week) and an antidepressant (2 subjects, on average 3 times a week). Medication was taken primarily to improve sleep. During the laboratory component of the study, medication intake was not allowed.

The control group of this study consisted of 13 healthy volunteers (7 men, 6 women; mean age ± SD: 44.9 ± 7.7; range 33-53 years) who were recruited through advertisements. History of medical or psychiatric illness and sleep times were obtained in a standardized telephone interview and questionnaires. No psychiatric or medical illnesses in recent years were reported. All control subjects were free from medication and were excluded if they reported excessive consumption of alcohol or other drugs. The self-reported sleep times of the control subjects were between 10:00 PM and 1:00 AM (in bed) and 6:00 AM and 9:00 AM (out of bed), indicating a normal sleep range. The control subjects were individually age- and sex-matched to the insomniacs.

After thorough explanation of the experiment, subjects gave consent to participate. This work was approved by the Medical Ethics Committee of the MCH Westeinde hospital in The Hague, The Netherlands.

Experimental Procedures

A constant-routine study was conducted in the Sleep Research Laboratory of the University of Leiden to record 24-hour physiology and performance, controlling for the masking effects of sleep, changes in light exposure, postural changes, and physical activity. Subjects remained awake in a semirecumbent position for a period of 26 hours under constant supervision (the first 2 hours served to acclimatize). Lighting conditions (30-50 lux, as measured in the horizontal angle of gaze) and ambient temperature (18°C) were kept constant throughout the laboratory experiment. Food intake was standardized by means of isocaloric snacks (100-120 kcal) provided every hour. Before each snack, subjective sleepiness and subjective fatigue were assessed.

The experiment started at 9:00 AM; recordings started at 11:00 AM after the 2-hour acclimatization period. Throughout the study, subjects were engaged in nonstrenuous activities in bed. Cognitive performance tests were administered every hour; details of the test battery are presented elsewhere. Between test bouts, participants were allowed to read, play board games, study, etc., but no vigorous activities were allowed.

Subjects were instructed to wear a wrist actigraph and keep a sleep diary during the 3 days prior to the experimental period. In this preexperimental period, subjects slept at home. A brief overview of the entire study protocol is provided in Table 1.

Measurements

Core body temperature was used as a biologic marker of...
Physiological Indexes in Chronic Insomnia—Varkevisser et al

Table 1—Overview of the Protocol

<table>
<thead>
<tr>
<th>Stage</th>
<th>Event</th>
<th>Time of Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening</td>
<td>Ambulatory polysomnography</td>
<td>2 x 24 h</td>
</tr>
<tr>
<td></td>
<td>(insomnia patients only)</td>
<td></td>
</tr>
<tr>
<td>Preexperimental (3 days)</td>
<td>Baseline questionnaires</td>
<td>Sometime during the 3 days</td>
</tr>
<tr>
<td></td>
<td>Sleep diary</td>
<td>Continuous</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>9:00 AM (2 h)</td>
</tr>
<tr>
<td></td>
<td>Cardiac activity</td>
<td>Continuous</td>
</tr>
<tr>
<td></td>
<td>Cortisol (saliva)</td>
<td>Every 3 h starting at 11:00 AM</td>
</tr>
<tr>
<td></td>
<td>Cognitive testing (not reported here)</td>
<td>11:00 AM</td>
</tr>
<tr>
<td></td>
<td>Questionnaires (not reported here)</td>
<td>11:00 AM</td>
</tr>
</tbody>
</table>

Cardiac activity was continuously recorded with the VU-AMS v4.6 ambulatory system (Free University, Amsterdam, The Netherlands), and preprocessed with dedicated software. The VU-AMS device used six Ag/AgCl electrodes to record both electrocardiography and impedance cardiography. Details on electrode placement, R-peak detection, and thoracic impedance (dZ) assessed by this device can be found in De Geus et al.1 The sampling rate for the interbeat-interval (IBI) time series was 1000 Hz. At 3-hour intervals, cardiovascular data were extracted from 30-minute periods preceding performance testing. Artifacts in these data were removed by rejecting outlier IBI values (<300 milliseconds or >1800 milliseconds). From the R-peak time series, heart rate average (HRA) was analyzed for each participant in 30-second segments. The root mean of the squared successive differences (rMSSD) of the IBIs was computed as an estimate of the short-term component of heart rate variability, which is an index of vagal tone.32,33 The rMSSD for consecutive beats in each 30-second segment was calculated as: \[ rMSSD = \sqrt{1/n \sum (IBI_i - IBi_{i+1})^2} \] Values exceeding 200 milliseconds were considered outliers and discarded. The preejection period (PEP), an index of sympathetic cardiac regulation,34 was assessed with the following procedure. The maximum velocity of ejection (dZ/dt), obtained from the thoracic impedance data and sampled at 250 Hz around each R-wave, was ensemble-averaged over each 30-second segment. The onset of the dZ/dt upstroke (opening of the aortic valves) was manually determined for each ensemble-averaged complex, and the PEP values were determined by adding a fixed Q-wave-to-R-wave interval of 48 milliseconds to the R-B interval time.31 From each selected 30-minute period, one 5-minute continuous and artifact-free block was extracted. The mean HRA, rMSSD, and PEP were averaged per subject over the 30-second segments in each 5-minute block.

To minimize invasive procedures during the experiment, saliva samples were used to measure free cortisol. Saliva was collected every 3 hours, prior to performance testing, in noncoated, absorbent swabs (Salivettes™; Sarstedt, Nümbrecht, Germany) placed under the tongue for 2 minutes. After centrifugation (4 minutes at 2500 rpm) the saliva was stored at -20°C until analysis. The concentration of free cortisol was determined by enzyme-linked immunosorbent assay (DSL, Etten-Leur, the Netherlands). Sensitivity of the assay was 1 ng/mL.

During the 3 preexperimental days, general motor activity was assessed with a wrist actigraph (Actiwatch; Cambridge Neurotechnology Ltd., UK). The activity data were analyzed with dedicated Actiwatch Sleep Analysis software (version 1.06). Habitual sleep times (total sleep time, time in bed, intermittent wake time), sleep efficiency, and sleep quality were calculated (for details see Mills and Waterhouse 25).

Finally, the Dutch versions of the Symptom Check List36 and Checklist Individual Strength were used in this study.35 The Symptom Checklist-90 was applied to evaluate possible psychiatric comorbidity, while the Checklist Individual Strength questionnaire was used to determine the coping strength of the insomniacs. The subscales of the Checklist Individual Strength questionnaire (fatigue, concentration, motivation, physical activity) are known to be sensitive to the impact of insomnia.36

Analyses

Descriptive statistics were performed for actigraphy measures and questionnaire scores. Group comparisons for these data were done by means of Wilcoxon signed ranks tests.

Primary analyses of the physiologic indexes of arousal were performed using SAS version 8.0 (SAS Institute Inc., Cary, NC). Body temperature data, cardiovascular parameters, and free cortisol measurements were subjected to mixed-model analysis of variance (ANOVA),6 which is a statistically more powerful analysis procedure for time series than conventional repeated-measures ANOVA (because it accounts for systematic interindividual variability). In mixed-model ANOVA, both fixed effects (reflecting changes over time) and random effects (reflecting interindividual variability around the fixed effects) are involved. Mixed-model ANOVA is robust to missing values, so there was no need to replace missing data points (no more than 6% of the data were missing).

For the HRA, PEP, rMSSD, and cortisol variables, which were determined at 3-hour intervals across the 24-hour recording period, the ANOVA model consisted of a fixed Time of Day effect (9 levels), a fixed Group effect (insomniacs vs controls), a fixed interaction term (Time of Day by Group), and an intercept with a random effect. The Time of Day and interaction effects were tested with an F test and the Group term effects were tested with a t test. For body temperature, which was assessed at 1-hour intervals across the 24 hours, the model had 24 levels for Time of Day and 9 levels for the between-subjects

SLEEP, Vol. 28, No. 12, 2005

Physiological Indexes in Chronic Insomnia—Varkevisser et al
standard deviation (i.e., the square root of the between-subjects variance) for the random effect on the intercept.

Contingent of the presence of a Time of Day main effect or interaction, 24-hour sinusoidal curves were fitted to the data by mixed-model harmonic regression to estimate the contribution of circadian rhythmicity in the physiologic indexes. This analysis considered the data from all subjects at once while explicitly considering group differences (fixed effect) and systematic interindividual differences (random effect). Planned contrasts (t tests) were implemented for overall level, circadian amplitude, and circadian phase to test differences between the 2 groups.

Finally, multivariate ANOVA (MANOVA) was carried out in SPSS version 9.0 (SPSS Inc., Chicago, IL) to assess overall differences in cardiac activity between insomniacs and controls during the daytime (11:00 AM-8:00 PM), during the nighttime (11:00 PM-8:00 AM), and during the whole 24-hour period. For this analysis, HRA, rMSSD and PEP were averaged for each time interval, so that 1 value per parameter remained for each subject. Homogeneity was tested by means of Box test of equality. Wilk’s $\lambda$ was used to determine statistical significance of the omnibus test.

### Power Calculations

Based on the results of comparing the insomniacs with the control group in terms of performance and fatigue in this study, as reported earlier, we performed power calculations to assess the probability that any differences in physiologic variables might be overlooked due to a lack of statistical power. Effect sizes were calculated for the overall level results from mixed-model harmonic regression. Effect sizes for performance variables varied from 0.966 (motor control accuracy) to 1.431 (working memory response latency); for subjective fatigue, the effect size was 2.069. Assuming a type I error threshold of 0.05 and using 2-sided t tests to compare groups, statistical power for the present investigation was calculated, using nQuery.

### RESULTS

Table 2 displays the sleep and personality characteristics of the 2 groups. Sleep efficiency showed lower values for the insomnia group, and almost all personality scores differed between groups as well. More details on the characteristics of sleep, subjective well-being, and performance for both groups have been published elsewhere.

Figures 1 to 3 illustrate the 24-hour patterns of body temperature, cardiac activity, and cortisol, respectively. Higher values of rMSSD represent more vagal activity (Figure 2b), and lower values of PEP represent more sympathetic activity (Figure 2c). Table 3 shows the results of the mixed-model ANOVAs, and Table 4 shows the results of the mixed-model harmonic regressions. Visual inspection of the figures suggested that, with the exception of core body temperature, all physiologic parameters in the insomnia group were changed in the direction of elevated arousal, but the differences with the control group were not statistically significant.

### Temperature

Figure 1 shows the temperature data for the insomniacs and the controls. Mixed-model ANOVA revealed a significant Time of Day effect in the temperature data. No significant Group effect or interaction was found (Table 3). There was circadian rhythm-
micity in the temperature data of both groups, as indicated by the significance of the amplitude parameters in the mixed-model harmonic regression (Table 4). The 2 groups were not significantly different in circadian amplitude or phase. Minimum temperature in both groups occurred at around 4:30 AM. There was no significant difference in overall level of the circadian pattern between the 2 groups, which is in agreement with the absence of a Group effect in the mixed-model ANOVA.

Cardiac Activity

Average Heart Rate

Mixed-model ANOVA indicated a Time of Day effect in the HRA data (Figure 2a). No statistical significance was reached in Group or interaction effects (Table 3). Mixed-model harmonic regression revealed that the amplitude parameters within the groups reached significance, indicating circadian rhythmicity (Table 4). The 2 groups did not differ significantly with respect to overall level, amplitude, or phase.

Root Mean of the Squared Successive Differences

Figure 2b indicates peak values during the nocturnal period in the rMSSD patterns of both groups. This is corroborated by the results of mixed-model ANOVA, which revealed a Time of Day effect in the data of the 2 groups. Neither the Group effect nor the Time of Day by Group interaction reached statistical significance (Table 3). A circadian rhythm was found in the HRA data of both groups, as indicated by the significant amplitude parameters observed in the mixed-model harmonic regression (Table 4). No differences were detected in overall level, amplitude, or phase between the groups.

Preejection Period

The results of mixed-model ANOVA showed no significant effects in the PEP data (Figure 2c) for Time of Day, Group, or interaction (Table 3). Because of the absence of a Time of Day

---

**Table 3—Interactions of Time of Day and Group on Temperature, Heart Rate, and Cortisol Levels**

<table>
<thead>
<tr>
<th>Time of Day</th>
<th>Group</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F(df1,df2)</td>
<td>P</td>
</tr>
<tr>
<td>Temperature,°C</td>
<td>19.96(23,429)</td>
<td>.00</td>
</tr>
<tr>
<td>HRA, bpm</td>
<td>7.67(8,162)</td>
<td>.00</td>
</tr>
<tr>
<td>rMSSD, ms</td>
<td>2.76(8,162)</td>
<td>.01</td>
</tr>
<tr>
<td>PEP, ms</td>
<td>0.78(8,162)</td>
<td>.62</td>
</tr>
<tr>
<td>Cortisol ng/mL*</td>
<td>7.27(8,167)</td>
<td>.00</td>
</tr>
</tbody>
</table>

The F columns show the F-values for the effect of Time of Day and for the interaction with Group, respectively, with degrees of freedom (df1, df2). The t column shows the t values for the effect of Group, with degrees of freedom (df). The P columns show the P values for each test. Mixed-model analysis of variance results. HRA refers to heart rate average; rMSSD, root mean of the squared successive differences; PEP, preejection period.

*Degrees of freedom differ from those for cardiac parameters due to a small difference in the number of missing data points (no more than 6%).

---

**Figure 2**—Group mean profiles of the cardiac variables (top panels) and group mean differences (bottom panels). Plotted are (a) heart rate average (HRA); (b) root mean of the squared successive differences (rMSSD); and (c) preejection period (PEP). In the top panels, the solid curves (open symbols) represent the insomniacs; the dotted curves (closed symbols) represent the controls. The curves in each of the bottom panels represent the differences between the 2 curves in the corresponding top panels; the error bars denote standard errors of the differences between the 2 groups as derived from mixed-model analysis of variance.

---

*SLEEP, Vol. 28, No. 12, 2005*
main effect and interaction, no mixed-model harmonic regression was performed.

Overall Cardiac Activity

The results of MANOVA showed no significant differences in overall cardiac activity (composite of HRA, rMSSD, PEP) between the 2 groups for the full 24-hour interval (Wilk’s λ = 0.92; F = 0.60; P = .62), for the daytime (Wilk’s λ = 0.91; F = 0.64; P = .60), and for the nighttime (Wilk’s λ = 0.95; F = 0.37; P = .77). Homogeneity of variance was met in each case (P > .05).

Cortisol

Although visual inspection (Figure 3) suggested an elevation in cortisol values of about 15% to 20% in the insomnia group, mixed-model ANOVA showed no significant differences between the cortisol patterns of the 2 groups (Table 3). A distinct Time of Day effect was found. Mixed-model harmonic regression revealed a significant circadian amplitude in both the insomnia and control groups, with the cortisol maximum in both groups occurring at around 9:00 AM (Table 4). No differences were detected between the circadian amplitudes, phases, or overall levels of the 2 groups.

Statistical Power

Power calculations showed that with the present sample size (11 insomniacs and 13 controls), any effect of insomnia in the physiologic variables with a standardized effect size equal to or greater than what we previously observed for subjective fatigue—i.e., the largest standardized effect size observed in Varkevisser and Kerkhof25—would have been detected with more than 99% power (at the conventional type I error threshold of .05). Even if standardized effect sizes in the present data set would have been equivalent to those previously observed for working memory and vigilance performance,25 the present statistical power would have been.

Table 4—Mixed-Model Harmonic Regression Results for Overall Level, Circadian Amplitude, and Circadian Phase

<table>
<thead>
<tr>
<th>Overall Level</th>
<th>Insomniacs</th>
<th>Controls</th>
<th>Difference</th>
<th>t</th>
<th>P</th>
<th>ES</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature, °C</td>
<td>36.89 ± 0.08</td>
<td>36.94 ± 0.08</td>
<td>0.05 ± 0.12</td>
<td>0.58</td>
<td>.57</td>
<td>0.38</td>
<td>0.80</td>
</tr>
<tr>
<td>HRA, bpm</td>
<td>69.5 ± 2.4</td>
<td>65.4 ± 2.2</td>
<td>4.1 ± 3.3</td>
<td>1.22</td>
<td>.24</td>
<td>1.04</td>
<td>0.82</td>
</tr>
<tr>
<td>rMSSD, ms</td>
<td>33.9 ± 5.3</td>
<td>40.6 ± 4.9</td>
<td>6.7 ± 7.2</td>
<td>0.92</td>
<td>.37</td>
<td>0.62</td>
<td>0.72</td>
</tr>
<tr>
<td>Cortisol, ng/mL</td>
<td>4.78 ± 0.69</td>
<td>3.97 ± 0.64</td>
<td>0.81 ± 0.94</td>
<td>0.86</td>
<td>.40</td>
<td>0.26</td>
<td>0.35</td>
</tr>
<tr>
<td>Amplitude</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>0.20 ± 0.01*</td>
<td>0.17 ± 0.01*</td>
<td>0.03 ± 0.02</td>
<td>1.38</td>
<td>.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HRA, bpm</td>
<td>2.3 ± 0.6*</td>
<td>2.2 ± 0.6*</td>
<td>0.1 ± 0.8</td>
<td>0.06</td>
<td>.95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rMSSD, ms</td>
<td>3.1 ± 1.5*</td>
<td>3.4 ± 1.6*</td>
<td>0.3 ± 2.2</td>
<td>0.11</td>
<td>.91</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol, ng/mL</td>
<td>1.54 ± 0.45*</td>
<td>1.52 ± 0.42*</td>
<td>0.02 ± 0.61</td>
<td>0.03</td>
<td>.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>04:47 ± 15</td>
<td>04:22 ± 17</td>
<td>25 ± 23</td>
<td>1.09</td>
<td>.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HRA, bpm</td>
<td>01:31 ± 59</td>
<td>03:05 ± 55</td>
<td>94 ± 81</td>
<td>1.17</td>
<td>.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rMSSD, ms</td>
<td>23:14 ± 121</td>
<td>04:08 ± 96</td>
<td>293 ± 154</td>
<td>1.91</td>
<td>.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol, ng/mL</td>
<td>08:59 ± 70</td>
<td>08:52 ± 66</td>
<td>7 ± 96</td>
<td>0.07</td>
<td>.94</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mixed-model harmonic regression results (estimate ± standard error) for overall level; circadian amplitude; and circadian phase (hh:mm ± min). The t column shows the t test values for the difference between the 2 groups. The P column shows the corresponding P values. The ES column shows the standardized effect size for the overall level difference between groups. The R² column in the overall level section shows explained variance as an index for goodness of fit. No harmonic regression analyses were performed for the preejection period because of the absence of a significant Time of Day effect in the mixed-model analysis of variance (Table 3). HRA refers to heart rate average; rMSSD, root mean of the squared successive differences.

*Statistically significant circadian amplitude within group (P < .05)
been at least 80%.

To get an indication of statistical power from independent sources, we looked for quantitative data from previous laboratory studies of insomniacs and controls, as reported in the literature. However, most laboratory studies investigating autonomic cardiac control either did not provide enough quantitative detail to calculate effect sizes or reported nonsignificant results. We found only 1 constant-routine study\(^9\) that could be used for an independent assessment of power in the present study. This study by Lushington and colleagues involved 8 elderly insomniacs who were compared with 8 control subjects.

Lushington et al documented a statistically significant difference in the overall level of core body temperature compared with controls (mean ± SEM: 36.84°C ± 0.06°C for the insomniacs; 36.66°C ± 0.06°C for the controls).\(^9\) The difference in the overall temperature level between the 2 groups was 0.18°C, which was modest relative to the reported standard errors (and accordingly, it is somewhat remarkable that the difference was found to be statistically significant). The temperature difference corresponded to an estimated standardized effect size of 1.06. Power calculations for conventional repeated-measures ANOVA revealed that, with the present sample size, we would have had 69% power to detect such temperature effects with statistical significance. However, since we employed the mixed-model ANOVA approach for time-series analysis, which takes into account systematic interindividual differences, our statistical power may have been greater than that.\(^40\)

These power calculations suggest that the consistent absence of statistically significant differences between insomniacs and controls across all measures considered in the present study may not have been due to a mere lack of statistical power.

### Systematic Interindividual Differences

Table 5 shows the magnitude of systematic interindividual differences as compared with the (nonsignificant) Group differences in the mixed-effects ANOVAs for all 5 physiologic measures. It is clear that the interindividual differences were considerably larger than the differences between the 2 groups in all cases.

### DISCUSSION

In the published literature, it has remained unclear whether hyperarousal is a primary contributor to the symptoms experienced by insomniacs, although the balance would appear to be in favor of such an explanation. We studied physiologic indexes of hyperarousal, all of which are known to be sensitive to fluctuations in the level of arousal\(^20,32\) in subjects with clinically defined chronic psychophysiolgic insomnia\(^27\) and matched healthy controls. The subjects were exposed to the rigorous experimental control of the constant-routine protocol for 24 hours, during which time they were not allowed to sleep. Under these unmasking circumstances,\(^23\) we found no statistically significant evidence for overall level differences or circadian rhythm differences between the insomniacs and the normal sleepers.

The physiologic indexes of arousal measured under constant-routine conditions were not significantly different from those observed in the control group. Even so, there were subtle, nonsignificant level differences between the 2 groups for most parameters, which pointed in the direction of relative hyperarousal for the insomniacs. The absence of significant findings could have been due to inadequate power, although power calculations did cast some doubt on this explanation. We used statistically powerful mixed-model analysis techniques, which accounted for systematic interindividual differences.\(^36,40\) We thus observed that any differences between the 2 groups were overwhelmed by the magnitude of systematic interindividual differences within the groups. These interindividual differences would make the physiologic measures of arousal, taken by themselves, highly unreliable as a diagnostic tool for insomnia because of a lack of (sensitivity and) specificity.\(^4\) Because of this limited specificity, it would appear to be unlikely that general hyperarousal was a critical underlying factor in our sample of chronic insomniacs.

Limitations of the present study include the moderate sample size (11 insomniacs and 13 controls), which limited our ability to investigate relationships among the different outcome variables. In addition, prior to enrollment in the study, 3 insomniacs occasionally took central nervous system-active medication (benzodiazepines and antidepressants) to improve their sleep. Hypothalamic-pituitary-adrenal axis activity, sleep quality, and performance can be affected by such medications.\(^29,42\) Although the 3 individuals were within the same physiologic range as the medication-free insomniacs and medication intake was not allowed during the study proper, it cannot be ruled out entirely that these specific insomniacs had some differential effect on the study outcomes.

A few previous studies have measured core body temperature in a group of insomniacs under strictly controlled conditions.\(^1,20,39\) In these studies, the level of body temperature was higher in the insomnia group than in the control group. Also, the circadian phase of body temperature was (somewhat) advanced in the insomnia group. These results were not supported by the present outcomes. A possible explanation for this discrepancy may involve the selection criteria used in the other studies, which recruited their subjects by means of public announcements. This may have resulted in heterogeneous groups of insomniacs ranging from those with mild to severe symptoms and with different etiologies and, possibly, also subjects with sleep-state misperception or circadian rhythm disorders.\(^47\) In the present study, the patient group consisted exclusively of individuals diagnosed with chronic psychophysicolgic insomnia by the criteria defined in the International Classification of Sleep Disorders.\(^27\) With this sample, the earlier evidence for hyperarousal from core body temperature data was not replicated.

To our knowledge, this was the first study to measure cardiac

---

**Table 5**—The Magnitude of Systematic Interindividual Differences as Estimated by Mixed-Model Analysis of Variance.

<table>
<thead>
<tr>
<th>Measure</th>
<th>(\sigma_{bg})</th>
<th>(\Delta_{bg})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature °C</td>
<td>0.28</td>
<td>0.06</td>
</tr>
<tr>
<td>HRA, bpm</td>
<td>8.30</td>
<td>4.06</td>
</tr>
<tr>
<td>rMSSD, ms</td>
<td>17.98</td>
<td>6.80</td>
</tr>
<tr>
<td>PEP, ms</td>
<td>17.55</td>
<td>4.03</td>
</tr>
<tr>
<td>Cortisol, ng/mL</td>
<td>2.16</td>
<td>0.84</td>
</tr>
</tbody>
</table>

\(\sigma_{bg}\) denotes the between-subjects SD for interindividual level differences. For comparison, \(\Delta_{bg}\) shows the corresponding overall level difference between the 2 groups. HRA refers to heart rate average; rMSSD, root mean of the squared successive differences; PEP, pre-ejection period.
autonomic activity in a group of subjects with chronic insomnia under strictly controlled constant-routine conditions with continuous wakefulness. In earlier studies during which sleep was allowed, autonomic cardiac control was shown to differ in insomniacs compared with good sleepers during sleep, possibly due to hyperarousal.\textsuperscript{9,10} We originally hypothesized that, in our study, when both groups were not allowed to sleep, sympathetic activity would still be higher and vagal tone lower in the insomniacs relative to the normal controls. Indeed, vagal tone seemed to show a modest decrease in the insomnia group throughout the experiment. Also, sympathetic control and heart rate appeared to be somewhat elevated. Yet, these differences did not reach statistical significance. Furthermore, the 2 groups responded similarly to the experimental protocol in terms of circadian rhythmcity. There were statistically significant circadian rhythms in HRA and rMSSD, which were comparable in the insomniacs and the controls and resembled those found in other studies.\textsuperscript{43,44} No clear circadian variation was present in either group for sympathetic influence, as measured by the PEP, in accordance with results of other studies measuring healthy subjects in a constant-routine setting.\textsuperscript{45,46}

When the cardiac data were averaged over time and combined in a multivariate analysis, neither overall nor nighttime- and nighttime-specific cardiac output differed significantly between the groups. It should be noted that we used a fixed time interval to define nighttime (11:00 PM to 8:00 AM) in this analysis. This was within the individual ranges of self-reported habitual bedtimes, but it is recognized that interindividual differences in the timing of subjective night may have contributed some error variance in this analysis. Even so, there were no convincing indications of hyperarousal in the cardiac measurements of the insomniacs regardless of how the data were analyzed.

In the insomnia group, there was a minor overall increase of approximately 1 ng/mL for free cortisol measured in saliva. In a study by Vgontzas et al with a similar sample size,\textsuperscript{7} a comparable increase was found in plasma cortisol. Considering the range of circadian variation (circa 6 ng/mL), the elevation of about 1 ng/mL in the insomnia group would not seem to be of physiologic importance. Yet, in the study by Vgontzas et al, the difference with control subjects was statistically significant (i.e., in the evening and in 24-hour pulses). In the present study, there was no statistically significant evidence either for overall 24-hour hypersecretion or specifically for a nocturnal or diurnal elevation of cortisol. The lack of statistically significant differences in our study could be due to the fact that cortisol data are noisier when measured in saliva than when measured in plasma.

On the other hand, the differences between the outcomes of the 2 studies could also be explained by differences in experimental design. In the study by Vgontzas et al,\textsuperscript{7} subjects were allowed to sleep. The hypersecretion of cortisol around the sleep period in the insomnia group studied by Vgontzas et al\textsuperscript{12} could have been due to an anticipatory reaction to sleep.\textsuperscript{48} Indeed, insomniacs are characterized by worrying about sleep, especially during the presleep period.\textsuperscript{49,47} Conditioned arousal to the bedroom and performance anxiety (i.e., trying too hard to sleep) are typical features in insomnia.\textsuperscript{52} Not allowing subjects to sleep, as in the present study, may have prevented the subjects from worrying about sleep, which may explain the absence of cortisol hypersecretion. In addition, it is possible that the insomniacs in the present study were more relaxed, even sleepy, because they were in a standardized, monotonous bedroom, whereas, in the Vgontzas study,\textsuperscript{7} the subjects were ambulatory during the daytime. Hence, the subjects in that study were exposed to changing or stimulating environments, which could have had a marked effect on cortisol secretion patterns.

In this line of reasoning, while it is possible that hyperarousal was not present at all in our sample of clinical insomniacs, it is also possible that the patients normally did experience arousal but solely as a result of presleep worrying. Thus, insomniacs may experience hypersensitivity to specific sleep-related matters.\textsuperscript{11} Insomniacs might also react differently to sleep-related instructions (e.g., “try to fall asleep”) than do control subjects. As such, sleepiness assessment tools like the Multiple Sleep Latency Test\textsuperscript{50,51} and Multiple Relaxation Test\textsuperscript{51} would seem to be of limited value in insomniacs—which could explain the inconsistent and potentially overinterpreted findings of reduced daytime sleepiness by sleep-latency criteria in these patients.\textsuperscript{52}

If the present findings under strictly controlled unmasking conditions can be replicated, they may have important clinical implications. Based on the study outcomes, we would not expect general relaxation therapy to be an effective treatment in insomniacs, as we found no compelling evidence of general hyperarousal. Rather, the focus of treatment should perhaps be on the sleep-onset period (sleep expectations, stimulus control, beliefs, etc.), as has also been suggested by others.\textsuperscript{53} Future research is needed to explore whether the hypothesized relationship between presleep worrying and insomnia is causal. When causality—or the absence thereof—is established, better inferences can be made with respect to treatment alternatives.

\textbf{ACKNOWLEDGMENTS}

The authors are grateful to Dr. Eco de Geus at the Department of Biological Psychology of the Free University in Amsterdam, the Netherlands, for his advice on the processing of the cardiovascular data. The Netherlands Organization for Scientific Research (NWO) is gratefully acknowledged for funding this project. The research was conducted while M. Varkevisser was supported by grant 580-02.401 of the Foundation for Behavioural and Educational Sciences of this organization, awarded to G.A. Kerkhof. H.P.A. Van Dongen was supported by NIH grant HL70154 and by the Institute for Experimental Psychiatry Research Foundation.

\textbf{REFERENCES}


SLEEP, Vol. 28, No. 12, 2005