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Estimation of Human Circadian Phase via a Multi-Channel Ambulatory Monitoring System and a Multiple Regression Model

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Abstract Reliable detection of circadian phase in humans using noninvasive ambulatory measurements in real-life conditions is challenging and still an unsolved problem. The masking effects of everyday behavior and environmental input such as physical activity and light on the measured variables need to be considered critically. Here, we aimed at developing techniques for estimating circadian phase with the lowest subject burden possible, that is, without the need of constant routine (CR) laboratory conditions or without measuring the standard circadian markers, (rectal) core body temperature (CBT), and melatonin levels. In this validation study, subjects ($N = 16$) wore multi-channel ambulatory monitoring devices and went about their daily routine for 1 week. The devices measured a large number of physiological, behavioral, and environmental variables, including CBT, skin temperatures, cardiovascular and respiratory function, movement/posture, ambient temperature, and the spectral composition and intensity of light received at eye level. Sleep diaries were logged electronically. After the ambulatory phase, subjects underwent a 32-h CR procedure in the laboratory for measuring unmasked circadian phase based on the "midpoint" of the salivary melatonin profile. To overcome the complex masking effects of confounding variables during ambulatory measurements, multiple regression techniques were applied in combination with the cross-validation approach to subject-independent prediction of circadian phase. The most accurate estimate of circadian phase was achieved using skin temperatures, irradiance for ambient light in the blue spectral band, and motion acceleration as predictors with lags of up to 24 h. Multiple regression showed statistically significant improvement of variance of prediction error over the traditional approaches to determining circadian phase based on single predictors (motion acceleration or sleep log), although CBT was intentionally not included as the predictor. Compared to CBT alone, our method resulted in a 40% smaller range of prediction errors and a nonsignificant reduction of error variance. The proposed noninvasive measurement method could find applications in sleep medicine or in other domains where knowing the exact endogenous circadian phase is important (e.g., for the timing of light therapy).

Key words human circadian rhythms, multi-channel ambulatory recording, skin temperatures, ambient light, melatonin, curve fitting, regression modeling

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The circadian system, driven by a circadian pacemaker located in the suprachiasmatic nuclei (SCN) of the hypothalamus, enables organisms to anticipate daily environmental changes by adjusting behavior, physiology, and gene regulation. A critical feature and the key to understanding the circadian clock and its control mechanisms is its synchronization to the external day (i.e., circadian entrainment). Under natural conditions, endogenous circadian rhythms are entrained to the 24-h external solar light-dark cycle (for a review, see Roenneberg et al., 2003). In humans, daily rhythms can be seen in a variety of molecular, physiological, and psychological measures ranging from gene expression, CBT, heart rate, the pineal hormone melatonin, cortisol secretion to subjective sleep and mood, as well as higher cognitive functions (for a review, see Schmidt et al., 2007). Variables such as CBT and endogenous melatonin and cortisol levels have been widely used to estimate parameters of the circadian system such as phase, amplitude, and endogenous period. However, in daily life situations, such estimation is complicated by "masking" effects (Minors and Waterhouse, 1989; Hiddinga et al., 1997) induced by sleep, physical activity, meals, emotional activation, and others, all of which have an effect on the measured variables to a different extent (Wilhelm and Grossman, 2010; Wilhelm et al., 2006). Melatonin is considered to be the best circadian marker and has least variability compared to CBT and cortisol (Klerman et al., 2002). However, measuring the secretion of hormones is very expensive and can be reliably done only under laboratory conditions so far. CBT is also a good circadian marker but is more sensitive to masking effects than melatonin or cortisol, and measurement of CBT is complicated because of the use of probes that must be worn inside the body (most often, these are rectal probes).

There are many reports that discuss the complexity of estimating the relationship between physiological data and endogenous circadian phase (Klerman et al., 1999; Minors and Waterhouse, 1989; Carrier and Monk, 1997; Eastman, 1992). However, in these works, only a limited number of physiological variables were used, and the demasking techniques were univariate or based on relatively simple heuristics using techniques such as subtracting or adding an amount to a circadian variable such as CBT, depending on the activity of the subject.

Thus, for accurate estimation of endogenous circadian phase using ambulatory data, it would be necessary to take multiple physiological and behavioral parameters into account that contain both circadian and masking components (e.g., skin temperatures, heart rate, motion acceleration) as well as measurements of ambient light as the "zeitgeber" that contributes most to the entrainment of the human circadian clock. The relation between the ambulatory measurements and endogenous phase measured in the laboratory under CR conditions could be established using a "gold standard" circadian marker such as melatonin. Upon collection of data from a representative sample of subjects, including a wide range of chronotypes, and after identification of ambulatory variables that provide the best accuracy of the estimation of endogenous circadian phase using statistical techniques, subject-independent prediction models could be obtained. With such models, estimation of circadian phase in real-life conditions could be possible without the need to stay in the laboratory.

The EU FP6 integrated project EUCLOCK (www.euclock.eu) extends previous research, taking into account these points. One of the most important aims of the human subproject is to estimate circadian phase using measurements provided by an ambulatory multi-channel circadian monitoring system and advanced modeling algorithms with low burden on the test subject, that is, without the need for staying in the laboratory under CR conditions and without invasive CBT and expensive melatonin or cortisol measurements.

In this article, we have used multi-channel ambulatory monitoring with multiple variables as well as multiple regression techniques for extraction of the underlying circadian rhythm in combination with numeric optimization procedures for waveform analysis (Van Someren and Nagtegaal, 2007). We describe the first results of prediction of ambulatory human circadian phase under entrainment and subject-independent prediction models developed within the project EUCLOCK.

MATERIALS AND METHODS

Protocol

Figure 1 illustrates the overall design of the ambulatory circadian monitoring validation study. The protocol involved 2 parts: first, the volunteers went about their usual everyday activities for approximately

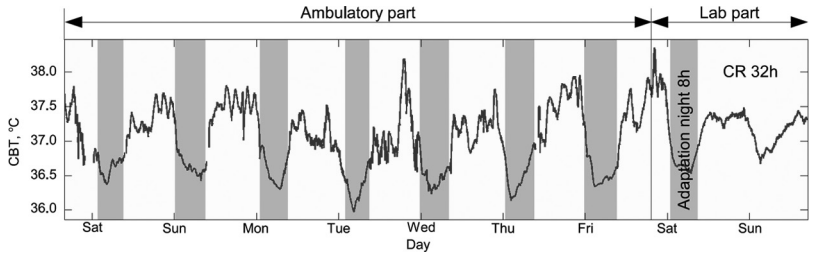

Figure 1. Overall protocol design and typical CBT measurements for one representative subject. Dark bars correspond to sleep, ticks on the x-axis correspond to midnights, and hatched area is the CR. The protocol starts on a Friday.

7 days, and then they reported to the chronobiology laboratory at the University Psychiatric Clinics (UPK Basel), where they spent about 44 h, including 1 night with sleep at habitual bed times (8 h) and 32 h of scheduled wakefulness under CR conditions.

The ambulatory measurements began between 1100 h and 1700 h, always on a Friday. During the ambulatory 7-day episode, subjects kept their usual bed-time regimen, registered by actimetry and sleep logs. In addition, they kept a daily log about their subjective well-being, fatigue, sleepiness, and so on. Besides the rest-activity cycle, the following physiological signals were recorded continuously with a wearable multi-channel ambulatory monitoring device: ECG, rectal temperature, and respiration. Skin temperatures from 11 locations were recorded with miniature autonomous loggers. In addition, ambient light was recorded by a miniature light sensor attached to the side of eyeglasses (either subjects' own or zero dioptric with a normal frame provided by the experimenters).

The laboratory part of the study started on the following Friday between 1900 h and 2100 h and ended on Sunday between 1500 h and 1800 h. All ambulatory recordings continued in the laboratory until the end of the protocol. The timing of the adaptation night (8 h) and of the immediately following CR (32 h) was scheduled according to the sleep midpoint during the ambulatory week as determined from sleep logs. Posture, food, ambient light, and temperature levels were kept constant or distributed uniformly across the CR protocol in order to minimize masking effects of those variables and to enable us to quantify phase and amplitude of the circadian markers. The light level was ≤8 lux. For further details on the CR procedure, see Cajochen et al. (2001).

The primary circadian marker during the CR was melatonin. Thus, saliva samples were collected every 60 min throughout the CR. CBT was continuously recorded throughout the protocol, that is, for 7 days during the ambulatory part and 2 days in the laboratory. CBT recordings were used for an additional comparison of predictions of circadian phase provided by the model proposed in this article, as were sleep logs acquired via electronic diaries. The study protocol was approved by the local ethical committee (Ethikkommission beider Basel) and conformed to the Declaration of Helsinki.

Subjects

Twenty-one male subjects were recruited. Participants were selected according to their habitual bed times to form a representative dataset including a wide range of chronotypes from evening to morning types. The chronotype of potential study participants was assessed as midsleep on free days corrected for sleep deficit (MSFsc) using the Munich chronotype questionnaire (Roenneberg et al., 2007), which they could download from the Web site of the Centre for Chronobiology (www.chronobiology.ch). Only healthy nonsmoking subjects 19 to 35 years old who lived on a normally entrained 24-h schedule and had no self-reported sleep problems were selected. The reason why only male subjects were studied was a practical one since in women, the entire timing of the protocol would have had to be adjusted to their menstrual cycle.

Study participants signed a written informed consent form before being enrolled in the study and underwent a medical examination at UPK Basel prior to participation. The complete protocol was carried out successfully by 16 subjects (mean age ± SD, 24.6 ± 4.3 years). Measurements with the other 5 subjects were interrupted because of initial technical problems like loose contacts and broken cables in the measurement devices (4 subjects) and noncompliance to the protocol (1 subject). Thus, the overall success rate in our difficult and time-demanding protocol was about 76%. Data were collected in the period from July 2008 to November 2009.

Figure 2. Ambulatory monitoring devices: 1) ClockWatcher: ECG, respiration, 3-dimensional acceleration, core body temperature; 2) Varioport: same channels as ClockWatcher; 3) Light sensor: ambient light, temperature, and 3-dimensional acceleration (for compliance check); 4) Palm computer: electronic diary (E-Diary); and 5) iButton: position of skin temperature measurements.

Ambulatory Monitoring Devices

A prototype of the ambulatory circadian monitoring device "ClockWatcher" for ambulatory studies within the EUCLOCK Project was developed by Personal Health Institute International (Phi-I) in Amsterdam (Fig. 2). The ClockWatcher is designed to record the following variables in real-life conditions over multiple days:

- CBT (measured with a disposable rectal probe);
- ECG (recorded with solid gel electrodes; each subject was given additional electrodes and instructed to replace them daily);
- respiration (recorded with 2 belts for thorax and abdomen);
- body movement and posture (measured with a 3-dimensional accelerometer);
- leg movement (measured with a 1-dimensional accelerometer); and
- event markers (going to sleep/taking shower).

Additionally, an "off-the-shelf" monitoring device (Varioport, Becker Meditec, Karlsruhe, Germany) was acquired in order to expedite the development of the ambulatory circadian models (Fig. 2). The Varioport device had custom-made signal preamplifiers that allowed them to be used for recording the same set of variables as with the ClockWatcher. Of 21 subjects who took part in our validation study, 8 wore the ClockWatcher and 13 the Varioport throughout the experiment. With the ClockWatcher, 5 complete datasets were obtained and 11 with the Varioport. Using calibration and our MATLAB software (MathWorks, Natick, MA), all data from both devices were brought to the same ranges to facilitate further model development irrespective of the device type. For details of our circadian software and data organization, see the supplementary online material.

For recording ambient light, a specialized miniature ambulatory monitoring device "LightWatcher" was also developed within the EUCLOCK Project by Sowoon (Lausanne, Switzerland) (Fig. 2). It has the following measurement channels:

- light in 5 spectral bands (infrared, red, green, blue, ultraviolet);
- 3-dimensional acceleration (motion along axes X, Y, and Z, used for compliance check); and
- ambient temperature.

Eleven miniature wireless temperature sensors (DS 1922L Thermochron iButtons, diameter × height: 17 × 6 mm, accuracy: 0.0625 °C, Maxim Integrated Products, Sunnyvale, CA) were used to record skin temperatures continuously in 2.5-min intervals throughout the protocol (Smith et al., 2010). The iButtons were fixed to the skin with thin, air-permeable adhesive surgical tape (Fixomull, Beiersdorf AG, Hamburg, Germany) on the left and right side of the body (except for thorax), as shown in Figure 2. The temperature sensors can be worn under normal life conditions including taking a shower and doing sports. The iButtons were applied by the experimenters at the beginning of the protocol and were worn throughout. Each subject was given approximately 50 pieces of adhesive tape 50 × 50 mm in size and instructed to replace them when necessary so that the temperature sensors stayed in good contact with the skin and at the same locations. All the sensors were numbered, and the subjects received a diagram showing the correct locations of the sensors on the skin according to the numbering, which were checked again in the laboratory before the CR.

Handheld computers of type Palm Tungsten E (Sunnyvale, CA) were used as an electronic diary. Questionnaires for sleep logs and a number of other scales and fill-in forms were programmed with Pendragon Forms software v. 4.0 (Libertyville, IL). For more details on the sleep logs, see the supplementary online material.

The clocks of all devices were synchronized at the beginning of the measurements with the master PC that was used for collecting and processing the data. The deviation of the clocks from the master clock after completing the entire protocol for each subject did not exceed ±3 min. Checking and transferring the measured data are explained in the supplementary online material.

Reference Circadian Phase

For model development, the reference circadian phase was determined based on salivary melatonin secretion in the laboratory part of the experiment under CR. Melatonin was used as a reference for the following reasons: it is known to be the most reliable circadian marker, also compared to CBT (Klerman et al., 2002); reliable measurements of melatonin secretion were available for all 16 subjects throughout the CR, whereas the CBT measurements for 4 subjects contained artifacts that complicated reliable detection of the underlying circadian rhythm. For 1 more subject, the CBT measurements in the laboratory were not available for more than half of the CR because of a technical problem.

First, the reference waveform of melatonin secretion was determined based on melatonin levels from 33 saliva samples taken every 60 min under CR conditions. This waveform was assumed to have a period of 24 h and was identified using the bimodal skewed baseline cosine function (BSBCF) (Van Someren and Nagtegaal, 2007). For details, see "Waveform analysis" in the supplementary online material.

The reference circadian phase was determined as the time corresponding to the center of gravity (COG) of the area (Wetterberg, 1998) under the periodic BSBCF curve for one period of 24 h (see "Calculation of circadian phase" in the supplementary online material). With the COG method, no thresholds need to be defined for determining circadian phase from the BSBCF curve because the threshold is always equal to the baseline, in contrast, for example, to the original work (Van Someren and Nagtegaal, 2007), where the phase of the BSBCF curve is defined as the midpoint at 25% level and may need to be adjusted if there is a narrow peak or 2 peaks very different in amplitude, with one of them below 25%.

Multiple Regression Modeling

The prediction model that we use is defined in a general form as

$$\begin{aligned}\hat{y}(t) = {} & \alpha_{1,0}x_1(t) + \alpha_{1,1}x_1(t-\Delta t) + \ldots + \alpha_{1,d_1}x_1(t-d_1\Delta t) \\ & + \alpha_{2,0}x_2(t) + \alpha_{2,1}x_2(t-\Delta t) + \ldots + \alpha_{2,d_2}x_2(t-d_2\Delta t) \\ & \ldots \\ & + \alpha_{n,0}x_n(t) + \alpha_{n,1}x_n(t-\Delta t) + \ldots + \alpha_{n,d_n}x_n(t-d_n\Delta t) + \beta,\end{aligned}$$

where $\hat{y}(t)$ is the predicted value of the circadian rhythm at time t; t is the time in hours; x_1, K, x_n are the predictor variables; d_1, K, d_n are their respective lags defined as a multiple of the sampling interval $\Delta t = 0.5$ h; $\alpha_{1,0}$, … , α_{n,d_n} are coefficients of the predictor variables; and β is the bias term. The parameters $\alpha_{1,0}$, … , α_{n,d_n} and β are identified using the least-squares method. The choice of lags d_1, K, d_n is described in the "Variable selection" section of the supplementary online material.

For determining the parameters of the prediction models (Fig. 3A), data including both the ambulatory and laboratory parts were used. The reference circadian rhythm for model identification was derived based on salivary melatonin secretion in the laboratory by fitting the BSBCF curve and extrapolating it onto the ambulatory part. To ensure subject-independent testing with data unseen during model identification, the models were identified using the so-called cross-validation approach (Hastie et al., 2001). For an explanation of this approach, see Figure 3B and the "Cross-validation" section of the supplementary online material.

For prediction of circadian phase, only data from the ambulatory part were used. For determining the predicted ambulatory phase, another BSBCF curve was fitted to the output of the prediction model $\hat{y}(t)$, and the respective predicted ambulatory circadian phase was determined again according to the COG method (see "Calculation of circadian phase" section in the supplementary online material) and compared to the reference phase determined by the COG method from the BSBCF curve of CR melatonin.

Final Model Structure

The inclusion of variables in the model was based on the accuracy of prediction of circadian phase in a cross-validation setting. Additionally, different lags for predictor variables were iteratively tried, and the best lags were identified, also based on the accuracy of prediction. For details, see the "Cross-validation" and "Variable selection" sections in the supplementary online material.

The variables that were selected with their respective lags are listed below:

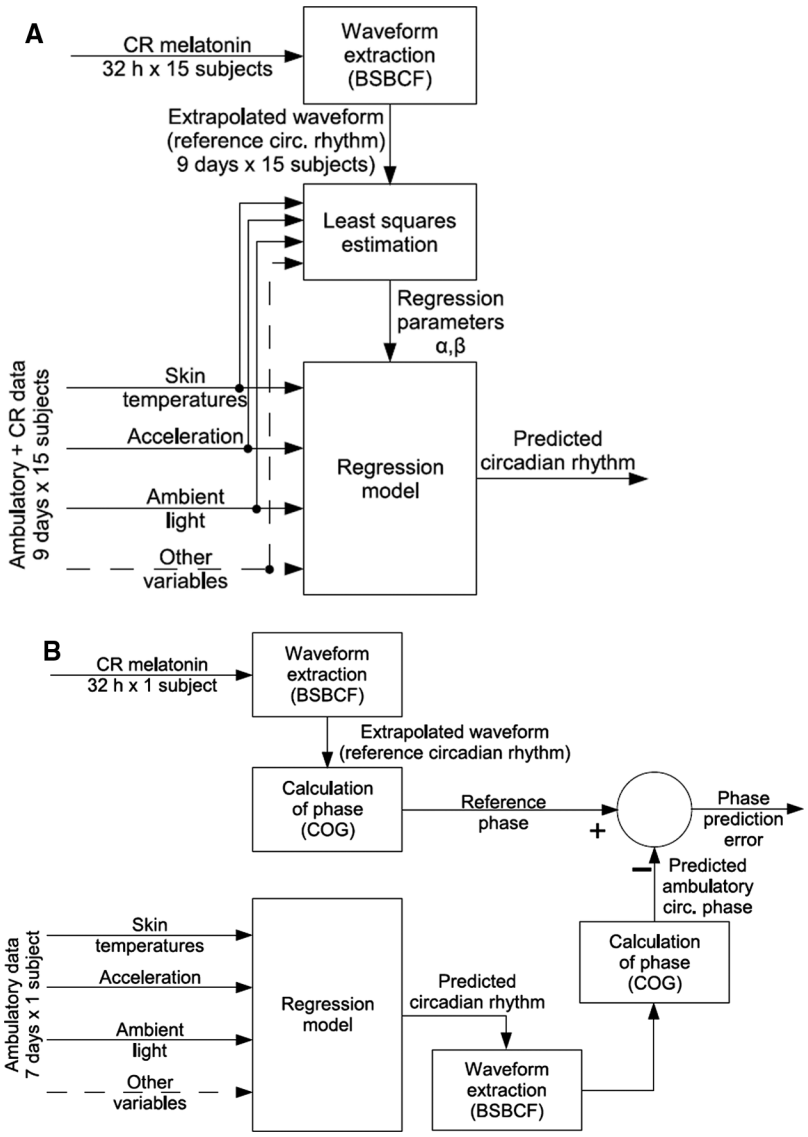

Figure 3. Modeling approach: (A) identification of a prediction model with ambulatory and CR data, and (B) validation of a prediction model with ambulatory data and CR melatonin for comparison of the predicted phase with the reference phase. The final structure of the regression model included only skin temperatures, motion acceleration, and irradiance for blue light as the input variables with their respective lags.

1. skin temperatures (6 variables for feet, hands, shoulders, thorax, upper legs, and lower legs) with lags of up to 5 h; we used the same lags for all skin temperatures;
2. motion with lags of up to 24 h; and
3. irradiance for ambient light in the blue spectral band with lags of up to 24 h.

Thus, 8 variables were selected (6 skin temperatures, motion, and blue light). With their respective lags, the structure of the resulting prediction models based on the cross-validation results was as follows:

$$\begin{aligned}\hat{y}(t) = {} & \alpha_{hands,0}T_{hands}(t) + \alpha_{hands,1}T_{hands}(t-0.5) + \ldots \\ & + \alpha_{hands,10}T_{hands}(t-5) + \alpha_{feet,0}T_{feet}(t) \\ & + \alpha_{feet,1}T_{feet}(t-0.5) + \ldots + \alpha_{feet,10}T_{feet}(t-5) \\ & + \alpha_{thorax,0}T_{thorax}(t) + \alpha_{thorax,1}T_{thorax}(t-0.5) \\ & + \ldots + \alpha_{thorax,10}T_{thorax}(t-5) \\ & + \alpha_{shoulders,0}T_{shoulders}(t) + \alpha_{shoulders,1}T_{shoulders}(t-0.5) \\ & + \ldots + \alpha_{shoulders,10}T_{shoulders}(t-5) \\ & + \alpha_{upper\ legs,0}T_{upper\ legs}(t) \\ & + \alpha_{upper\ legs,1}T_{upper\ legs}(t-0.5) + \ldots \\ & + \alpha_{upper\ legs,10}T_{upper\ legs}(t-5) \\ & + \alpha_{lower\ legs,0}T_{lower\ legs}(t) \\ & + \alpha_{lower\ legs,1}T_{lower\ legs}(t-0.5) + \ldots \\ & + \alpha_{lower\ legs,10}T_{lower\ legs}(t-5) \\ & + \alpha_{motion,0}M(t) + \alpha_{motion,1}M(t-0.5) \\ & + \ldots + \alpha_{motion,48}M(t-24) \\ & + \alpha_{light,0}L(t) + \alpha_{light,1}L(t-0.5) + \ldots \\ & + \alpha_{light,48}L(t-24) + \beta,\end{aligned}$$

where T_{hands}, T_{feet}, T_{thorax}, $T_{shoulders}$, $T_{upperlegs}$, and $T_{lowerlegs}$ were the respective skin temperatures, M was the integrated variable for motion, and L was blue light. All data were down-sampled to 30-min bins, and all lags were given in hours with a step of $\Delta t = 0.5$ h. The models contained 165 parameters each: 164 coefficients $\alpha_{hands,0}$, K, $\alpha_{light,48}$ for the predictor variables with their respective lags plus the bias term β. CBT was intentionally not included in the prediction variable set because the goal of the validation study is to develop a device that imposes minimal subject burden.

Comparison of Prediction of Ambulatory Circadian Phase with Different Methods

For comparison, we also computed predictions of ambulatory circadian phase using 3 other variables that are most often used as ambulatory circadian markers: CBT, motion, and sleep midpoint. For CBT and motion, circadian phase was determined by the minimum of a waveform with a period of 24 h that was computed from ambulatory recordings using harmonic regression. For both CBT and motion, 1, 2, and 3 harmonics were tried, and the best results (lowest standard deviation of prediction errors of CR melatonin phase) were with 1 harmonic for CBT and 2 harmonics for motion. Sleep midpoint was computed using the respective entries for "Lights-On"

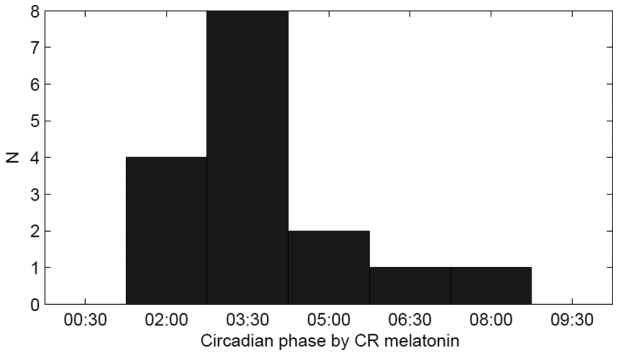

Figure 4. Distribution of circadian phase by melatonin midpoint of study participants.

and "Lights-Off" in the electronic diary during the ambulatory part of the experiment. In order to see if the proposed prediction model provides a statistically significant improvement over the single predictors, we performed the Pitman-Morgan test of differences in variance (Mudholkar et al., 2003) of predictions by different methods.

RESULTS

In our study, we collected a representative dataset of a range of chronotypes. This is shown in Figure 4 as a distribution of circadian phases of study participants as determined by CR melatonin midpoint using the BSBCF and COG techniques. Data for CR melatonin, blue light exposure, CBT, motion, and shoulders and feet skin temperatures for the subjects with the earliest and the latest phase of entrainment as determined by CR melatonin are shown in Figure 5. Table 1 provides information on the quality of recordings from our monitoring devices as percentage of time with acceptable data.

Predictions of the circadian rhythm for the 2 subjects with the earliest and the latest phase of entrainment as determined by CR melatonin phase are shown in Figure 6. The prediction of the ambulatory circadian rhythm is available for 6 days starting on the second day of the ambulatory part of the study because of the lag of the maximum lag 24 h in the prediction model. Figure 6 also shows the reference circadian rhythm in the form of a BSBCF curve for CR melatonin extrapolated onto the ambulatory part and scaled to [0, 1] and the BSBCF curve extracted from the prediction. Phases of these BSBCF curves determined using the COG method determine the reference phase from CR melatonin and the predicted ambulatory circadian phase.

Table 2 depicts the results of comparisons between the developed prediction models and conventional approaches using CBT, sleep log, and motion, as well as the chronotype of participants as midsleep on free days corrected for sleep deficit (MSFsc) (Roenneberg et al., 2007) determined during screening prior to participation in the study. Additionally, mean wake-up times during the ambulatory part of the study with respective standard deviations are shown to give an idea of the variability in sleep timing of study participants with different chronotypes.

As it can be seen from Table 2, the regression models with multiple predictors provided the most accurate prediction of circadian phase of CR melatonin. The prediction based on multiple regression had the lowest mean prediction error, lowest standard deviation of error, highest correlation to CR melatonin phase, and smallest error range. The prediction error was determined as the reference phase by CR melatonin minus the predicted phase by the respective method.

We compared the improvement of standard deviation of predicted circadian phase by the multiple regression approach over CBT, sleep midpoint determined from the sleep log, and motion acceleration using the Pitman-Morgan test at $\alpha = 0.05/3 \approx 0.016$ with the conservative Bonferroni correction (Curran-Everett, 2000). The improvement over CBT (standard deviation of error of 41 min v. 49 min) was not significant ($p = 0.486$), although the use of multiple regression techniques resulted in a 40% smaller range of prediction errors (125 min v. 174 min). At the same time, significant improvements were achieved over sleep midpoint ($p = 0.013$) and motion acceleration ($p = 0.005$). This is particularly noteworthy because the CBT variable was not used for constructing the prediction model.

Scatter plots demonstrating the relationship of ambulatory circadian phase predicted by the different methods versus the reference phase computed from laboratory melatonin are shown in Figure 7. It can be easily seen that the best prediction is provided by the regression models (upper left plot in Fig. 7): the points in this plot lie closest to the identity line, representing the ideal prediction of zero error.

We also computed correlations with circadian phases by CR melatonin for chronotype defined as midsleep corrected for sleep deficit (MSFsc) (Roenneberg et al., 2007) determined during screening and mean wake-up times from the sleep logs. The correlations were 0.555

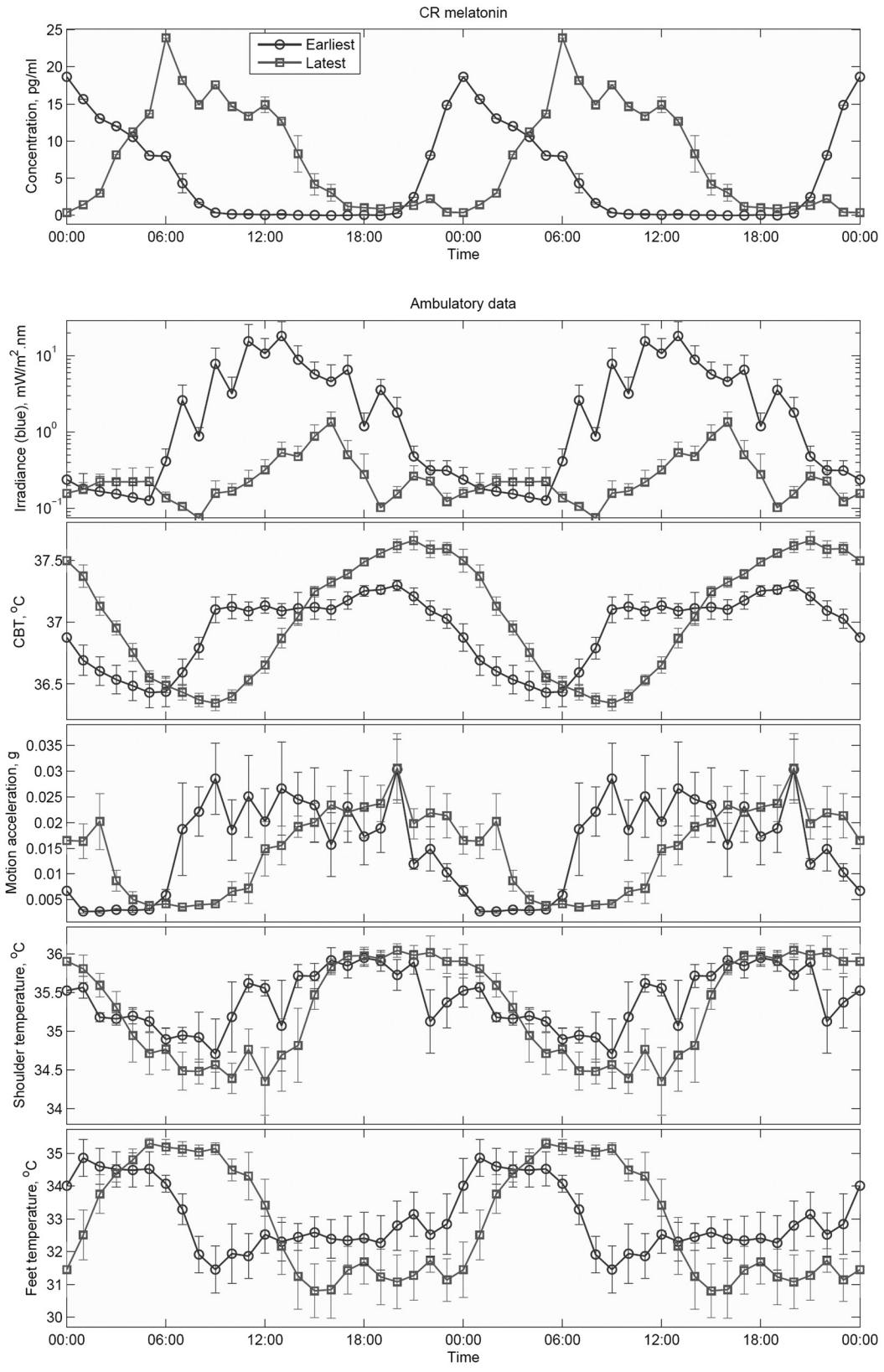

Figure 5. Salivary melatonin level under CR for 32 h (top) and ambulatory data for 7 days (blue light irradiance, CBT, motion, shoulders and feet temperature): double plotted hourly mean ± SE for 2 individual subjects with the earliest and the latest phase of entrainment.

($p = 2.56 \times 10^{-2}$) for MCTQ and 0.806 ($p = 1.63 \times 10^{-4}$) for mean wake-up time; that is, both correlated at a significant level with CR melatonin phase. The correlation of the mean wake-up time with CR melatonin phase was approximately equal to that of sleep midpoint (r = 0.798), while using mean wake-up time for predicting the circadian phase resulted in an error range of 267 min versus 261 min for sleep midpoint as indicated in Table 2 (SD = 65 min for sleep midpoint and mean wake-up time). At the same time, the range of prediction errors for circadian phase with MSFsc was 360 min (SD = 81 min).

DISCUSSION

Our results indicate that reliable detection of circadian phase in real-life conditions is feasible based on a multiple regression model and data gathered from a multi-channel

Table 1. Percentage of time with acceptable data for all monitoring devices with respect to the entire time of the protocol averaged for all subjects.

ClockWatcher & Varioport	Time with Acceptable Data (%)
Heart rate	92.5
Respiratory rate	93.4
Rectal temperature	89.1
Motion, posture	96.3
iButtons	
Skin temperatures	99.1
LightWatcher	
Ambient light	95.7

ambulatory monitoring system, even without including the "classic" circadian CBT marker as a predictor. The predicted circadian phase compares very accurately to that of salivary melatonin secretion obtained in the laboratory under CR conditions. The variables that were included in the prediction models were obtained under real-life conditions from noninvasive small devices, which imposed little subject burden, and compared to the same variables measured under CR laboratory conditions. Possible reasons for inclusion of the particular variables in the model are discussed below.

Skin temperatures exhibit periodic patterns throughout the day that are related to circadian rhythms but also to thermoregulation and are affected by many masking factors. It is known, for example, that the so-called distal-proximal temperature gradient (DPG) is a better indicator of the endogenous circadian rhythm than distal or proximal skin temperatures taken alone. DPG is less affected by masking occurring, for example, due to thermoregulation (Kräuchi et al., 1999; Kräuchi, 2007). We did not simply use a linear combination of skin temperatures with fixed weights of 1 or –1, as is the case with DPG, but included 6 separate skin temperature variables computed from 11 multiple locations in the prediction model with lags of up to 5 h. Thus, the model revealed individual weights of the separate skin temperature variables for best prediction of the endogenous circadian rhythm.

Motion as measured with a portable accelerometer has been known to be an important marker of circadian rest-activity rhythms for many years and became a standard technique for noninvasive measurements of chronotype-related rest-activity cycles as well as in patients with sleep-wake disturbances (Ancoli-Israel et al., 2003; Wirz-Justice, 2007). Conversely, locomotor activity can influence circadian rhythms (Mrosovsky, 1996). Therefore, in our analysis, we included motion as a predictor in the regression model with lags corresponding to one complete 24-h period of activity. The motion variable helped in demasking the endogenous circadian rhythm from the skin temperatures.

Light is responsible for entraining the circadian clock to the external day. Therefore, it is very important to measure daily light exposure in order to predict the day-to-day variations in circadian phase. Light in the blue part of the spectrum appears to be most important for entraining the circadian clock in humans (Lockley et al., 2003; Cajochen et al., 2005; Smith et al., 2009). In our regression model, we included irradiance measurements for ambient light corresponding to the blue spectral band with lags corresponding to one complete 24-h day-night cycle. It was also confirmed by cross-validation that blue light was a better predictor of circadian phase compared to the other 4 spectral bands (infrared, red, green, and ultraviolet), the combinations of all or all the visible spectral bands. Blue light was also the only variable in our model that reflected season via the intensity of lighting and day length.

The result of skin temperatures, motion, and ambient light being the optimum predictors is also advantageous for the following reasons: some variables such as heart rate and respiratory rate are heavily influenced by artifacts that are inevitable in multiple-day recordings because of electrodes coming off (for ECG) or respiratory belts sliding down. This explains a lower percentage of acceptable data for heart rate and respiratory rate in Table 1 compared to data from motion, posture, ambient light, and skin temperatures. Besides that, wearing additional ECG electrodes, respiratory belts, or a leg movement sensor is less comfortable than wearing only small skin temperature sensors, an accelerometer on a soft belt at the waist, and spectacles or a headset with a light sensor. Additionally, respiratory belts require calibration, and the processing of respiratory channels as well as ECG requires more sophisticated algorithms with adjustable parameters for correct extraction of parameters such as heart rate and respiratory rate (Grossman et al., 2010). Extraction of additional variables from ECG (e.g., heart rate variability) imposes yet higher requirements on data quality that can be hardly feasible for ambulatory measurements over multiple days.

In general, the developed regression model can be considered as a multivariate dynamic demasking technique because it is based not just on a single variable like CBT or DPG, taken at a single time point for

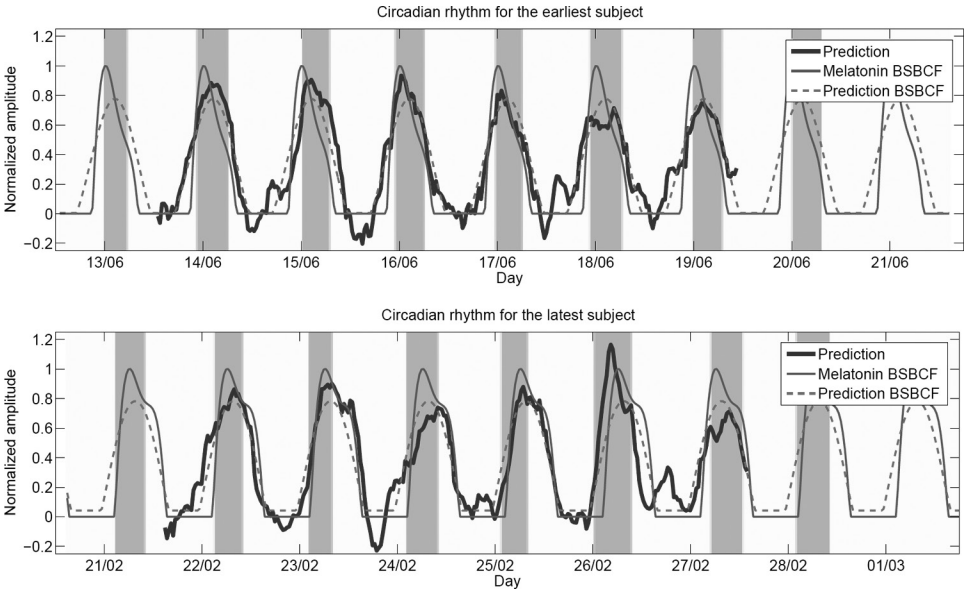

Figure 6. Prediction of ambulatory circadian rhythm for the subjects with the earliest and the latest phase of entrainment. [AQ: 1] represents the prediction for the ambulatory part of the study, thin solid line represents the extrapolated BSBCF curve of CR melatonin scaled to [0, 1], dashed thin line represents the BSBCF curve extracted from the prediction, dark bars correspond to sleep, and ticks on the x-axis correspond to midnights.

determining the current value of the underlying circadian rhythm, but rather on a combination of multiple variables on a moving window. The model extracts the circadian variation from multiple skin temperature variables "filtering out" the masking influences by taking into account the rest-activity cycles, as given by the motion variable, and the most important factor of entrainment, as given by blue light. The improved accuracy of prediction of the entrained circadian phase compared to CBT, actimetry, DPG, or sleep logs, even without including CBT as a predictor, can be explained by the use of the multiple regression approach using carefully selected multiple predictor variables with respective lags and CR melatonin as the target variable, in combination with advanced curve-fitting techniques (Van Someren and Nagtegaal, 2007), even though the prediction model for melatonin rhythm is linear in structure.

At the beginning of our modeling, we first tried a different and more straightforward approach that was to try and build a model for prediction of melatonin levels in the CR using the measured data (temperatures, motion, light, etc.) from the ambulatory week with lags of 0 to 7 days without any backward extrapolation of the melatonin-based reference circadian rhythm. However, we failed to obtain any reasonable prediction that would be better than simpler methods such as actimetry or sleep logs. A possible reason for that was the overfitting effect because the number of data points for CR melatonin is small. As a result, we developed a different approach presented in this article in which the target variable used for modeling is the melatonin secretion profile extrapolated on to the ambulatory week, under the assumption that the timing of sleep and daily activities does not change considerably throughout the ambulatory part and that prior light history affects melatonin secretion in the CR (Wehr, 1998; Hébert et al., 2002; Smith et al., 2004).

The proposed approach needs to be further validated with a larger number of test subjects of different chronotypes and ages, also including female participants. The best results can be expected for late and extremely late chronotypes that are subject to "social jet lag" due to the discrepancy between their entrained circadian phase and the phase of their rest-activity cycles as dictated by work or study hours, indicating the putative clinical applications in circadian sleep disorders. For persons whose daily schedule corresponds well to their own chronotypes, simpler methods such as actimetry or sleep logs would provide sufficient accuracy of determination of circadian phase. Therefore, in order to reveal the advantages of our method, we would need a dataset with a larger proportion of late chronotypes than is usually encountered in the general population.

Additionally, the next step would be to investigate the dependence of accuracy of prediction of circadian phase on the number of days for ambulatory measurements as well as on the number of skin temperature sensors. Another interesting direction of research would be the use of nonlinear modeling techniques, for example, artificial neural networks (Haykin, 1999), which might better approximate the relation between the input variables and the reference circadian rhythms. Thus, a yet smaller range of prediction error might be achieved.

Table 2. Comparison of accuracy of different methods for prediction of ambulatory circadian phase versus CR melatonin phase.

Subject No.	MSFsc[a] (h:min)	End Date (d.mo.y)	Ambulatory Mean Wake-up Time (h) ± SD (min)	CR Melatonin Phase (h:min)	Prediction Error (min) (Error = CR melatonin phase – Predicted phase)			
					Regression Models[b]	CBT	Sleep Midpoint	Motion
1	4:53	13.07.08	0817 ± 125	3:54	45	–66	–14	60
2	3:32	27.07.08	0800 ± 31	3:31	17	–38	–8	–14
3	5:30	17.08.08	0815 ± 25	3:51	–19	–117	–49	–100
4	5:47	31.08.08	0833 ± 44	5:43	69	–122	65	43
5	4:27	16.11.08	0745 ± 20	3:56	27	–97	2	–6
6	7:15	18.01.09	0941 ± 71	5:55	5	–51	–6	15
7	6:18	01.03.09	0954 ± 98	8:29 (latest)	56	–27	132	34
8	5:53	29.03.09	0818 ± 49	4:57	82	–73	17	34
9	4:02	26.04.09	0728 ± 30	3:00	–1	–49	–21	–33
10	3:51	12.06.09	0631 ± 36	1:46 (earliest)	–31	–176	–71	–71
11	4:48	12.07.09	0830 ± 54	2:37	–43	–201	–129	–188
12	6:32	23.08.09	0811 ± 69	2:47	–40	–127	–127	–147
13	4:11	13.09.09	0615 ± 35	1:56	–15	–49	–66	–80
14	5:00	27.09.09	0802 ± 100	4:06	21	–94	–19	5
15	4:13	11.10.09	0736 ± 69	2:35	63	–106	–43	–73
16	7:32	08.11.09	0908 ± 20	3:44	–40	–125	–80	–72
				Mean ± SD (min)	12 ± 41	–94 ± 49	–26 ± 65	–37 ± 61
				Error range (min)	125 (–43 to 82)	174 (–201 to –27)	261 (–129 to 132)	248 (–188 to 60)
				Correlation of predicted phase with CR melatonin phase	0.915 $p = 6.86 \times 10^{-7}$	0.875 $p = 9.12 \times 10^{-6}$	0.798 $p = 2.12 \times 10^{-4}$	0.717 $p = 1.79 \times 10^{-3}$

a. Midsleep on free days corrected for sleep deficit, determined during screening using the MCTQ questionnaire prior to study.
b. 16-fold cross-validation.

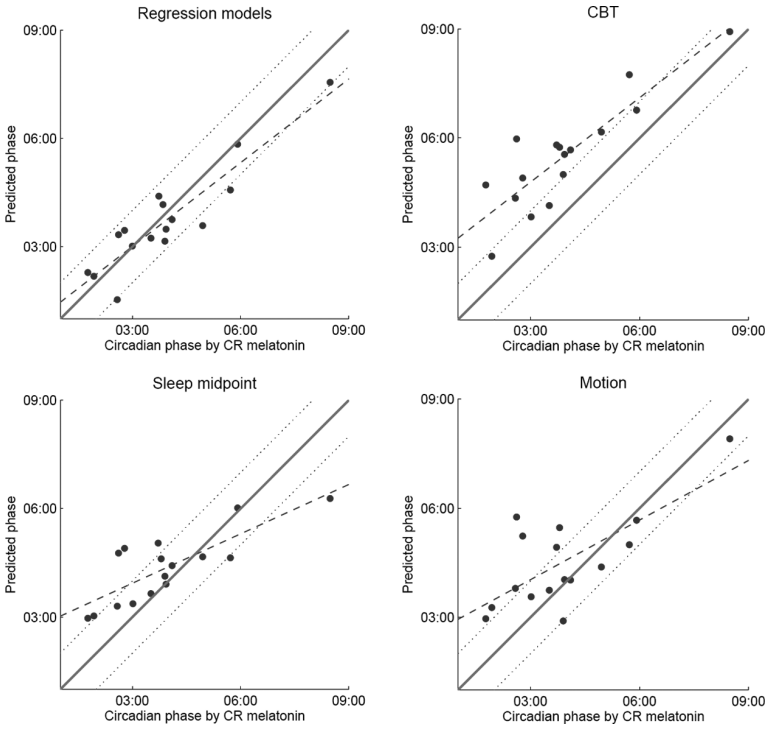

Figure 7. Prediction of ambulatory circadian phase for 16 subjects by 4 different methods versus the circadian phase by melatonin in the laboratory under CR. Thick solid line represents the identity line of ideal prediction with zero error, dashed line is the best fit line, and dotted lines are errors ±1 h.

In a practical application of our method for prediction of circadian phase, the equipment would include only tiny skin temperature sensors (iButtons), a 3-dimensional accelerometer worn on a belt at the waist to minimize the influence of artifacts like, for example, hand movements for wrist-worn actimeters, and a light sensor for the blue spectral band. The accelerometer and the light sensor can be further miniaturized compared to the multi-channel versions that were used in our validation study because most of the channels in the multi-channel devices proved to be redundant, including those that required high sampling rates (ECG and respiration) and most power consumption. The prediction model could be predefined in advance such that the regression coefficients would be fixed and no CR measurements, not even melatonin assays, would be required. This noninvasive measuring equipment and the proposed prediction method hold promise in sleep medicine or psychiatric illness or other clinical domains where knowing the exact endogenous circadian phase is important

for accurate treatment timing, for example, with light or melatonin, and where more invasive measurements and/or undergoing laboratory investigation are not possible.

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NOTE

Supplementary material for this article is available on the journal's Web site: http://jbr.sagepub.com/supplemental.

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