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Internal interactions within the human circadian system: the masking effect

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Summary. In the realm of human circadian rhythms, the masking effect is defined as the change in the course of deep body temperature induced by changes in the degree of physical activity, or by the alteration between sleep and wake. This effect is particularly obvious during internal desynchronization where the rhythms of deep body temperature, and the sleep-wake sleep cycle – i.e. one of the masking factors – run with different periods.

Every sleep onset is accompanied by a rapid drop, and wake onset by a rapid rise in deep body temperature, each one with an overshoot of about 50% of the steady state variations. When rhythms are calculated, with the dominant temperature period as the screening period, exclusively from data obtained during sleep episodes, on the one hand, and from those obtained exclusively during wake, on the other, two average cycles emerge: the *'sleep temperature curve'* and the *'wake temperature curve'*. Both run in parallel but are separated by the *'masking effect'*. As derived from many experiments, the mean masking effect amounts to 0.28 ± 0.06°C. The masking effect also depends to some extent on the phase of the temperature rhythm; it is larger than average around the temperature maximum and during the descending phase of the temperature cycle, where the alertness commonly is highest and the probability to sleep, in general, and the REM sleep propensity, in particular, are smaller than average. This also can be interpreted to indicate that the sleep temperature curve is phase advanced relative to the wake temperature curve; this, on the average, by 0.9 ± 0.3 h.

If the individually determined amount of masking is added to the temperature data obtained during sleep, or substracted from the temperature data obtained during wake, a temperature curve emerges that can be thought of as being 'purified' of the masking effect. Analyses of this artificial curve allow estimation of that part of the internal interactions uninfluenced by the masking effect. On the average, about half of the amount of interaction between the rhythm of sleep-wake and that of deep body temperature is explained by the masking effect, whereas the other half is *'oscillatory interaction'*. Both types of interaction are inherent and inseparable parts of the circadian clock mechanism, as can be deduced from model considerations.

Key words. Circadian rhythms; sleep temperature curve; wake temperature curve; masking effect.

Introduction

Originally, the 'masking effect' (ME) has been defined as the change of a biological variable in response to a change in the relevant environmental conditions. It was first discovered to be manifested by the change in locomotor activity induced by a change in an environmental condition, such as intensity of illumination or ambient temperature[1]. For instance, if a light-active bird was exposed to light at a time of the day when it usually was dark and when the bird was resting, it was bound to respond with a burst of locomotor activity ('positive masking'). In turn, when darkness was made to set in earlier than usually and earlier than the bird would go to rest, it would terminate its locomotor activity immediately after light-off ('negative masking'). The masking-induced changes in locomotor activity appear to be superimposed on the usual changes of the circadian variations of activity. Consequently, the masking effect is most obvious under conditions where the environmental stimuli vary in a fashion which deviates from the normal schedule. This is the case, for instance, under the influence of artificial zeitgebers with periods considerably deviating from 24 h, or, though only temporarily, immediately after phase shifts of the zeitgeber. The masking effect does not apply only to locomotor activity but to a variety of other biological variables as well, such as, e.g. deep body temperature. If the concept of ME initially was applied to locomotor activity only, this was due to the fact that at that time locomotor activity was virtually the only variable measured in those early experiments.

In the science of human circadian rhythms, 'masking' primarily means the effect of the sleep-wake alternation, and/or the direct influence of physical activity, on deep body temperature and other variables. Bodily activity is bound to elevate deep body temperature; in turn, sleep, or plainly rest, leads to a drop of deep body temperature, independent of and superimposed on the circadian rise and decline of this variable. In the (normal) 24-h day, the masking effect becomes manifest if one compares the circadian variations of deep body temperature during a normal activity-sleep cycle, on the one hand, and during sleep deprivation with continuous activity or during continuous bedrest with an alternation between rest and sleep, on the other (fig. 1). When a subject is active at nighttime where he normally would be asleep, his rectal temperature is elevated during the night in comparison to its normal course, while it is normal during daytime; in turn, when a subject continuously stays in bed and rests, his temperature shows the normal course during the night but is reduced during day. Consequently, as depicted in figure 1, the rhythm in rectal temperature shows the largest amplitudes in the normal activity-sleep condition where circadian variations and the masking effect amplify each other, but reduced amplitudes during sleep deprivation as well as during continuous bedrest where the masking effect is eliminated.

Figure 1 shows, the masking effect, as derived from sleep-wake alternation on deep body temperature, can be of similar magnitude as the normal circadian variation of deep body temperature. Consequently, the masking effect can either completely obscure, or drastically enhance, the circadian rhythmicity of deep body temperature, depending on the temporal relation – i.e. the phase relationship – between circadian variations and masking effect. In some special cases, the masking effect can simulate an alteration in the phase relationship of the temperature rhythm relative to other rhythms or an external zeitgeber. Such derangements of hypothetically 'pure' circadian rhythms cannot be expected in healthy subjects living under normal conditions. In turn, such rhythm disorders can be expected in subjects following unusual activity-rest routines (e.g. in shift workers) or in patients suffering from a variety of diseases (e.g. sleep disorders). They are the rule, however, in subjects living under very artificial conditions, e.g. internal desynchronization under temporal isolation. The elucidation of the temporal and quantitative influence of the masking effect is a precondition for the proper understanding of human circadian rhythmicity, both, in healthy subjects living under the unusual conditions as just mentioned and in patients with rhythm disorders. In the following, we shall present and discuss some analyses that aim at the understanding of the masking effect; these analyses will be based on some special experiments performed with healthy subjects under temporal isolation. The analyses were performed on data obtained in long-term experiments that have been run in a special underground station. Details of this station and the performance of human experiments under temporal isolation have been described earlier[4].

Quantitative analyses of the masking effect on deep body temperature induced by sleep-wake alternations are hard to perform in internally synchronized rhythms, as in these rhythms the circadian variations and the masking-induced variations of temperature run more or less in phase and, hence, cannot be kept apart without any special hypotheses. Thus, investigations of the masking effect are better based on states where the two types of variation run, at least temporarily, with different periods, so that they vary their phase relationship from cycle to cycle[4]. Such investigations, therefore, should usually be performed under internal dissociation and, in particular, in experiments with internally desynchronized rhythms[7]. The state of internal desynchronization can occur spontaneously under constant conditions; or else, this state can be enforced under the influence of a strong zeitgeber with a period distinctly deviating from the freerunning period[4]. In the following, both types of internal desynchronization will be employed.

Rhythms under constant conditions

Quantitative separations of the circadian variations and of the masking effect in internally desynchronized rhythms are possible in two different ways that complement each other, i.e. with reference to the sleep-wake cycle and with reference to the temperature cycle. In the following, both these ways will be illustrated with a representative experiment as the base. In this experiment, a subject lived under constant conditions for 4 weeks; he showed internal desynchronization during the entire course of the experiment, with an overt period of rectal temperature variations of 25.0 h and a sleep-wake periodicity of 30.2 h. The first way to demonstrate special aspects of the masking effect is illustrated in figure 2. This figure shows the average course of rectal temperature to be synchronized during all subsequent cycles, to the instants of sleep onset and wake onset respectively; these instants have been determined from polygraphic sleep recording (EEG, EOG, EMG). The courses during the several hours before and after these instants (in absolute time) were averaged (means ± SD). Since rectal temperature constitutes only a residual component within the mean period of the sleep-wake rhythm (see below), there is no marked temperature rhythm. Rather, there are steep drops of body temperature accompanying sleep onset and steep rises accompanying wake onset; in both cases, there are clear overshoots in temperature up to about 50% of the steady state alteration. The remaining parts of the temperature course are uncharacteristic. The diagram of figure 2 then makes it rather obvious that the apparent rhythm of body temperature running with the period of the sleep-

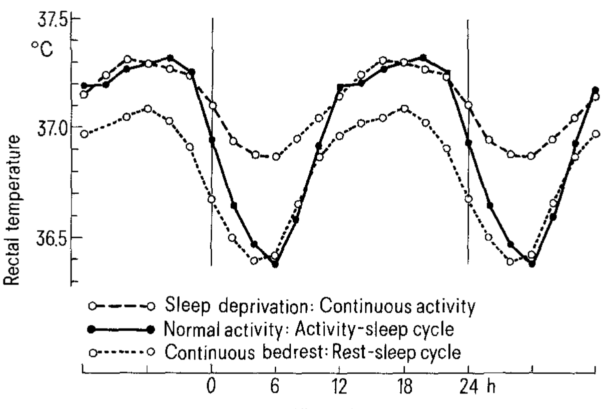

Figure 1. Average courses of rectal temperature in healthy subjects (averaged from many different studies) in the normal living routine (i.e. with alternation between activity and sleep), during sleep deprivation (i.e. with continuous activity), and during continuous bedrest (i.e. with alternation between rest and sleep).

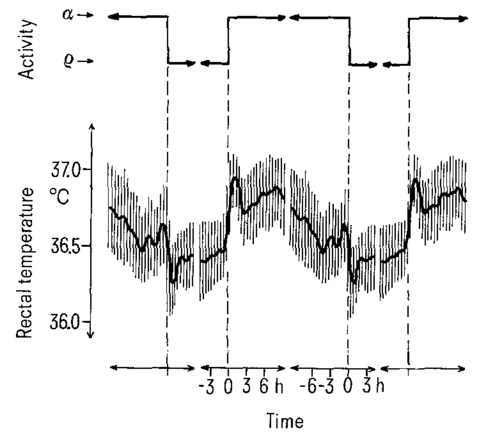

Figure 2. Educed cycles of sleep-wake and rectal temperature (with SD), originating from a 28-day experiment under constant conditions, with a subject showing internal desynchronization. The data are synchronized to the instants of sleep onset and wake onset, with several hours before and after these instants each.

wake rhythm is generated predominantly by the masking effects including the overshoots.

The other way used to demonstrate aspects of the masking effect is illustrated in figure 3; it shows, using data from the same experiment, educed cycles of the rectal temperature rhythm with the dominant period of this rhythm, calculated separately from temperature data obtained exclusively during sleep episodes (lower trace) and from those obtained exclusively during wake episodes (upper trace). Since, in the state of internal desynchronization, the rhythms of sleep-wake and temperature proceed with different overt periods, the temperature data obtained during successive sleep episodes, or successive wake episodes respectively, cover different overlapping sections of the mean sleep or wake temperature cycles. Figure 3 shows that the educed 'sleep temperature cycle' and 'wake temperature cycle' run remarkably parallel, including individual peculiarities such as the split minima, or the maxima with three primary and one secondary peaks each. The difference between the two curves constitutes the masking effect; it is separately drawn in the lower diagram. In this special experiment its mean amounts to 0.36°C. However, as the diagram shows, the masking effect is not independent of the phase of the temperature rhythm; it is larger during the section with dropping temperature than during the section with rising temperature. This phase dependence can also be interpreted to indicate that the sleep temperature cycle is phase advanced relative to the wake temperature cycle by about 1 h. This latter interpretation is favored because all individual details of the temperature cycles can be reduced to a phase shift by very similar amounts. In summary, one can state that the amount of the masking effect depends not only on the phase of the sleep-wake cycle as demonstrated by the overshoots (cf. fig. 2) but also on the phase of the temperature cycle.

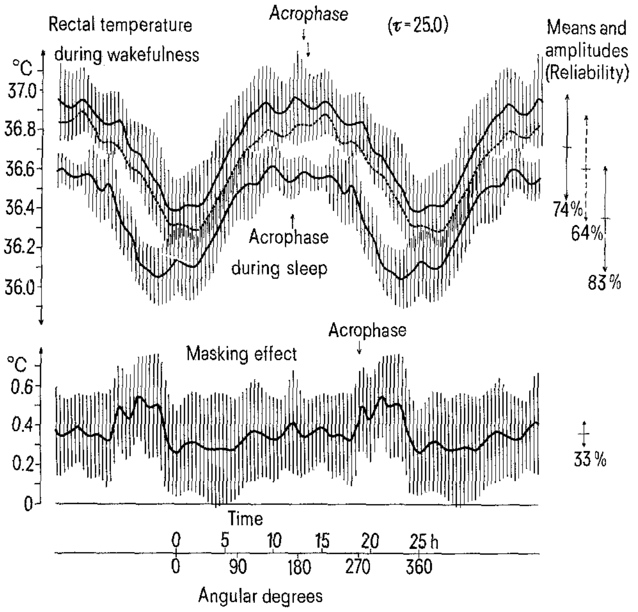

Figure 3. Educed cycles of rectal temperature (with SD), originating from the same experiment as in figure 2. The averaging procedure is calculated with the dominant period of the temperature rhythm as the scanning period, separately from temperature data obtained during wake episodes only (upper trace) and during sleep episodes only (lower trace). Dotted line: educed cycle calculated from all temperature data. Lower diagram: interval (with SD) between the calculated wake and sleep temperature cycles ('masking effect'). Acrophases, mean values and amplitudes, and levels of reliability are indicated for the three educed cycles and the masking effect.

Figure 3 also shows SD around the means of the sleep and wake cycles. These intraindividual variabilities had shown, as depicted in figure 2, that the overshoots are unambiguously relevant. In figure 3, there is virtually no overlap in the sleep and wake data; it must be kept in mind that these variabilities are enlarged by including the systematic overshoots. The significant separation of the educed sleep and wake temperature cycles becomes also obvious in the variabilities of the masking effect (fig. 3, lower diagram). Figure 3 also includes the common educed temperature curve as derived from all data (dotted line). The asymmetric position of this common cycle between the two separated cycles points towards the generally higher probability to hit on a wake episode than a sleep episode; and the change in position indicates that this probability is greater around the maximum and the descending section of the temperature cycle (where the masking effect is larger than average) than it is around the minimum and the ascending section of the temperature cycle (where the masking effect is smaller than average). This frequency distribution of sleep and wake episodes within the temperature cycle is in accordance with that found in previous evaluations[12]. Furthermore, this frequency distribution shows that the dependence of the masking effect on the phase of the temperature cycle cannot be simulated by the overshoots in the masking effect (cf. fig. 2).

The right part of figure 3 also depicts the parameters of the fundamental periods of the three educed cycles, from the sleep-, the common and the wake-temperature cycle. The mean is higher in the wake than in the sleep cycle by 0.36°C (as mentioned before); the mean of the common cycle is somewhat closer to that of the wake cycle than to that of the sleep cycle, whereas the amplitudes are nearly identical in all three cycles. The numbers indicate the levels of reliability of the rhythms[4]. It is obvious that those reliabilities of each one of the separated sleep and wake cycles are greater than that of the common cycle. The reason for this discrepancy can be seen in the fact that the masking effect which engenders a distinct increase in the variability, is included exclusively in the common cycle, whereas the separated cycles reflect rather the pure circadian component. Moreover, the reliability of the sleep cycle is greater than that of the wake cycle; this discrepancy again is due to the smaller variability of the sleep than the wake cycle, i.e. the greater 'homogeneity' of sleep as compared with wake with regard to the level of physical activity which influences deep body temperature via the masking effect.

After having established quantitative aspects of the masking effect, the influence of this factor on the course of rectal temperature can now be evaluated. For better consistency in the description, again the data from the same subject as shown in figures 2 and 3 are used. The middle trace of figure 4 depicts the originally recorded course of rectal temperature over the 4-week period of

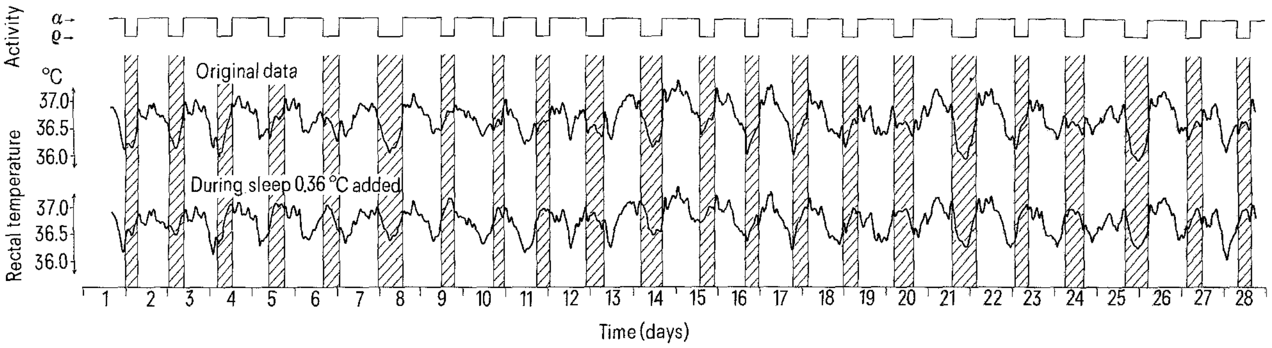

Figure 4. Circadian rhythms in an experiment under constant conditions with internal desynchronization (same experiment as in figs 2 and 3). Presented are (from top to bottom) the courses of sleep-wake, rectal temperature as originally recorded, and rectal tempertature with all data obtained during sleep elevated by 0.36 °C (mean amount of the masking effect; cf. fig. 3).

the experiment. The uppermost trace presents the sleep-wake alternation; in addition, sleep periods are indicated also by the shaded areas. It is obvious that the difference in the periods of the two rhythms leads to a beat phenomenon: The amplitude of the temperature rhythm varies systematically depending on the internal phase relationship between the two rhythms, sleep-wake and rectal temperature. The bottom trace of figure 4 shows again the temperature data as plotted in the middle trace, but with 0.36 °C added whenever the subject was asleep (as determined from the polygraphic sleep recording). This latter curve can now be interpreted as being the temperature curve 'purified' from the masking effect (the inclusion of the overshoots would not noticeably change the picture); i.e. the hypothetical course of rectal temperature during continuous sleep deprivation, or, when lowered by a constant amount of 0.36 °C, the hypothetical temperature course during continuous sleep. The obvious impression from this modified temperature course is its relative consistency; with regard to its amplitude it looks now more like a temperature record from an internally synchronized rather than from an internally desynchronized rhythm. This is in spite of the difference in the periods of the sleep-wake and temperature rhythms which is even more obvious after the 'purification' from the masking effect.

A detailed analysis of the three time series depicted in figure 4 is shown in figure 5, i.e. the analysis of the sleep-wake pattern, the original temperature data and the temperature data after elimination of the mean masking; it is based on a periodogram analysis[4]. The left-side diagram shows results from period analyses. Sleep-wake shows a dominant period at 30.2 h and a secondary period at 32.5 h, whereas a period of 25.0 h is only weakly marked. The analysis of the original temperature data shows a dominant period at 25.0 h and a secondary but still significant period at 30.2 h (i.e. coinciding with the dominant period of the sleep-wake rhythm). The analysis of the 'purified' temperature data shows again a dominant period at 25.0 h but now with considerably higher reliability, whereas the secondary period at 30.2 h is nearly abolished. Here, it must be added that the separate analyses of the temperature

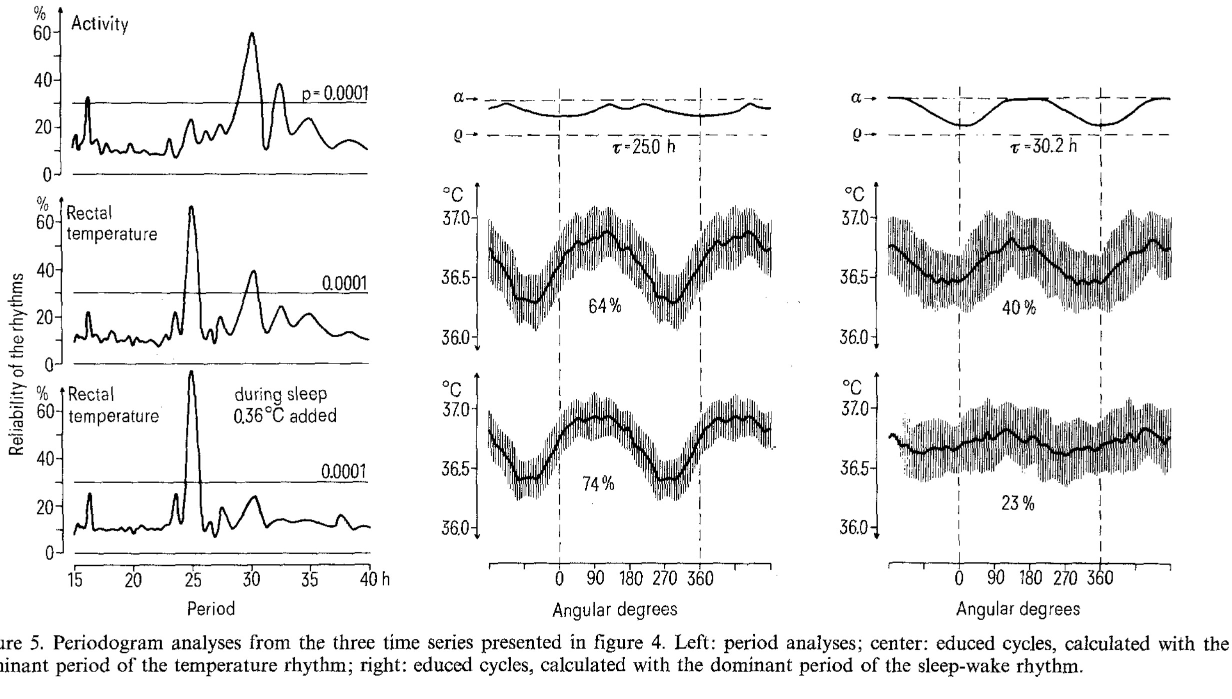

Figure 5. Periodogram analyses from the three time series presented in figure 4. Left: period analyses; center: educed cycles, calculated with the dominant period of the temperature rhythm; right: educed cycles, calculated with the dominant period of the sleep-wake rhythm.

data from sleep episodes only and those from wake episodes only (cf. fig. 3) reveal one dominant and highly reliable period at 25.0 h each. The other two diagrams show the educed wave shapes (with SD), calculated with the two separate periods. In the middle diagram the educed cycles are based on the period of 25.0 h which is the dominant period of the temperature rhythm. The sleep-wake cycle shows no relevant rhythmicity; the original temperature data show a reliable average cycle; and the 'purified' temperature data show an average cycle with clearly smaller variability than that of the original data, resulting in a distinctly higher reliability of the purified than that of the original rhythm. In the right diagram the educed cycles are based on the period of 30.2 h which is the dominant period of the sleep-wake rhythm. The sleep-wake cycle shows a highly reliable rhythmicity; the original temperature data likewise show a rhythmicity of fair reliability ($p < 10^{-6}$); the 'purified' data, however, show only random fluctuations; this indicates that the temperature period at 30.2 h as it is included in the original data, is predominantly generated by the masking effect.

A cursory inspection of the two time series of rectal temperature shown in figure 4 may suggest that the internal interaction with the sleep-wake rhythm is based only on the masking effect. However, this conclusion would be incomplete even in this special experiment. Figure 6 shows the temporal courses of the rhythm parameters, calculated from these two time series as the results of successive harmonic analysis in overlapping intervals. The courses of the mean values run parallel for both time series. The amplitude of the original time series (calculated with the dominant period of 25.0 h) shows regular fluctuations, originating from beat phenomena according to the difference in the periods of 25.0 h (rectal temperature) and 30.2 h (sleep-wake); the amplitude of the modified time series (calculated with the same period) shows only residual fluctuations indicating nearly temporally constant amplitudes. The acrophases show a markedly scalloping pattern in the original time series. On the average, the slope of the trace corresponds to a period of 25.0 h; this indicates that, on the average, the acrophase is 1.0 h later every day. However, individually considered, the delay in the acrophase is mostly distinctly greater than 1.0 h/day, and for compensation, after several days there is a phase advance each, and, characteristically, at such days the amplitude of the temperature rhythm is minimal. The acrophases of the modified, or 'purified' temperature rhythm show a much more regular course; but, the average slope is exactly the same as with the origial data. However, a scalloping pattern is still present, without interposed phase advances, but with the smallest phase delays at the same days as the phase advances in the original data. This clearly shows that a smaller part of the interaction between the rhythms of rectal temperature and sleep-wake is based on mechanisms different from that of the masking effect; in this special experiment, it amounts to about a third of the total interaction. This remaining interaction constitutes the 'oscillatory interaction'[7] which leads to the phenomenon of 'internal relative coordination'[3].

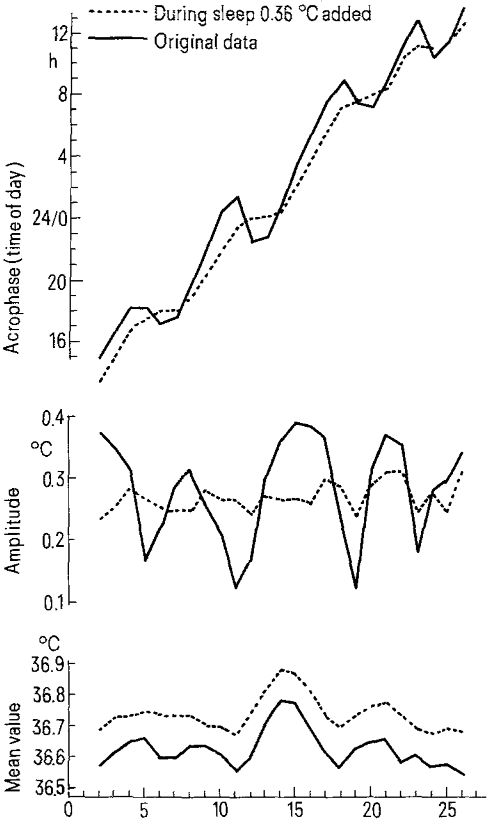

Figure 6. Course of three rhythm parameters of the two temperature series shown in figure 4, as the result of consecutive overlapping harmonic analyses.

The considerations dealt with sofar are based on the establishment of a mean masking effect, determined as the mean interval between the separated sleep and wake temperature cycles (cf. fig. 3). It could be argued, however, that such a definition is arbitrary and is based on a special hypothesis about masking. Consequently, it would be hasty to draw further conclusions from such an estimate, like that concerning the participation of different mechanisms in the overall interaction between the rhythms of sleep-wake and deep body temperature. To test this argument, the procedure of adding a fixed amount of temperature to the sleep temperature data, in order to 'purifiy' the time series from masking (cf. fig. 4), has been repeated many times with increasing amounts of temperature, up to a reasonable limit; from each of the artificially modified time series, numerous analyses have been performed corresponding to those applied in the original times series. The endpoints of these analyses are plotted in figure 7, as functions of the temperature increment during sleep; for comparison, the same procedure has been applied to temperature data from a typical experiment with internally synchronized rhythms (dotted lines in fig. 7).

The first analysis concerns the total time series regardless of any period estimation. It shows that the SD of all data around the overall mean decreases initially with increasing temperature increment during sleep in both rhythm types (fig. 7, left diagram). In the internally desynchronized rhythm, the variability reaches a minimum when 0.36 °C is added to the sleep temperature data, i.e. just when the mean masking amount as determined previously from the same subject is added during sleep; with further elevation of the sleep temperatures, the variability increases again. In the internally synchronized rhythm, on the other hand, the variability of the total time series decreases steadily with increasing sleep increments. This indicates that the amount of the masking effect can be estimated by calculating the variability of the time series, independent of any period estimation and independent of any hypothesis about masking; this method is applicable to internally desynchronized but not to internally synchronized rhythms. In a second step, period analyses have been calculated, based on the common periodogram algorithm[4]. The dominant period turned out to be independent of the amount of temperature added to the sleep data. As the right diagram of figure 7 shows, the reliability of the resulting rhythm increases initially with increasing elevation of the sleep temperature data, reaches a maximum with an increment of 0.36 °C, and decreases eventually. Such a course passing an optimum, however, is present only in the internally desynchronized rhythm; in the internally synchronized rhythm, the reliability decreases steadily with increasing elevation of the sleep temperatures. Again, the mean amount of the masking effect can be estimated independently with this method. There remains the consideration of the rhythm's amplitude (fig. 7, middle diagram). Whereas the amplitude decreases steadily with increasing elevation of the sleep temperatures in internally synchronized rhythms, the amplitude is virtually independent of this elevation in internally desynchronized rhythms. As the general result, the method of analyzing time series after arbitrarily adding various amounts of temperature to the sleep data, leads to independent estimates of the masking effect from sleep-wake on body temperature, confirming the previous result. But, to be sure, this method is effective only in internally desynchronized but not in internally synchronized rhythms.

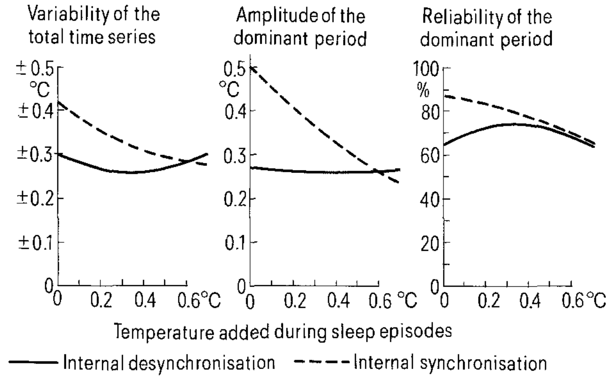

Figure 7. Parameters of rectal temperature rhythms, originating from two typical experiments, with internally desynchronized rhythms (solid lines; same experiment as in figs 2–6) and internally synchronized rhythms (dotted lines), depending on the amount of the elevation of the sleep temperature data.

Rhythms under strong zeitgebers

The state of internal desynchronization, the prerequisite for proper evaluation of the quantitative properties of the masking effect, is not restricted to constant conditions where the rhythms are freerunning. It can also be induced in every subject by exposure to an artificial zeitgeber with a period within the range of entrainment of the sleep-wake rhythm but outside this range of the

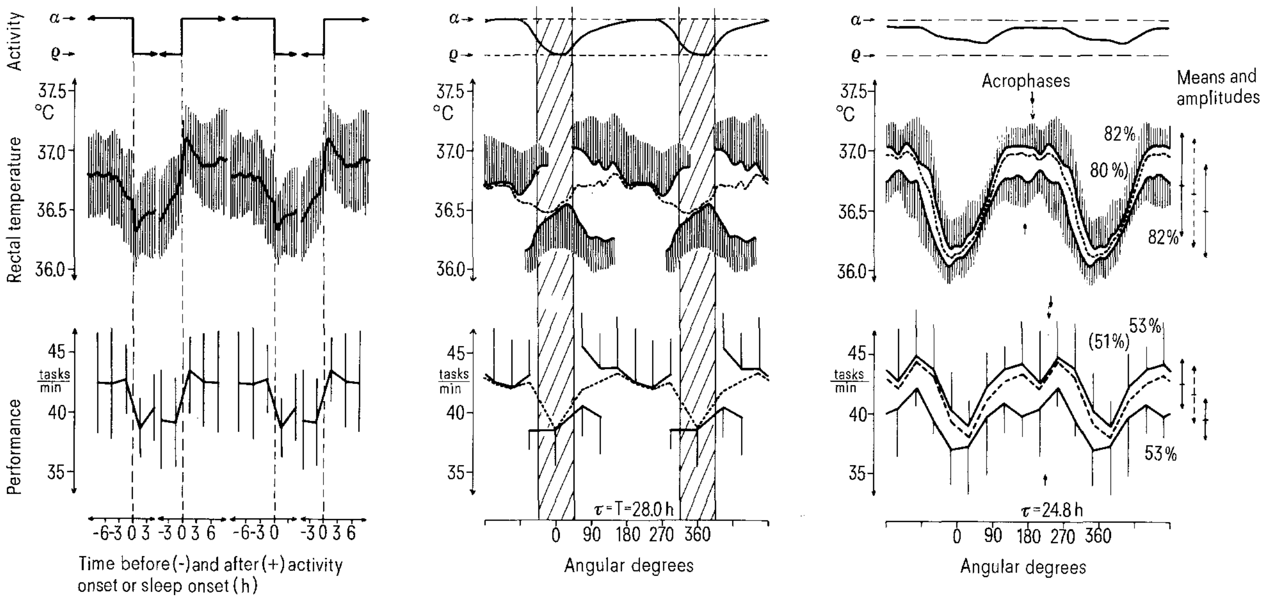

Figure 8. Educed cycles of sleep-wake, rectal temperature and computation speed (with SD), originating from a 25-day experiment with a subject living under the influence of a strong 28-h zeitgeber and, hence, showing forced internal desynchronization. Left: synchronized to the instants of sleep onset and wake onset, with several hours before and after these instants each; center: educed with the zeitgeber period and, hence, the dominant period of the sleep-wake rhythm; right: educed with the dominant period of the temperature and the performance rhythms. In the two latter diagrams, the educed cylces are calculated separately from temperature (or performance) data obtained during wake episodes only (upper traces) and during sleep episodes only (lower traces).

body temperature rhythm[4]. Such partial (sleep-wake) synchronization occurs, for instance, with the exposure to a strong artificial zeitgeber with a 28-h period. Figure 8 shows data from a subject living for 25 days under the influence of such a condition. Since under the influence of a zeitgeber the subject could be awakened from sleep in regular intervals without jeopardizing the aims of the experiment, additional measurements of psychical performance (computation speed) were feasible[6]; also this latter variable is expected to become masked by the sleep-wake alternation. Consequently, not only data from rectal temperture are presented as in the experiment under constant conditions, but also performance data. Again, the presentation of the different variables characterizes the different aspects of the masking effect. The left diagram of figure 8 shows educed rhythms, synchronized with the instants of sleep and wake onset; this diagram corresponds to figure 2 but includes now also the performance data. Again the main changes in the variables occur immediately after the transitions from sleep to wake and vice versa; both, temperature and performance decrease with sleep onset and increase with wake onset, with marked overshoots in all cases. Rectal temperature again is lower during sleep than during wake, and the speed in solving the computation tasks is lower during the sleep episode – when the subject must be awakened for the tests – than during the wake episode.

The right diagram of figure 8 shows again educed rhythms calculated for the most prominent period in these variables (which is 24.8 h in this experiment); the average cycles are calculated separately from data obtained exclusively during the sleep episodes (lower traces) and data obtained exclusively during wake episodes (upper traces); this diagram corresponds to figure 3. In case of performance, the separation means that the 'sleep values' have been obtained each a few minutes after enforced awakening the subjects from sleep. The result obtained with this separation is essentially the same as in the case of spontaneously occurring internal desynchronization: The sleep and wake cycles in temperature and performance run parallel up to individual details; but they are vertically separated, on the average, by 0.22°C, or 3.0 tasks/min respectively (mean amounts of the masking effects). The values of the masking effects for both variables are larger than average during sections where sleep occurs more rarely than average (around the maximum values and the descending sections of the cycles); they are smaller than average where sleep occurs more frequently than average (around the minimum values and the ascending sections of the cycles). Again, the interpretation is in place that the sleep cycles are phase advanced relative to the wake cycles by about one hour each, as indicated by the uniform shift of the individual patterns around the maximum and minimum values in react 1 temperature. This is obvious also, in case of performance for the 'post lunch dip' which is not only independent of the meals (which succeed one another with another sequence than the temperature period) but which is even manifest during the sleep episodes. Consequently, it may be adequate to speak of a dip in performance around the maximum in deep body temperature, independent of the phase of the sleep-wake cycle and, hence, of the meals.

In this particular experiment, the value of the masking effect of rectal temperature is considerably smaller than in the experiment depicted in figure 3, whereas the circadian amplitude is considerably larger. While the mean masking effect in the experiment under constant conditions (cf. fig. 3) amounted to 68% of the circadian range of the temperature cycle, it amounts to only 24% in the experiment with the strong zeitgeber (cf. fig. 8); however, for performance, it amounts to 68%, i.e. this ratio is clearly larger than for temperature. In turn, the phase advance of the sleep cycle relative to the wake cycle in the latter experiment, concerning the temperature data and the performance data, is rather similar to that in the previous experiment. To indicate these comparisons, the parameters of the average cycles are given beside the right diagram of figure 8. Finally, the numbers in the figure indicate the higher reliabilities of the separated cycles of sleep and wake data in comparison with the common cycle.

With the mean masking amount determined in this way, one can again 'purify' the original temperature rhythm from masking due to sleep-wake, by adding 0.22°C to all temperature data obtained during the sleep. The artificial time series thus generated appears nearly unchanged. In particular, it shows beat phenomena nearly as markedly as does the original time series. The regular fluctuations in successive amplitudes still reach 88% of that in the original time series, and not only 28% as in the case of the rhythm under constant conditions (cf. fig. 6). The scalloping in the rhythm's phases is nearly as marked in the modified rhythm as it is in the original rhythm. The short-term variability among successive acrophases as a measurement of this scalloping pattern in the modified rhythm still amounts to 98% of that in the original rhythm, and not only to 44% as in the case of the rhythm under constant conditions (cf. fig. 6). From these results, it can be deduced that the portion of the masking effect from the total interaction between the rhythms of sleep-wake and deep body temperature is rather small, i.e. about a quarter of the interaction, and thus much smaller than that portion in the other rhythm (cf. fig. 6) where it amounted to about two thirds. This means, in the case of the rhythm under the influence of the strong zeitgeber (cf. fig. 8) the oscillatory interaction is the dominant mechanism in the overall interaction, whereas in the case of the rhythm under constant conditions (cf. figs 2–6) the masking effect was the dominant mechanism.

In the middle diagram of figure 8, the same procedure for the separate evaluation of the sleep and wake data is used but employing the zeitgeber period and, hence, sleep-wake as the scanning period. The most striking difference between the middle and the right side diagram concerns the fact that the sleep and wake data do not cover a full cycle each, because, in this case, scanning and the mean sleep-wake period are identical. To be sure, due to the variabilities of sleep onset and, even more so, of wake onset as deduced from the uppermost trace, there is some overlap between sleep and wake data. The dotted lines again indicate the common educed cycles. They coincide with the educed sleep cycles during the dark episode because there are no wake data; and they coincide with the educed wake cycles during a section out of the wake episode because there are no sleep data. It is striking that the mean intervals between the sleep and wake partial cycles during the overlaps are substantially larger than the intervals between the sleep and wake cycles when educed with the period of the temperature rhythm (cf. fig. 8, right side diagram). This discrepancy may suggest differential amounts of the masking effect when deduced from the same data but with different methods. This apparent contradiction is based on the fact that, with the sleep-wake period as the scanning period, during the relatively small overlaps mainly the overshoots in the masking effect (cf. fig. 8, left side diagram) are responsible for the intervals mentioned, whereas, with the temperature period as the scanning period, the overshoots contribute only insignificantly to the intervals.

The results worked out from figure 8 may have practical implications with regard to data analysis. For various reasons, data for several variables are obtained only during the wake but not during the sleep episodes; in particular, this restriction concerns performance measurements. For instance, with freerunning rhythms under constant conditions subjects cannot be awakened for measurements without disturbing the constancy of the conditions. In the diagram of figure 8, the time courses of the values obtained exclusively during the wake episodes can be compared with data obtained during wake and sleep, in the same experiment. As the right-side diagram of figure 8 shows, inferences drawn from wake data only do reflect conclusions drawn from the consideration of all data, except for mean values; this holds true especially when the scanning period deviates sufficiently from the sleep-wake period. In turn, as the middle diagram of figure 8 shows, the conclusions from the analysis only of wake data about the parameters of the emerging rhythm are completely misleading when the scanning period is of the same (or is close to the) length of the sleep-wake period. In this experiment, in particular, the acrophases calculated with the two procedures differ widely. The table summarizes the parameters of the educed cycles of the rectal temperature and the performance obtained with both scanning periods, from wake data only, on the one hand, and from all data, on the other. Again, it is obvious that, while the amplitudes turn out to be similar with both procedures, the acrophases are similar only when the scanning period deviating from the sleep-wake has been used, but are very dissimilar when the scanning period identical to the sleep-wake period has been used. The slight differences in the mean values between analyses with the two different scanning periods is due to the different weights of the various sections of the educed cycles with the two periods. It must be emphasized that the results obtained from this special experiment can be generalized in the sense that, as long as the scanning period is close to the sleep-wake periods, no inference is possible from the cycles educed from wake data to the cycles educed from wake and sleep data. In other experiments though, in particular in those with internally synchronized rhythms, the amplitudes of the oscillation of the performance measure turn out to be very small when only wake data are used, but considerably larger when sleep data are included[6]. Consequently, a full spectrum analysis calculated from wake data only, on the one hand, and a corresponding analysis from wake and sleep data, on the other, are similar in special ranges of periods but very dissimilar in other ranges; this inconsistency must be considered when rhythm endpoints are evaluated from variables like performance which have been measured only during the wake episodes.

Parameters of educed rhythms in their dependence on the measuring mode: Data obtained during wake (W) and sleep episodes (S), or during wake only

Scanning period	Variable	Data mode	Mean value	Amplitude	Acrophase
24.8 h	Temperature	W + S	36.65°C	0.46°C	186°
		W only	36.72°C	0.44°C	188°
		△	0.07°C	0.02°C	2°
	Performance	W + S	41.7 t/m	2.4 t/m	234°
		W only	42.6 t/m	2.1 t/m	237°
		△	0.9 t/m	0.3 t/m	3°
28.0 h	Temperature	W + S	36.65°C	0.13°C	157°
		W only	36.84°C	0.16°C	57°
		△	0.19°C	0.03°C	− 100°
	Performance	W + S	41.7 t/m	1.6 t/m	177°
		W only	43.9 t/m	1.6 t/m	42°
		△	2.2 t/m	0.0 t/m	− 135°

Conclusions

The experimental examples shown so far are individual cases. Yet, they are representative for all cases of internal desynchronization, regardless whether desynchronization occurs spontaneously under constant conditions (cf. figs 2–6), or is forced by the exposure to a strong artificial zeitgeber with a period sufficiently deviating from the freerunning period (cf. fig. 8). In all cases, deep body temperature (and, in addition, the level of performance, as in the latter instance) rises rapidly with wake onset and drops rapidly with sleep onset, with overshoots by about 50% beyond the steady state alteration (cf. fig. 2, and fig. 8, left diagram). Also, in all cases the educed cycles calculated either from data obtained during the sleep episodes or from data obtained during the wake eposides, are clearly separated but run virtually parallel (cf. fig. 3, and fig. 8, right diagram). The mean masking effect (± SD) of rectal temperature as calculated from 10 experiments amounts to 0.28 ± 0.06°C; it deviates significantly from zero ($p < 10^{-6}$). Finally, the masking effect depends on the phase of the temperature rhythm. This can be expressed by a phase advance of the sleep versus the wake temperature cycle by, on the average, 13 ± 4°C; or, if converted into absolute time in a 24-h day, by 52 ± 15 min. This phase advance is also statistically significant ($p < 10^{-5}$).

The question arises as to the interrelation between the exact value of the masking effect, on the one hand, and individual peculiarities of the subject and/or the experimental condition, on the other. For instance, from the two representative experiments the impression arises that the masking amount observed under constant conditions (0.36°C; cf. fig. 3) is larger than the one observed under zeitgeber exposure (0.22 °C; cf. fig. 8, right diagram). This impression, however, is not supported by the results of other experiments. To elucidate the variability of results, figure 9 shows results from another experiment under constant conditions where the subject exhibited internal desynchronization during the entire duration of the 4-weeks experiments, as did the subject whose data are shown in figure 3; this figure again presents cycles educed separately for temperature data obtained during sleep and wake (as did fig. 3). The mean amount of the masking effect (0.22 °C), however, is distinctly smaller than in the other experiment under constant conditions (0.36 °C; cf. fig. 3), and it coincides, by chance, with the one of the experiment under zeitgeber exposure (0.22 °C; cf. fig. 8, right diagram). In summary, the mean masking amount of the five experiments under constant conditions (0.29 ± 0.06 °C) cannot be differentiated from the mean masking amount of the five experiments under zeitgeber exposure (0.27 ± 0.05 °C). Likewise the circadian amplitude under constant conditions is smaller in the second (cf. fig. 9) than in the first experiment (cf. fig. 3). The relative amount of the masking effect (i.e. relative to the circadian range of temperature oscillation) in the second experiment (69 %) is similar to that in the first experiment under constant conditions (68 %), and it is distinctly greater than that in the experiment under zeitgeber exposure (24 %). The presently available results support the notion that the relative (but not the absolute; see above) amount of the masking effect in experiments under constant conditions is greater than in experiments under zeitgeber exposure, though not to a level of statistical significance; just the relative masking seems to be an interesting measure[5].

Still the suggested difference between the relative amount of masking in experiments under constant conditions and that in experiments under zeitgeber exposure must not necessarily be due to the difference in the experimental conditions; the relative masking may depend on personality trends. In fact, it is well established that the propensity of the spontaneous occurrence of internal desynchronization under constant conditions depends significantly on the personality of the subjects, whereas internal desynchronization can be forced by exposure to a suitable zeitgeber in every subject, independent of his personality[4]. Therefore, the hypothesis offers itself that the relative masking effect depends on items similar to those enhancing the probability of the spontaneous occurrence of internal desynchronization. That is to say, both are larger than average, for instance, under behavioral stress, in elder subjects, and in subjects with increased scores of neuroticism[4,6]. Yet, it is up to future research to examine such possible interdependencies, whose knowledge may indeed be of practical importance in the diagnosis of special personality trends and diseases.

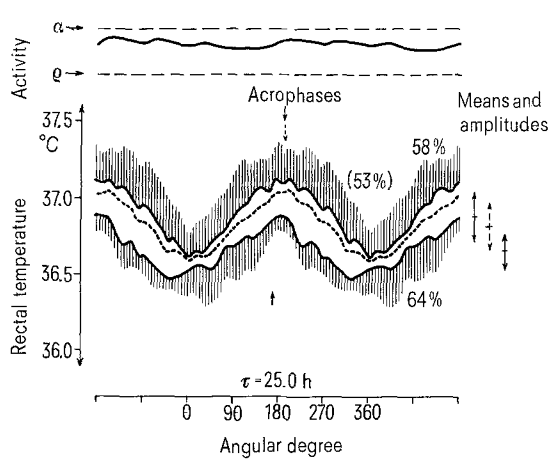

Figure 9. Educed cycles of sleep-wake and rectal temperature (with SD), originating from another 28-day experiment under constant conditions, with a subject also showing internal desynchronization. The averaging procedure is calculated with the dominant period of the temperature rhythm as the scanning period, separately from temperature data obtained during wake episodes only (upper trace) and during sleep episodes only (lower trace). Dotted line: educed cycle calculated from all temperature data.

The consistent phase dependence of the masking effect on the sleep-wake cycle is well demonstrated by the overshoots of the rapid rises and declines of temperature accompanying wake and sleep onset. Here, the dependence of the masking effect on the phase of the temperature cycle is of special interest. The masking effect arising from sleep-wake on body temperature is generally largest around the maximum values and the descending sections, and it is smallest around the minimum values and the ascending sections of the temperature cycle. This means, the masking amount is negatively correlated with the general probability to sleep or with the self-rated fatigue, and it is positively correlated with the self-rated alertness. Moreover, the amount of the masking effect is smaller than average during phases of the temperature cycle where preferably REM sleep occurs, and it is larger than average during phases of the temperature cycle where the REM sleep propensity is lower than average[6,10,11].

All the analyses aiming at the quantification of the masking effect, as discussed above, are applicable only to internally desynchronized rhythms. However, the consideration of masking-induced rhythm distortions would be of special interest also under natural conditions where all rhythms are externally synchronized to the 24-h day and, hence, are also internally synchronized. It may be possible to transfer, by analogy, the results obtained in internally desynchronized rhythms to internally synchronized rhythms. Indirect procedures suggest that the mean amount of the masking effect is similar in both cases; this is particularly true in those 33 experiments of ours that include a section with internally synchronized rhythms and another one with internally desynchronized rhythms, where each one was of sufficient duration to draw pertinent conclusions[9]. Under the probable but not directly testable precondition that also the parallelity between sleep and wake temperature curves are similar in both rhythm states, the influence of the masking effect on internally synchronized rhythms can be predicted. With increasing amount of the masking effect relative to the circadian amplitude, the overt rhythm amplitude increases in externally synchronized 24-h rhythms as well as in freerunning rhythms. The internal phase relationship between the rhythms of sleep-wake and deep body temperature, however, changes in opposite directions in both rhythm states: In 24-h rhythms where the temperature minimum is commonly late within the sleep episode, increasing masking effect leads to a phase advance of the temperature rhythm relative to the sleep-wake rhythm; in freerunning rhythms where the temperatur minimum is commonly early within the sleep episode, the same increasing masking effect leads to an internal phase delay. Both internal phase shifts are relevant only in the fundamental period determined by means of an harmonic analysis. The actual changes in the wave shape around the temperature minimum can be interpreted also as being a direct effect of masking on that section of the temperature cycle; with this latter interpretation, however, any additional statement about the rhythm's phase is meaningless.

The masking effect is an important mechanism in the interaction between different overt rhythms in one organism, but it is not the only one. Another mechanism is the 'oscillatory interaction' where one overt rhythm (or one basic oscillator) affects another one in the same sense as an external zeitgeber affects a rhythm. Consequently, the first rhythm can be considered as an 'internal zeitgeber' with regard to the second one[7]. The second mechanism is relevant, in the sense that it is the only one exerting dynamic forces and, hence, is the only one that is capable of inducing mutual synchronization of the two rhythms. Since, under constant conditions, the different rhythms mostly also run mutually synchronized, this mechanism is generally effective. The masking effect, on the other hand, can alter the wave shape and, hence, rhythm parameters such as phase and amplitude; but it can never alter the period of a rhythm. The relevant rhythm parameter that determines coupling, responsible for the oscillatory interaction between different overt rhythms or basic oscillators, is the amplitude of these rhythms[8]: The larger the amplitude of a forcing rhythm, the stronger its internal zeitgeber effectiveness to another rhythm. In turn, the larger the amplitude of a forced rhythm, the higher its resistence to perturbations and, thus, external and internal zeitgebers. Consequently, the amplitude of a rhythm is a measure of its oscillatory impact. This means, the ratio of the rhythm's amplitude to the masking effect, as determined previously, reflects also the ratio of the oscillatory component to the masking component of the internal interaction. This theoretical consideration confirms the previous evaluation of the ratio between the different interaction mechanisms as exemplified in the individual experiments.

In the present discussions, the masking effect had been treated as though it were superimposed, more or less independently, on the circadian variations. This would mean that environmental stimuli affecting the circadian system, or stimuli generated by another circadian rhythm of the same organism, operate in two different modes using different physiological pathways: In the one mode, the stimulus affects, as a zeitgeber, the 'circadian clock' which then controls the biological variable; and in the other mode the stimulus affects directly the biological variable by-passing the 'clock'. This way of considering the masking effect had been selected because it elucidates the actions of the masking effect in an obvious manner. However, this way of considering the masking effect does not constitute the only hypothesis about masking. Rather, there is the alternative hypothesis which states that there is an intrinsic and inseparable relation between circadian variations, or 'oscillatory action', on the one hand, and masking effect, on the other. Both these actions may be considered to be complementary aspects of the same clock mechanism. The ratio between both actions is subject to individual variations, or variations depending on internal or external conditions, also according to the second hypothesis. A discrimination between both hypotheses concerning the relation between oscillatory interaction and masking effect is possible on the basis of model considerations. A simple mathematical model of circadian rhythmicity includes inherently the masking effect as an inseparable part of the clock mechanism[2]. This had been shown as an effect of an external zeitgeber in a single-oscillator model; and it had been shown in a combined two-oscillator model where either oscillator affects the other as an internal zeitgeber[8]. In developping this model, it had been modified in various ways, and each modification had been tested according to its agreement with basic biological results, applying 'trial and error' methods[8]. In the course of these investigations, also the model had been modified in a way that the masking effect was abolished and, then, in a second step, a function of the controlling stimulus as a representative of the masking effect had been added to the model solution after 'purification' from the masking effect. As the result, only the original model, which included the masking effect inherently, was consistent with various biological results in all respects tested; on the other hand, the modified model with separate oscillatory interaction and masking effect was inconsistent with various biological results, except, of course, the description of the masking effect[8]. The model considerations force us to conclude that the hypothesis suggesting that a separate mechanism, bypassing the 'clock', is responsible for the masking effect, is not in agreement with basic biological results and, hence, has to be rejected. The other hypothesis stating that the masking effect is an inherent part of the clock mechanims and a complementary aspect to circadian variations, is in agreement with all known facts and, hence, has to be favored. The acceptance of this second hypothesis cannot exclude the possibility that there can be external or internal impulses that influence a biological variable directly; it only means that every impuls that operates as a zeitgeber, simultaneously releases a masking effect.

Finally, it must be emphasized that the restriction in our considerations of the masking effect to actions from sleep-wake to deep body temperature, is an arbitrary one in this paper; there is a masking effect too from deep body temperature to sleep-wake: Every rise in temperature enhances the probability of the subject to stay awake, and every drop in temperature is attended by an increased probability of the subject to sleep. It was the aim of this paper to exemplify the quantitative deduction of a masking effect from experimental data obtained under special conditions. The same method can also be applied to deduce other types of masking effects, e.g. between variables different from sleep-wake and deep body temperature.

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The effect of surgery and anesthetic agents on granulocyte-chemiluminescence in whole blood

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Summary. The effect of anesthesia and major abdominal surgery on zymosan-induced chemiluminescence (CL) of neutrophil granulocytes was evaluated. CL was measured in diluted whole blood taken at distinct intervals within the perioperative period. In addition, blood samples from healthy volunteers were supplemented with ether and halothane to investigate the in vitro effect of these agents. The phagocytosis-induced CL was not found to be depressed by anesthesia and surgery. Only at supranarcotic concentrations was CL reduced. Surgery and anesthesia, therefore, do not appear to impair this defense system significantly under the conditions of this investigation.
Key words. Chemiluminescence; anesthesia; surgery, abdominal; granulocytes; perioperative period; immunological resistance.

Introduction

The question of impaired immunological resistance following general anesthesia and surgery, and its biological significance, has been raised frequently. To date, the influence of anesthesia and surgery on the humoral-phagocyte defense system is still a subject of controversy. The phagocytosis-induced chemiluminescence (CL) of polymorphonuclear leukocytes (PMN) in whole blood depends on both the humoral and the cellular components of the phagocyte defence system[1,2]: Opsonization of particulate matter or activation of the complement cascade (humoral factors) can lead to the activation of the PMN-membrane (cellular factors). This in turn results in the production of activated oxygen species (O_2^-, $\cdot OH$, $^1O_2$, H_2O_2) which mediate the PMN-killing function and the generation of CL. Although the biological significance of CL is not yet understood it can be used to measure these functionally important oxygen products of PMN-activation. CL measurements in whole blood samples make it possible to follow the activity of the humoral-phagocyte system under nearly physiological conditions, because they reflect the in vivo interaction of cellular and humoral factors.

In the present study, the influence of general anesthesia and limited surgical stress on the humoral-phagocyte defense system was investigated by phagocytosis-induced CL-measurements. CL in diluted whole blood samples was determined before and several times during and after surgery. In addition to that, CL was measured in whole blood samples from healthy volunteers, equilibrated in vitro with volatile anesthetic agents (halothane, ether).

Methods

Chemiluminescence-assay. The reaction mixture contained in a final volume of 0.5 ml heparinized whole blood (0.1 ml), Dulbecco's modified Eagle's medium (0.4 ml), luminol (20 µg, 0.23 mM) and zymosan (500 µg). CL was measured at 37 °C in a photon counter (Biolumat, model LB 9700 or 6-channel-Biolumat LB 9505, Berthold, Wildbad, FRG) over a 30-min period after the initiation of the reaction by non-preopsonized zymosan. The mean activity during this period expressed as counts per minute (cpm) was related to both units of whole blood (whole blood (WB) activity: cpm/µl WB) and to the number of polymorphonuclear granulocytes (PMN) in the system (specific activity: cpm/1000 PMN). Details of chemicals, media and procedure were as published previously[2].

In vitro experiments. For ether and halothane experiments, the usual polystyrene vials were replaced by inert glass vials which were dark-adapted 2 h prior to the experiment.