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REPEATED ASSESSMENT OF THE ENDOGENOUS 24-HOUR PROFILE OF BLOOD PRESSURE UNDER CONSTANT ROUTINE*

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ABSTRACT

The impact of environmental and behavioral factors on the 24-h profile of blood pressure (BP) has been well established. Various attempts have been made to control these exogenous factors, in order to investigate a possible endogenous circadian variation of BP. Recently, we reported the results of the first environmentally and behaviorally controlled laboratory study with 24-h recordings of BP and heart rate (HR) during maintained wakefulness. In this constant-routine study, a pronounced endogenous circadian rhythm of HR was found, but circadian variation of BP was absent. This result suggested that the circadian rhythm of BP observed in earlier controlled studies, with sleep allowed, was evoked by the sleep–wake cycle as opposed to the endogenous circadian pacemaker. In order to verify our previous finding during maintained wakefulness, we repeated the experiment five times with six normotensive, healthy young subjects. Statistical analyses of the hourly measurements of BP and HR confirmed the replicable presence of an endogenous circadian rhythm

*We dedicate this paper to the memory of Dr. Dolf Bobbert.

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of HR, as well as the consistent absence of an endogenous circadian variation of BP. Thus, this study provided additional evidence that the 24-h profile of BP—as observed under normal circumstances—is the sole result of environmental and behavioral factors such as the occurrence of sleep, and has no endogenous circadian component. (*Chronobiology International*, *18*(*1*), *85–98*, *2001*)

Key Words: Blood pressure; Heart rate; Endogenous circadian rhythm; Constant routine; Repeated experiments.

INTRODUCTION

The origin of the circadian variation observed in blood pressure (BP) has been the subject of fierce discussion. In the literature (1–5), a substantial portion of the circadian variation has been attributed to exogenous factors, resulting from environmental and behavioral state. Several attempts have been made to eliminate these "masking" influences on BP. For instance, Mann et al. (6), Van den Meiracker et al. (7), and Van de Borne et al. (8) recorded subjects during 24 h of bed rest, and Athanassiadis et al. (9) studied accident ward patients immobilized by orthopedic plaster casts. These attempts usually led to a reduced circadian variation of BP, as compared with natural ambulatory conditions. However, the existence of an endogenous circadian rhythm of BP, driven by the biological clock, was not ruled out.

When investigating a possible endogenous circadian rhythm of BP, it is important to consider that various studies have revealed a significant effect of the timing and intensity of sleep on BP (see (10) for a review). Thus, merely restricting subjects' mobility is not sufficient to eliminate all exogenous influences. Furthermore, it has been shown that many overt circadian rhythms can be affected (i.e., masked) and even induced by such factors as ambient light intensity and food intake (11). Therefore, a firm conclusion about the endogenous 24-h profile of BP can only be drawn from experiments conducted under the most rigorously controlled conditions, while subjects must maintain wakefulness.

Recently, we reported the first 24-h BP data obtained under the strictly controlled laboratory conditions of the constant routine (12). The constant routine (13,14) differs from other paradigms that have been used to measure the endogenous 24-h profile of BP in three important respects. Firstly, the environmental conditions are kept constant throughout the experiment; secondly, meals are evenly distributed across the 24 h of the day; and thirdly, sleep is not allowed. Under these unmasking conditions, we found *no* circadian variation of BP. However, the presence of a pronounced circadian rhythm of heart rate (HR), measured simultaneously and with the same recording device, confirmed the validity of the constant routine in investigating circadian rhythmicity in the cardiovascular system. Thus, we concluded that there was *no endogenous* circadian variation of BP.

Our finding that the endogenous 24-h profile of BP is essentially flat, refueled a long-standing debate about the circadian regulation of BP. Therefore, we

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refined existing statistical methodology to enhance our ability to detect circadian rhythmicity. Furthermore, we conducted a *within-subject* study consisting of repeated constant-routine experiments, using a subset of the group of subjects participating in our earlier between-subjects study. This enabled us to investigate the replicability of our previous finding, on which we report in the present paper.

METHODS

Subjects and Recordings

Out of 25 subjects who participated in our earlier study (12), six were invited to continue their participation into the present constant-routine study. These normotensive, healthy, nonsmoking, nonmedicated males (age range 17–19 years) reported no sleep complaints, and were neither morning-nor evening-types as verified with a validated questionnaire (15). The subjects were paid for their participation, and gave written informed consent.

In the constant-routine paradigm, subjects were kept awake in a near supine position during 26 h of bed rest, from 11:00 h until 13:00 h the next day, in the isolated environment of a sleep laboratory with constant ambient temperature (21°C) and illumination (less than 100 lx). Subjects were occupied with nonstrenuous activities such as reading, watching video tapes, and casual conversation with the experimenter. No visitors were admitted to the laboratory. Subjects were allowed to leave the bed only once every 3 h to visit the toilet. Their daily nutritional and liquid intake was divided into hourly equicaloric quantities (alcohol and caffeine were prohibited). Every hour before mealtime, a total of 24 times from 13:00 h until 12:00 h the next day, systolic BP (SBP), diastolic BP (DBP), and HR were recorded during restful wakefulness. Recordings were made with SpaceLabs 90207 monitors (Redmond, WA) using the oscillometric method. Subjects kept the same monitor during the whole constant routine.

In March 1996, the subjects participated in the constant-routine experiments for the first time. The data from this set of constant routines were included in the results reported in (12). In June, September, and December 1996 and in March 1997 the constant routines were repeated, so that each subject participated five times. The experiments in March and September were completed before the daylight saving time transitions. The present paper comprises the data of all five sets of constant routines. The experimental protocol was approved by the Medical Ethical Committee of Leiden University Medical Centre, The Netherlands.

Statistical Analyses

As described above, SBP, DBP, and HR were recorded hourly before mealtime, from 13:00 h until 12:00 h the next day. Twenty-three (about 3%) out of a total of 720 hourly recordings (24 h by five constant routines by six subjects) were missing

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(concurrently for BP and HR). Missing values were not replaced, but were properly taken into account during the statistical analyses. A periodogram pseudowindow analysis (16,17) was applied to verify that the temporal distribution of missing values did not by itself generate or conceal circadian variation in the time series of SBP, DBP, and HR. It is important to note that the time series were also adequate in both sampling frequency and duration for the detection of circadian variation. Further increasing the sampling frequency would not have enhanced the expected detection probability [(18); cf. (19)].

Two approaches to statistical analysis of the SBP, DBP, and HR time series were undertaken. The first approach utilized mixed-model analysis of variance (ANOVA). The ANOVA model was estimated separately for SBP, DBP, and HR. Initially the model included fixed effects of time of day (Time; 24 levels), constant routine (Trial; 5 levels), and Time by Trial interaction; random effects of Subject, Subject by Time, and Subject by Trial; and residual error. Parameter estimates were obtained using the restricted maximum-likelihood (REML) method (20), useful for estimating variance components in the ANOVA model. The fixed-effect Time by Trial interaction was removed if not significant (F-test at $\alpha = 0.1$), and the model was re-estimated and examined for a Time main effect (F-test) and a Subject by Time interaction (Wald Z-test). If the Time by Trial interaction was significant, however, then Time effects within each trial (i.e., simple effects) were estimated. The proportions of the total variance attributable to random Subject by Time, Subject by Trial, and Subject effects were computed to assess stability among subjects in the data.

In order to investigate the likelihood that real 24-h variation existed but our study failed to find it as statistically significant (i.e., the study resulted in considerable type II error), we used the noncentral F statistic for the Time main effect resulting from the mixed-model ANOVA. The implied effect size f^2, as defined by Cohen (21, pp. 410–14), was computed as $f^2 = (u/v)F$, where u and v are the numerator and denominator degrees of freedom of the F statistic, respectively. The F statistic noncentrality parameter λ was computed as $\lambda = (u + v + 1)f^2$. By comparing this parameter to values listed in Table 9.4.3 in (21), statistical power to detect a Time effect could be determined.

The second approach, more parsimonious from a statistical point of view, utilized a two-stage data analysis scheme (separately for SBP, DBP, and HR). The first stage entailed fitting a sinusoid with a period of 24 h to each 24-h time series. Thus, for each of the 30 time series (six subjects by five constant routines) comprising 24 (t_i, y_i) pairs, we fitted the model:

$$A \sin\left(\frac{2\pi t_i}{24\text{ h}} - \phi\right) = \hat{y}_i - \bar{y} = a \cos\left(\frac{2\pi t_i}{24\text{ h}}\right) + b \sin\left(\frac{2\pi t_i}{24\text{ h}}\right),$$

with $\bar{y}$ denoting the average y_i value across time of day, and $i = 1, 2, \ldots, 24$. Parameters A and ϕ represent the amplitude and phase, respectively, of the sinusoid in the left-hand expression. Use of the right-hand expression permits parameter estimation by linear regression (22) using least-squares estimation, and the statistical

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distributions of parameters a and b are normal (23) under conditions of random error (i.e., white noise).

The 30 pairs (a_{jk}, b_{jk}), with $j = 1, 2, \ldots, 6$ for subjects and $k = 1, 2, \ldots, 5$ for constant routines, were subjected to second-stage analysis as follows. Using mixed-model ANOVA, which included a fixed effect of constant routine (Trial), a random effect of Subject, and residual error, we tested whether subject-specific parameters a_{jk} and b_{jk} (analyzed separately) were stable over trials (F-test). If this was the case, summary statistics were computed as $a_{j\cdot} = \sum_k a_{jk}$ and $b_{j\cdot} = \sum_k b_{jk}$, and the mean values $\mu(a_{j\cdot})$ and $\mu(b_{j\cdot})$ were calculated. Furthermore, the mixed-model ANOVA was reformulated defining Trial as a random effect in order to estimate the within-subject variances $\sigma^2(a_{j\cdot})$ and $\sigma^2(b_{j\cdot})$. These were used to test our twofold null hypothesis of *no* circadian variation:

$$H_0(\text{I}): \begin{cases} \mu(a_{j\cdot}) = 0, \\ \mu(b_{j\cdot}) = 0, \end{cases}$$

$$H_0(\text{II}): \begin{cases} \sigma_s^2(a_{j\cdot}) = 0, \\ \sigma_s^2(b_{j\cdot}) = 0, \end{cases}$$

using two-sided single-sample t-tests for H_0(I) and one-sided Wald Z tests for H_0(II). Homogeneous circadian variation among subjects implies that H_0(I) is false and H_0(II) is true. Subject-specific variation among the circadian parameter pairs (a_{jk}, b_{jk}) implies that H_0(II) is false (assuming that stability over trials was not rejected). Thus, any presence of circadian variation implies that either H_0(I) or H_0(II) or both are false.

Both approaches to statistical analysis were adapted to the fact that the experiments were conducted to replicate the results of our earlier study (12), in which we found a circadian rhythm of HR, but *no* endogenous circadian variation of BP during constant routine. In order to be liberal in rejecting the null hypothesis of *no* circadian variation, we set the type I error threshold to $\alpha = 0.1$ for all analyses of SBP and DBP, thus increasing statistical power to contradict our hypothesis. For HR analyses, a conventional $\alpha = 0.05$ was used.

RESULTS

Table 1 gives the grand means and the 95% confidence intervals for the BP and HR values observed in this study. The SBP and DBP means and confidence intervals were well within the ranges for normotensive subjects [(24); Tab. 2]. Figure 1 shows the overall 24-h profiles of SBP, DBP, and HR. These were very similar to those obtained in our earlier study [(12); Fig. 1], in which a clear endogenous circadian rhythm of HR was observed, but no endogenous circadian variation of BP was found. In Figure 2, the individual subjects' 24-h profiles for SBP and DBP are superimposed for each constant routine. This figure does not suggest any circadian

Table 1. Least-Squares Estimated Grand Means ± Standard Errors (S.E.) and 95% Confidence Intervals (C.I.) for SBP and DBP (in mm Hg) and HR (in bpm)[a]

	Mean ± S.E.	95% C.I.
SBP	122.4 ± 2.3	117.8–127.0
DBP	65.7 ± 2.1	61.5–69.9
HR	63.3 ± 2.9	57.5–69.0

[a]Grand means were estimated from fixed effects and standard errors were derived from linear combinations of random effects using the mixed-model ANOVA described in the statistical analyses section. This enabled derivation of the lower and upper bounds of the 95% confidence intervals for the true means. There were 697 recordings per variable–out of a total of 720 (24 h by five constant routines by six subjects) there were 23 missing values for which the results presented in this table were appropriately weighted. The BP results demonstrate that the subjects were normotensive based on the criteria provided by Staessen et al. (24).

variation in BP values either. Figure 3 shows the distribution of the 23 (about 3%) missing values across time of day and over constant routines for each subject. Periodogram pseudowindow analysis (17) of each of the available recordings did not reveal any significant artificial effects on circadian variation due to these missing values ($P > 0.5$; 25).

For the SBP data, mixed-model ANOVA yielded a significant effect for Time by Trial ($F_{92,437} = 1.28$, $P = 0.053$), but not for Time ($F_{23,115} = 1.38$, $P = 0.13$) and for Subject by Time ($Z < 0.001$, $P > 0.95$). A further analysis per individual trial revealed that only the data recorded during the third constant routine contained a significant effect of Time ($F_{23,437} = 2.23$, $P = 0.001$; for the other trials we found $P > 0.15$). Figure 4 shows the individual subjects' SBP time series for the third constant routine. Visual inspection of these data suggested that the significant Time effect for this trial—while yielding evidence for SBP changes

Table 2. Between-Subjects Variance Components (as percentages of total variance) for SBP, DBP, and HR, Estimated with Mixed-Model ANOVA using the Restricted Maximum-Likelihood (REML) Method as Described in the Statistical Analyses Section[a]

	Subject (%)	Subject by trial (%)	Subject by time (%)
SBP	27.0	12.1	0.0%
DBP	32.3	9.6	1.4%
HR	49.1	19.0	2.1%

[a]Subject by Time contributions to the total variance were very small, underlining that individual differences in the 24-h profiles of BP and HR were insubstantial.

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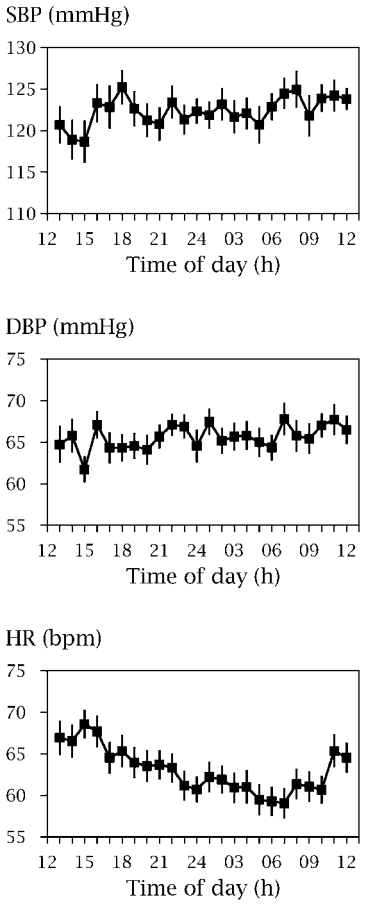

Figure 1. Means (boxes) ± standard errors (vertical bars) of SBP, DBP, and HR for each of the 24 hourly recordings during the constant routine, averaged over the five constant routines and over the six subjects. These averaged curves merely serve to illustrate; they were not used for analyses of circadian variation.

over the 24 h—may not be reflective of any circadian rhythmicity. This was confirmed by the results of the two-stage analysis of circadian rhythmicity presented below.

For the DBP data, mixed-model ANOVA yielded no significant effects for Time ($F_{23,115} = 1.11$, $P = 0.34$), Time by Trial ($F_{92,437} = 1.18$, $P = 0.14$), nor Subject by Time ($Z = 0.73$, $P = 0.47$). Thus, there was no evidence for circadian variation of DBP at all. For the HR data, mixed-model ANOVA yielded a significant effect for Time ($F_{23,115} = 5.19$, $P < 0.001$), but not for Time by Trial ($F_{92,437} = 1.06$, $P = 0.36$) nor for Subject by Time ($Z = 1.77$, $P = 0.077$). Thus, there was strong evidence for circadian variation of HR, and the 24-h profiles appeared to be stable across constant routines, with near-negligible between-subjects differences. Table 2 shows the relative between-subjects variance components in the data. The

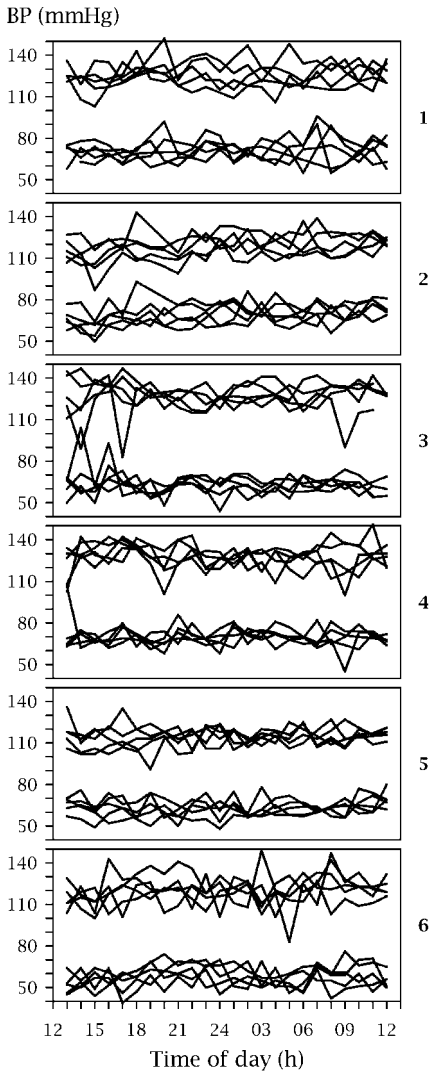

Figure 2. Raw BP data for individual subjects; subject numbers are indicated on the right of each panel. The 24-h time series for each of the five constant routines are superimposed within each panel—upper curves are SBP and lower curves are DBP.

Subject by Time contributions to the total variance were very small, underlining that the absence of Time main effects in the BP values could not be explained as an artifact due to individual differences.

The implied effect size of the Time effect in the HR data was $f^2 = (u/v)F = (23/115) \times 5.19 = 1.038$, and the noncentrality parameter was $\lambda = (u + v + 1)f^2 = (23 + 115 + 1) \times 1.038 = 144.3$. From Table 9.4.3 in (21) it follows that statistical power (at $\alpha = 0.1$) to detect Time main effects for SBP and DBP—if they were as

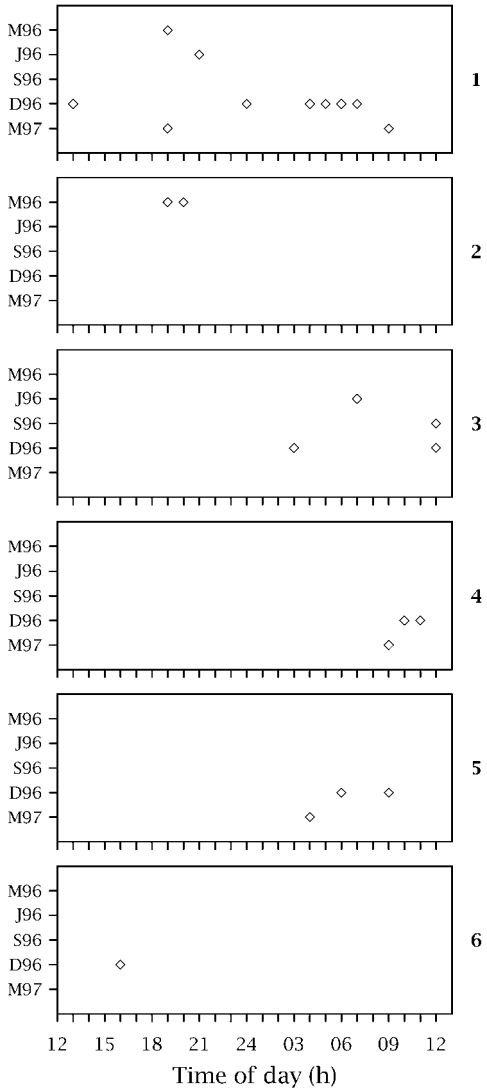

Figure 3. Distribution of missing values across times of day (on the abscissas) and constant routines (on the ordinates). Each panel represents one subject; subject numbers are indicated on the right. The labels on the ordinates indicate the months in which the constant-routine experiments were conducted (M96 = March 1996, J96 = June 1996, S96 = September 1996, D96 = December 1996, M97 = March 1997). Twenty-three out of 720 hourly recordings (about 3%) were missing in total, concurrently for SBP, DBP, and HR.

large as the one detected for HR—was more than 99%. Using the same table, we computed the minimum value for f^2 that was detectable with at least 80% power. The value for λ detectable with 80% power was approximately 18.7, implying that the minimum f^2 value detectable with 80% power was 0.135, which is less than 15% of the implied effect size for the Time effect in the HR data. Thus, if the Time

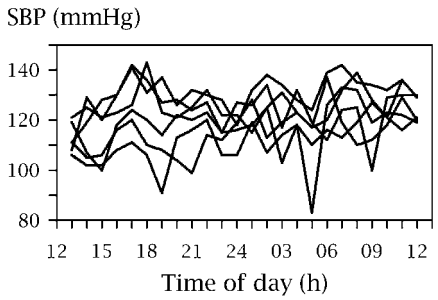

Figure 4. SBP time series for each individual subject during the third of five repeated constant routines. This was the only trial for which a significant time simple effect was found in SBP (but not in DBP).

main effect in the SBP and DBP data was only 15% as large as that observed for HR, our study was likely to reject the null hypothesis of equal means across times of day, that is, *no* endogenous circadian variation in BP.

In the mixed-model ANOVAs—with Trial as a fixed effect—of the two parameters a and b for the more parsimonious two-stage analysis of circadian rhythmicity, no significant effect of Trial was found for SBP (for a_{jk}: $F_{4,20} = 0.98$, $P = 0.44$; for b_{jk}: $F_{4,20} = 1.60$, $P = 0.21$), for DBP (for a_{jk}: $F_{4,20} = 0.60$, $P = 0.66$; for b_{jk}: $F_{4,20} = 1.90$, $P = 0.15$), nor for HR (for a_{jk}: $F_{4,20} = 0.79$, $P = 0.54$; for b_{jk}: $F_{4,20} = 2.30$, $P = 0.095$). Thus, we proceeded by repeating these mixed-model ANOVAs with Trial as a random effect, in order to test the twofold hypothesis of *no* circadian variation consisting of H_0(I) and H_0(II) defined previously.

For SBP, there were no significant deviations from zero for $\mu(a_{j\cdot})$ ($t_5 = -0.48$, $P = 0.65$), for $\mu(b_{j\cdot})$ ($t_5 = 0.48$, $P = 0.65$), for $\sigma_s^2(a_{j\cdot})$ ($Z < 0.001$, $P > 0.95$) and for $\sigma_s^2(b_{j\cdot})$ ($Z = 0.66$, $P = 0.51$). Similarly, for DBP, there were no significant deviations from zero for $\mu(a_{j\cdot})$ ($t_5 = 0.15$, $P = 0.89$), for $\mu(b_{j\cdot})$ ($t_5 = 1.41$, $P = 0.22$), for $\sigma_s^2(a_{j\cdot})$ ($Z < 0.001$, $P > 0.95$) and for $\sigma_s^2(b_{j\cdot})$ ($Z < 0.001$, $P > 0.95$). Furthermore, for HR, there were no significant deviations from zero for $\sigma_s^2(a_{j\cdot})$ ($Z = 0.61$, $P = 0.54$) and for $\sigma_s^2(b_{j\cdot})$ ($Z = 1.35$, $P = 0.18$). However, the HR mean parameter values $a_{j\cdot}$ and $b_{j\cdot}$ were both significantly different from zero (for $\mu(a_{j\cdot})$: $t_5 = -3.34$, $P = 0.021$; for $\mu(b_{j\cdot})$: $t_5 = -3.75$, $P = 0.013$). Thus, in this approach to statistical analysis, we found circadian rhythmicity for HR only, with no evidence for individual differences. Circadian rhythmicity in the 24-h profile of BP could not be detected at all, even with the liberal choice of $\alpha = 0.1$.

CONCLUSION

This study demonstrated the absence of endogenous circadian variation of BP during repeated assessment under constant routine. Several points can be made to show that this finding was reliable.

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Firstly, signal theory predicts that sampling occurred sufficiently frequent (i.e., one measurement per hour) and sufficiently long (i.e., 24 h) during each constant routine to reveal any circadian variation of the signals at hand (26). Thus, if there was any circadian variation of BP at all, it should have been present still in the sampled data.

Secondly, endogenous circadian variation of SBP and/or DBP could not be detected even when a novel, powerful two-stage statistical analysis of circadian rhythmicity was employed with a liberal choice of the type I error threshold (i.e., $\alpha = 0.1$).

Thirdly, with mixed-model ANOVA of the full set of 697 hourly recordings (24 h by five constant routines by six subjects minus 23 missing values) of SBP and DBP, a significant time effect was found only for the SBP data of the third constant routine (in a series of five). This singular finding could not be interpreted as a first-time, last-time, or adaptation effect; nor could it be readily explained by some hypothetical seasonal modulation, given that the experiments were placed evenly across the four seasons (cf. 27). Upon visual inspection of the SBP data of the third constant routine (see Fig. 4), the significant time effect did not appear to reflect a circadian rhythm. This was confirmed by the results of the two-stage statistical analysis of circadian rhythmicity.

Fourthly, we were able to demonstrate that if the time main effects in the SBP and DBP data were only 15% as large as the time effect observed for HR, our study was sufficiently powered to reject the null hypothesis of *no* endogenous circadian variation in BP. Nevertheless, no main time effects were detected for SBP and DBP.

Fifthly, the constant routine was designed to eliminate the confounding effects of exogenous factors on the 24-h profiles of variables of interest (13,14). The presence of a circadian rhythm of HR, which was also observed under constant routine by Kräuchi and Wirz-Justice (28), Burgess et al. (29), Kerkhof et al. (12), and Khalsa et al. (30), corroborated the potential of the constant-routine paradigm for exposing endogenous circadian rhythmicity in the cardiovascular system. Thus, the absence of circadian variation of BP could not be explained as a result of an inappropriate experimental design.

Sixthly, HR and BP were measured simultaneously with the same recording device, and analyzed with the same statistical methodology. As a consequence, the presence of a circadian rhythm of HR in both statistical analysis approaches demonstrated that the absence of circadian variance of BP could not be explained as a measurement problem or a weakness in the statistical design. Note also that the 95% confidence intervals for SBP and DBP in this study (see Tab. 1) were well within the ranges for normotensive subjects reported by Staessen et al. (24).

Seventhly, trial-to-trial variations in HR measurements were nonsignificant, irrespective of the statistical analysis approach. The same was found for all the BP data, with only the SBP time series of the third constant routine causing a deviation from this stability across trials (as discussed previously). Furthermore, individual differences in the 24-h profiles of HR and BP were insubstantial (see Tab. 2). Consequently, the averaged curves in Figure 1 were representative of the

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overall data set. Importantly, any trial- or subject-related variance clearly could not have significantly diminished the statistical power to detect any circadian variation in the BP data.

Finally, the present results, based on rigorous within-subject analysis of repeated measures, replicated the results of our earlier study (12), which had a between-subjects design. In that study, endogenous circadian variation of BP was absent under constant routine also, while a pronounced endogenous circadian rhythm of HR was observed simultaneously as well.

Having established the reliability of our finding that there was *no* endogenous circadian variation of BP during repeated assessment under constant routine, it is remarkable that this result did not match those of other studies during which the environmental and behavioral conditions were standardized (6–9). In these studies a circadian rhythm of BP was observed consistently, albeit with a smaller amplitude than under natural, ambulatory conditions. None of these studies, however, maintained wakefulness throughout 24-h recordings. Thus, the occurrence of sleep may have confounded these studies. Indeed, evidence for substantial effects of sleep and sleep architecture on BP was reported by Mancia (31), Somers et al. (32), Bursztyn et al. (33), and Van de Borne et al. (8).

Given the absence of circadian variation of BP under constant routine and, in contrast, the presence of such variation when circumstances change over the 24 h of the day (e.g., when sleep is allowed, or during ambulatory recordings), it appears that on a circadian time scale, BP may be fine-tuned exclusively to the prevailing behavioral and environmental conditions. It is quite conceivable that the cardiovascular system would not be well-served by an additional independent, endogenous circadian variation of BP. The observed circadian rhythm of HR during the constant routine could reflect compensation of the effects on BP due to other endogenous circadian rhythms that may occur in the cardiovascular system [e.g., circulating blood volume, viscosity, and peripheral resistance; see (34)]. This explanation may clarify the paradoxical conclusion of the present study: While there is a pronounced circadian rhythm of HR under constant routine, there appears to be no endogenous circadian variation in the 24-h profile of BP.

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