Endogenous and exogenous components in the circadian variation of core body temperature in humans

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SUMMARY
Core body temperature is predominantly modulated by endogenous and exogenous components. In the present study we tested whether these two components can be reliably assessed in a protocol which lasts for only 120 h. In this so-called forced desynchrony protocol, 12 healthy male subjects (age 23.7 ± 1.4 y) were subjected one by one to an artificial light/dark cycle of 20 h (10 lux vs. darkness). Core body temperature was measured continuously. The temperature data were analysed by an iterative method based on the assumption that the endogenous and exogenous components contribute to body temperature in an additive way. The results show that the average temperature curve is an almost perfect addition of the two components. The endogenous component differs from a sinusoid, and the relative contributions of the endogenous and exogenous components to the raw temperature curves differ substantially between the subjects. The average amount of unexplained variance in the individual data was 17%. Averaging of the body temperature curves over subjects reduced the unexplained variance to only 2%. This reduction in unexplained variance upon averaging over subjects must be due to the fact that most of the variance is either differently dependent on circadian phase for the various subjects or not dependent on circadian phase at all. The circadian pacemaker component revealed an average value of tau of 24.30 ± 0.36 h, which is consistent with recent findings in the literature. We conclude that a short forced desynchrony protocol is sufficient for the distinction between the masking and pacemaker components of core body temperature. The same protocol can be used to study the influence of these components on all kinds of other physiological and psychological signals.

KEYWORDS  circadian rhythm, core body temperature, forced desynchrony protocol, masking effect

INTRODUCTION
Core body temperature is predominantly modulated by the circadian pacemaker and by so-called masking activities (Wever 1979). The term masking refers to environmental influences as well as to factors within the body which modulate the overt rhythm (Minors and Waterhouse 1989). Masking activities, like sleep (Barrett et al. 1993), physical activity (Gander et al. 1986; Davenne and Lagarde 1995), postural changes (Minors and Waterhouse 1989), ambient temperature (Moran et al. 1995), meals (Dauncy and Bingham 1983), and the environmental light/dark cycle (Badia et al. 1991; Dijk et al. 1991) mask the characteristics of the endogenous temperature cycle. Under normal circumstances it is difficult to separate the two main contributions to the course of core body temperature because the period of the circadian pacemaker and the period of our daily pattern of activities are identical, and the phase relationship is virtually constant. The separation of the two components is also difficult in circumstances where the rest/activity cycle has a period different from that of the core body temperature rhythm, as occurs temporarily during shift-work and jet-lag. This is due to the fact that ‘masking’ consists of a variety of influences that all may vary under jet lag or shift-work conditions. In order to investigate the effects of a manipulation on the circadian pacemaker it is therefore necessary...
necessory to experimentally control or mathematically correct for the amount of masking. Several methods have been designed to this end.

A popular experimental way is the constant routine procedure, first proposed by Mills et al. (1978), in which the masking component is kept as constant as possible. This is carried out by keeping all conditions known to influence the circadian variable under consideration as constant as possible. It is assumed that the remaining circadian variation of all investigated variables is due to the circadian pacemaker. However, the increasing sleep deprivation that is inherent to the constant routine procedure may modify circadian patterns and put restrictions on the interpretation of the results (Mills et al. 1978; Czeisler et al. 1985; Minors et al. 1996).

Another study design used to quantify circadian pacemaker effects is to compare core body temperature data collected during continuous bedrest with data collected during continuous activity and sleep deprivation (Weyer 1985). On the basis of such observations Weyer concluded that about half the amplitude of the observed temperature waveform may be attributable to masking by ‘the rest/activity cycle’. Weyer also noticed that the masking effect depends on the phase of the core body temperature rhythm. According to this author, the masking effect was larger than average around the temperature maximum and during the descending phase of the circadian core body temperature cycle. However, the interpretation of such data is difficult because the activities responsible for the masking effects were spontaneous activities. It is conceivable, perhaps even likely, that circadian phase has an impact on spontaneous activity. Consequently, it is possible that the dependency of masking on circadian phase is the result of circadian variation in activity.

A third method is the purification of masked data. This method was used originally to correct temperature data for the effects of sleep (Folkard 1989). Since then the method has been used to correct for different types of activity (Minors and Waterhouse 1993). The analysis makes use of an activity record to correct each value of body temperature. Several assumptions are made to this end. It is assumed that the masking effect of sleep and masking component is kept as constant as possible. This is carried out by keeping all conditions known to influence the core body temperature rhythm. According to this author, the masking effect was larger than average around the temperature maximum and during the descending phase of the circadian core body temperature cycle. However, the interpretation of such data is difficult because the activities responsible for the masking effects were spontaneous activities. It is conceivable, perhaps even likely, that circadian phase has an impact on spontaneous activity. Consequently, it is possible that the dependency of masking on circadian phase is the result of circadian variation in activity.

A fourth alternative method to sort out masking and pacemaker effects is to experimentally force the pacemaker and the sleep/wake cycle to desynchronize (Kleitman and Kleitman 1953) and then mathematically disentangle the two components. This desynchronization between the core body temperature cycle and the sleep/wake cycle is reached by forcing individuals to live on a sleep/wake schedule which is out of the range of entrainment of the circadian pacemaker. Under circumstances of low light intensity the circadian pacemaker will free-run with a period close to 24 h (Klerman et al. 1996). Under those circumstances forced periods of 20 or 28 h are very suitable. Recently, this technique of forced desynchronization has been shown by Czeisler’s group, in Boston, to provide excellent possibilities to distinguish between the two components (Dijk et al. 1992; Johnson et al. 1992; Dijk and Czeisler 1995). However, the protocol of these authors

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Figure 1. Forced desynchrony protocol using an imposed period of 20 h. The first 4 d are baseline days at home. The interval for sleep was scheduled from 24.00 until 08.00 hours. Subsequently each subject spent 6 d in an isolation unit. After one baseline night subjects were scheduled to the 20 h d. The free-running circadian pacemaker is indicated in the dotted line.
is very laborious. The sleep/wake cycle is 28 h and the forced desynchronization is continued for four beat periods of 7 days each. Thus the total duration of the experiment is 4 weeks. In the present study we tested whether the two components can also be reliably obtained in a forced desynchrony protocol which lasts only 120 h, by using an imposed period of 20 h and restricting the study to approximately one beat period (Fig. 1). One beat period is the minimal interval required to study a full set of phase relationships between the two rhythmic processes. This set of phase relationships, in turn, is required to enable the separation of the contributing components.

METHODS

Subjects

Twelve healthy male subjects (age 23.7 ± 1.4 y, range 21–25) participated in this study. The subjects responded to an advertisement in the local newspaper. Subjects were selected on the basis of the following criteria. Subjects had to be male, aged between 20 and 25 y and in good physical health [screened by a physician (A.E.H.)]. They had to report regular sleeping habits with bedtime near midnight (mean 23.45, range 23.00–01.00 hours) and wake up time near 08.00 hours (mean 7:45, range 07.00–09.00 hours) and with an average sleep duration of 8 h (range 7–9 h). Exclusion criteria were smokers, the use of medication in the month prior to the experiment, chronic disease, a history of psychiatric treatment and reported sleep disturbances of any kind.

In order to determine psychopathology and depression the Beck Depression Inventory (Beck et al. 1961; Beck et al. 1979; Bouman et al. 1985) and the General Health Questionnaire (Koeter et al. 1987; Goldberg and Williams 1988) were used. A BDI rating over 8 or a GHQ rating over 2 lead to exclusion of the subject. Morning and evening types were determined with the Morningness/Eveningness Questionnaire, M/A (Kerkhof et al. 1985) and excluded (M/A<30 or M/A>70).

Every subject gave informed consent. No subject reported psychological difficulties during or after the experiment.

Study design

Before admission to the laboratory the subjects spent 4 baseline days at home.

During these days they were not allowed to perform physical exercise or to drink alcohol. They were allowed to go outside. The interval for sleep was scheduled from 24.00 until 08.00 hours, which corresponds to their habitual sleep times. The subjects wore an actometer on their non-dominant arm to verify the compliance with the requested sleep schedule.

Subsequently each subject spent almost 6 d and nights in an isolation unit. This is a sound and light shielded apartment, in which no information on time of day is available. Subjects lived under an artificial light/dark schedule with a period of 20 h, 13.5 h of dim light (<10 lux) during which they had to stay awake and 6.5 h of darkness, during which they had to be in bed. By reducing the available time for sleep to somewhat less than 33% we tried to increase sleep efficiency to the level that all nights would have little intermediate waking, but without generating a cumulative sleep deprivation effect. A similar sleep efficiency in all nights improves the quality of the deconvolution procedure to be explained below. All daily activities like taking a shower, meals and performing tests were scheduled in such a way that all subjective days had exactly the same temporal structure. In their spare time subjects were allowed to study, read or to watch video. The subjects were allowed to consume caffeine during their subjective morning (till 4 h after waking up, with a maximum of 4 cups). For each subject the number of cups was held constant per subjective day during the experiment. During the experiment the staff members entered the room in order to tell the subject to wake up, to eat, to take a shower or to perform some tests. These were brief contacts. Before the subject had to go to bed a staff member entered the room to attach the electrodes in order to record the electroencephalogram. Staff members were trained to give no information about the time of day or the nature of the experimental manipulation. The subjects had no knowledge of time.

Throughout the experiment, core body temperature was measured continuously by a rectal thermometer and the data were stored at 1-min intervals. Due to technical failures and personal hygiene of the subjects, 13% of the temperature data were missing. Intervals of missing data shorter in duration than 1 h and 30 min were interpolated linearly. Intervals longer in duration were treated as missing data. Every 2 h during wakefulness subjects performed a battery of tests and filled out questionnaires. These data will be presented elsewhere (A.E. Hiddinga, D.G.M. Beersma and R.H. van den Hoofdakker, in preparation).

RESULTS

The analysis applied in this study is based on one main assumption (Folkard and Åkerstedt 1987), i.e. that the endogenous and exogenous components contribute to body temperature in an additive way. Figure 2(a) shows the average core course of body temperature of the 12 healthy male subjects over the period of 120 h, 5 ‘real’ 24-h days and 6 ‘subjective’ 20-h days. The obvious modulation of amplitude in the figure is due to the interference between the imposed 20-h oscillation and the circadian oscillation of the pacemaker. In order to quantify the separate contributions of these two oscillations to the average course of raw data (Fig. 2a) are folded at the imposed period of 20 h and the average time course of body temperature in the 20-h day is calculated (Fig. 2b). This step in the procedure exploits the fact that all subjective days have the same temporal structure, and that the ‘masking’ contributions to core body temperature simultaneously scan through all circadian phases. The circadian contribution therefore is largely averaged out in the folding procedure, while the masking profile remains.

For each 20-h interval the resulting average curve was subtracted from the raw data. The result of this subtraction
Variation of body temperature in humans

Figure 2. (a) The average course of core body temperature of the 12 healthy male subjects over a period of 120 h. The modulation of amplitude over time is due to the interference between the 20-h d and the endogenous oscillation of the pacemaker. (b) The time course of core body temperature in the 20-h day, averaged over the 6 subjective d and nights, replotted six times. (c) The residual variation, resulting after subtracting Fig. 2 (b) from Fig. 2 (a). (d) The residual variation, resulting after subtracting the results of Fig. 3 (a) and Fig. 3 (b) from Fig. 2 (a).

was a roughly sinusoidal curve with almost five oscillations over the 120 h of the recording (Fig. 2c). The period, tau, of this oscillation was estimated by autocorrelation. The data from Fig. 2(c) were folded at the period tau and averaged. The averages represent a first estimation of the endogenous component of core body temperature. The resulting curve is similar to Fig. 3(b) and differs from a precise sine function. Its period differs from 24 h. As a consequence the presumption that the clock component would average out in the first step of the procedure is not completely justified. Having some knowledge on the clock component, it is possible to improve on the estimates of the two components. For that purpose, the estimate of the clock component is subtracted from the original raw data, enabling a better estimation of the masking component of body temperature in the remaining data. These data in turn were subtracted from the original raw data, etc.

The procedure was repeated until no further change in the fits was obtained. Figures 3(a,b) present these stable results. They are the best estimates of the variations in body temperature that occur in every 20-h day and every circadian day, respectively. In other words Fig. 3(a) shows a doubleplot of the effects on body temperature of the imposed schedule; Fig. 3(b) presents a doubleplot of the dominant rhythm in the residual temperature data, which turned out to have a period of 24.28 h. As this period is much longer than the imposed 20 h sleep/wake schedule, the protocol was successful in desynchronizing the sleep/wake cycle and the endogenous circadian oscillation. The curve in Fig. 3(b) is interpreted as the reflection of the effects of the circadian pacemaker. When the best estimates of both the effects of the exogenous and the endogenous component are subtracted from the raw data the residual variation is left (Fig. 2d).

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Regardless of the face value of the results presented in Figs 3(a,b), it is important to verify the resolving power of the technique. For that purpose we simulated temperature curves by the addition of various types of smooth circadian components and square wave masking components, and applied the deconvolution method to retrieve the original components. It turned out that the resulting curves very closely approached the components used for the addition.

All individual data were analysed according to the explained procedure. The average period of the biological clock of all subjects was 24.30 ± 0.36 h (Table 1). Two of the 12 subjects had a period of the biological clock shorter than 24 h.

The data were z-transformed for each individual in order to study the similarities in shape of each component. For each individual curve of the biological clock component a reference phase was assessed. The midpoint of the interval during which the core body temperature was below the mean value was used as the reference phase. Then the data of all individuals were averaged for each circadian phase and corresponding standard deviations were calculated (Fig. 4a). The same procedure was repeated for the data on the effects of the sleep/wake component (Fig. 4b). Here, time since lights-on served as the reference time. Obviously the individuals show very similar patterns of masking and very similarly shaped circadian pacemaker contributions. Finally, the variance over the entire recording was calculated of the masking component, the biological clock component, and of the residual data.

Table 1 shows the absolute amounts of variance. The average amount of variance explained in the individual body temperature data by masking is 0.069 (56%), and by the circadian pacemaker 0.034 (31%). The standard deviation of the amount of variance explained by masking is 49% of the average value, and the standard deviation of the amount of variance explained by the clock component is 32% of the average value. Apparently, while the shape of the curves is very similar for all individuals (Fig. 4a,b) the amplitudes differ substantially between subjects. The amount of unexplained variance is 0.018 (17%). The amount of variance explained by
Table 1  Individual values of tau, the total amount of variance and the amount of variance explained by the masking and the clock component, and the residual unexplained variance. Mean subj. represents the average temporal course calculated over 12 subjects. This average signal was analysed in the same way as the data from the individual subjects.

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Tau in h</th>
<th>Total variance</th>
<th>Masking variance</th>
<th>Clock variance</th>
<th>Residual variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>9402</td>
<td>23.63</td>
<td>0.188</td>
<td>0.133</td>
<td>0.039</td>
<td>0.018</td>
</tr>
<tr>
<td>9403</td>
<td>24.38</td>
<td>0.123</td>
<td>0.053</td>
<td>0.060</td>
<td>0.013</td>
</tr>
<tr>
<td>9404</td>
<td>24.78</td>
<td>0.071</td>
<td>0.031</td>
<td>0.031</td>
<td>0.015</td>
</tr>
<tr>
<td>9405</td>
<td>24.52</td>
<td>0.104</td>
<td>0.042</td>
<td>0.044</td>
<td>0.021</td>
</tr>
<tr>
<td>9406</td>
<td>24.25</td>
<td>0.058</td>
<td>0.031</td>
<td>0.020</td>
<td>0.011</td>
</tr>
<tr>
<td>9407</td>
<td>24.45</td>
<td>0.117</td>
<td>0.073</td>
<td>0.029</td>
<td>0.021</td>
</tr>
<tr>
<td>9408</td>
<td>24.18</td>
<td>0.181</td>
<td>0.136</td>
<td>0.031</td>
<td>0.021</td>
</tr>
<tr>
<td>9409</td>
<td>24.00</td>
<td>0.144</td>
<td>0.087</td>
<td>0.044</td>
<td>0.015</td>
</tr>
<tr>
<td>9410</td>
<td>23.65</td>
<td>0.129</td>
<td>0.082</td>
<td>0.030</td>
<td>0.023</td>
</tr>
<tr>
<td>9411</td>
<td>24.67</td>
<td>0.099</td>
<td>0.048</td>
<td>0.034</td>
<td>0.021</td>
</tr>
<tr>
<td>9412</td>
<td>24.42</td>
<td>0.101</td>
<td>0.065</td>
<td>0.018</td>
<td>0.021</td>
</tr>
<tr>
<td>9413</td>
<td>24.72</td>
<td>0.096</td>
<td>0.050</td>
<td>0.032</td>
<td>0.018</td>
</tr>
<tr>
<td>mean</td>
<td>24.30 ± 0.36</td>
<td>0.118 ± 0.037</td>
<td>0.069 ± 0.034</td>
<td>0.034 ± 0.11</td>
<td>0.018 ± 0.004</td>
</tr>
<tr>
<td>mean subj.</td>
<td>24.28</td>
<td>0.088</td>
<td>0.058</td>
<td>0.029</td>
<td>0.002</td>
</tr>
</tbody>
</table>

masking, clock and residual data in all individuals is slightly more than 100%. Simulations demonstrated that this is due to the presence of intervals of missing data.

As was shown on the basis of Fig. 2, the circadian pacemaker component of the average core body temperature curve of the 12 subjects has a period of 1457 min, or 24 h and 17 min. The amount of variance explained by the masking component is 0.088 (66%) and by the biological clock is 0.058 (33%). The amount of unexplained variance is only 0.002 (2%).

Apparently the unexplained variance within each individual subject is almost entirely independent from the unexplained variance within the other subjects. In the case of total independency one would expect that averaging over subjects would reduce the amount of variance by a factor \(\sqrt{N} = \sqrt{12} = 3.5\). The 17% average value within the individual subjects would then reduce to about 5%. The observed 2% seems to suggest such an intersubject independency. However, the averaging over the subjects might actually imply averaging out some of the circadian variance due to differences in phase between the subjects. The phase differences between the subjects changed only slightly during the experiment. The standard deviation of the times at which the endogenous component reached its minimum was 1.18 h (mean: 04.52 hours). During the experiment the standard deviation increased to 1.25 h (mean: 06.23 hours). With this narrow range of phase angles, averaging over subjects can not substantially lead to averaging out the circadian contributions.

DISCUSSION

The purpose of this study was to test whether the effects of the endogenous circadian and exogenous masking component on the temperature rhythm can be reliably distinguished in a forced desynchrony protocol which lasts for only 120 h, while applying an imposed period of 20 h.

The results show that the separation of these components was successful. The average period tau was found to be 24.30 ± 0.36 h which is much longer than the imposed 20 h sleep/wake schedule. Tau was shorter than observed in previous experiments with a self-selected L/D cycle (24.9 ± 0.2 h; Wever 1985). However, the shorter period is consistent with recent findings. Czeisler et al. (1995) and Klerman et al. (1996) reported shorter tau values (24.1–24.2 h) for the endogenous circadian pacemaker under dim light conditions (<15 lux), in a forced desynchrony protocol. Klein et al. (1993) reported shorter tau values (range 24.22–24.27 h) in a blind subject who lived on a regular 24-h schedule. Middleton et al. (1996) reported also shorter tau values (24.26 ± 0.049 h) in a constant dim light study (8 lux). Klerman et al. (1996) concluded that classical free-run studies in humans who were allowed to select their own L/D schedules probably resulted in an observed period longer than the intrinsic period. They simulated the influence of the imposed period on the observed period during a forced desynchrony protocol based on theoretical considerations of pacemaker sensitivity to light. Under low light conditions (<10 lux) this influence is very small. Especially when the period of the L/D-cycle differs ± 4 h from the intrinsic period. The simulated observed period was found to be within 0.2% of the intrinsic period.

The shape of the endogenous component of core body temperature in our experiment is an approximation to a cosine curve with a wider maximum than a minimum. The shape is very similar to the shape of the endogenous component found by Johnson et al. (1992), Dijk et al. (1992), Dijk and Czeisler (1994, 1995) and Boivin et al. (1997). Apparently the period of the imposed sleep/wake schedule (20 or 28 h) and the length of the experiment (5 d or 28 d) have limited influence on the shape of the endogenous component. The results indicate that the endogenous circadian rhythm is not sinusoidal. This will have implications on the purification method proposed by Minors and Waterhouse, because one of the assumptions made in that method is that the shape of the endogenous circadian...
changes in masking. In an additive approach possible phase dependent changes simply contribute to the amount of unexplained variance. Given the average of 17% unexplained variance, phase dependency of masking could in principle be substantial. However, averaging of the body temperature curves over subjects reduces the unexplained variance to only 2%. This demonstrates that the average curve is an almost perfect addition of the effects of a 20 h component and a 24.3 h component. Apparently the unexplained variance in one subject is independent from the unexplained variance in another subject. At the start of each experiment the subjects do not show a wide range of circadian phase angles. In spite of the differences in tau (Table 1) the range of circadian phase angles is still not very large at the end of the 6 d in isolation. So the reduction in unexplained variance through averaging over subjects is likely to be due to the fact that most of the variance differs from subject to subject. This strongly suggests that the unexplained variance is independent of circadian phase.

The small amount in residual variation is also a strong argument in favour of the conclusion that the circadian component is adequately determined, even although circadian phase and shape are determined simultaneously. Further evidence in favour of this conclusion stems from the experiment by Dijk et al. (1995) showing that synchrony exists between the thus determined circadian body temperature component and the independently determined circadian cycle derived from constant routine measurements performed prior to and subsequently after the forced desynchrony experiment.

Given the relatively small standard deviations in Fig. 4(a,b) it can be concluded that the shape of each component is rather similar for all individuals. Nevertheless, the amount of variance explained by these components varies considerably over subjects (Table 1). Apparently the amplitude of the components is strongly dependent on the subject. This finding is at variance with assumptions of similarity that are being made in purification methods applied to estimate the circadian pacemaker component of core body temperature (Minors and Waterhouse 1992; Minors et al. 1996).

Wever concluded on the basis of his experiment (1985) that the masking effect depends on the phase of the core body temperature rhythm. The masking effect was found to be the smallest during the ascending phase of the pacemaker component of the core body temperature cycle. However, the activities that are responsible for the masking effects were spontaneous activities. Therefore it is conceivable that circadian phase has an impact on spontaneous activity and that the observed circadian phase dependency of masking is simply due to this circadian variation in activity. Minors and Waterhouse (1989) found inconsistent results in an experiment considering postural changes throughout the 24 h. Dijk and Czeisler (1995) analysed temperature data derived from a forced desynchrony protocol with a sleep/wake cycle of 28 h. They concluded that during sleep the circadian waveform of core body temperature changed as a function of time since the start of the sleep episode. However, the interaction between circadian phase and the time since the start of the sleep episode was not significant.

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Figure 4. (a) The z-transformed temperature data on the effects of the biological clock of all individuals were averaged and plotted together with the curves which deviate one standard deviation from the mean. (b) The z-transformed temperature data on the effects of the sleep-wake component of all individuals were averaged and plotted together with the curves which deviate one standard deviation from the mean.

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rhythm is sinusoidal (Minors and Waterhouse 1992; Minors et al. 1996).

The method of analysis used in this study was based on the assumption that the endogenous and exogenous components contribute to body temperature in an additive way. Obviously, such an approach is not suited to discovering phase dependent
So during sleep, when there is no spontaneous activity, the interaction between the two components is not significant. This finding supports the idea that circadian phase has an impact on spontaneous activity. In our study spontaneous activity was minimized, due to the strictly scheduled protocol. This could explain the minimal amount of variance in the residual data. In conclusion we are convinced that the method of forced desynchronization is capable of distinguishing between the effects of masking and those of the pacemaker on the body temperature signal. The same protocol enables the study of the influence of the pacemaker on all kinds of other physiological and psychological variables, including alertness and mood (Dijk et al. 1992; Johnson et al. 1992; Boivin et al. 1997). These latter variables are the topic of present investigations.

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