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Neuroscience Letters 218 (1996) 153–156

NEUROSCIENCE LETTERS

# Morning-type and evening-type individuals differ in the phase position of their endogenous circadian oscillator

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Received 23 September 1996; accepted 2 October 1996

## Abstract

In dealing with inter-individual phase differences in overt circadian rhythms, it is often difficult to distinguish the impact of the endogenous circadian oscillator from that of an individual's habitual lifestyle. In an attempt to resolve this uncertainty about the role of masking influences, two groups of subjects, morning-types and evening-types, were selected and monitored during entrained, habitual sleep-wake conditions and during 24 h of controlled wakefulness in a laboratory-based constant-routine procedure. Under both conditions significant differences were observed in the circadian phases of body temperature and subjective alertness. During constant routine mean between-group differences for these two variables were 2.12 and 4.28 h, respectively. Thus, evidence is provided for the endogenous nature of morningness-eveningness.

*Keywords:* Human circadian rhythms; Morningness-eveningness; Constant routine; Masking; Body temperature; Subjective alertness

The form of overt circadian (~24 h) rhythms may vary considerably between individuals, which may be indicative of the condition of the underlying circadian oscillator. For example, inter-individual differences in the amplitude and/or the phase of the body temperature rhythm have been implicated in circadian clock dysfunction associated with affective disorders [18], chronic forms of insomnia [15], and intolerance for shiftwork [14]. The assessment of the phase of circadian rhythms is of particular relevance because it provides information about the temporal organization of the body's regulatory processes. Various morningness-eveningness questionnaires have been put forward in an attempt to estimate the characteristic phase position of an individual (e.g. [8]). The difficulty in dealing with the concept of morning-type (M-type) and evening-type (E-type) individuals, however, lies in the uncertainty about the impact of the endogenous circadian oscillator versus that of an individual's lifestyle and its ensuing masking effects upon an overt rhythm's waveform [19]. In an attempt to solve this problem, M-types and E-types were selected and monitored, both in the presence and in the absence of masking influences, i.e. during habitual, entrained sleep-wake conditions and during 24 h of controlled wakefulness in a constant routine procedure.

Volunteer subjects with no reported sleep complaints were recruited from students and were paid for their participation in the study. In a two-step procedure, care was taken to screen subjects as habitual M-types or E-types by employing criteria which were derived from both questionnaire and sleep-wake log data of similar groups of students, who had participated in an earlier study [9].

Candidate subjects who obtained extremely low or high scores on a Dutch morningness-eveningness questionnaire were instructed individually how to use a sleep-wake logbooklet, in which they kept daily records of their bedtimes and times of final awakening, and with a mean frequency of five to six times per day, measurements of their oral temperature (calibrated electronic thermometer) and subjective alertness (five-point rating scale) for a period of 2 consecutive weeks. In order to promote a uniform distribution of measurements, the subjects were instructed to make these self-measurements about once every 3 h, except when asleep. Subjects were told not to measure their temperature within half an hour after ending activities such as eating, drinking, bathing or heavy physical exercise.

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*PII* S0304-3940(96)13140-2

For each subject, the values of oral temperature and subjective alertness were folded into one 24 h period and fitted with a third-degree polynomial. The validity of this 'ensemble averaging' procedure was verified by applying periodogram analysis to the temperature and alertness data (Lomb method [10]). Except for the alertness data of one E-type subject, all data-sets gave evidence of a pronounced 24 h periodicity. Next, the $t_{max}$ (i.e. the time at which the fitted curve reaches its maximum value) of the oral temperature was identified. The subject was selected if his/her $t_{max}$ (oral temperature) fell earlier than 1730 h (for M-types) or later than 1930 h (for E-types) [9]. In total, seven M-types (mean age 23.9 years, range 22–27; three females) and seven E-types (mean age 23.6 years, range 20–28; three females) were included, after obtaining their written informed consent.

The difference between the mean $t_{max}$ (oral temperature) values of the two groups amounted to 4.6 h (see Table 1). Significant differences were also obtained for the other phase estimates derived from the log-booklet, i.e. the mid-sleep times (midpoint of estimated period of sleep) and the $t_{max}$ (alertness) values.

The group difference in sleep-wake behavior could be verified by the use of actigraphy [17] for five M-types and five E-types. During a period of 11 days overlapping the 2 weeks of log measurements, a continuous recording was made of spontaneous body movements by means of an activity monitor, worn on the wrist of the non-dominant arm. On the basis of half-hour values a mean 24 h pattern was calculated for each subject, and fitted with a 24 h harmonic sinusoidal curve. The $t_{max}$ (actigraphy) values differed significantly between the two groups (see Table 1), and correlated significantly with the mid-sleep times (Spearman rank correlation, 0.77, $n = 10$, $P < 0.01$). Thus, a consistent between-group difference in the habitual 24 h sleep-wake pattern was apparent.

Halfway the 2 weeks of log measurements an ambulatory 24 h recording of rectal temperature was made, sampling one value every 2 min. A combined 24 h fundamental and a 12 h harmonic sinusoidal curve [3] was fitted to these data and the $t_{min}$ (ambulatory rectal temperature) calculated. A comparison between the $t_{min}$ (ambulatory rectal temperature) values of the M-types and the E-types showed a mean difference of 2.6 h (see Table 1).

Table 1

Mean values (h) ± SD (h) of the estimates of circadian phase position.

| Phase estimate | M-types | E-types |
|---|---|---|
| Mid-sleep time | 0331 ( ± 0037) | 0601 ( ± 0047) |
| $t_{max}$(Oral temperature) | 1537 ( ± 0136) | 2013 ( ± 0055) |
| $t_{max}$(Alertness) | 1152 ( ± 0145) | 1752 ( ± 0148) |
| $t_{max}$(Actigraphy) | 1425 ( ± 0104) | 1723 ( ± 0043) |
| $t_{min}$(Ambulatory rectal temperature) | 0323 ( ± 0028) | 0557 ( ± 0112) |
| $t_{min}$(CR rectal temperature) | 0438 ( ± 0046) | 0645 ( ± 0053) |
| $t_{max}$(CR alertness) | 1525 ( ± 0142) | 1942 ( ± 0041) |

$t_{max}$ and $t_{min}$ denote the times at which the curve fitted to the data reaches its maximum and minimum values, respectively. All comparisons were tested (two-sided) with the Mann–Whitney test, and gave significant ($P \leq 0.01$) results.

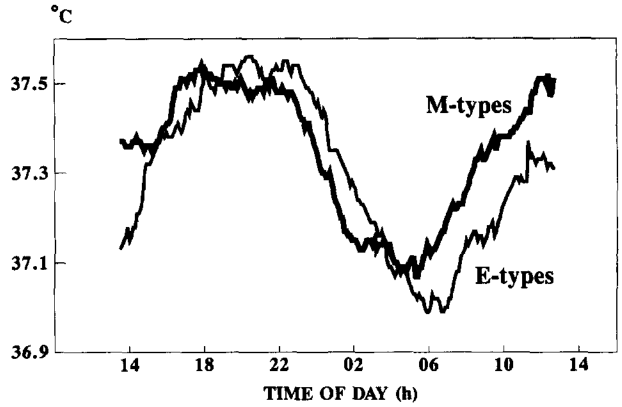

Fig. 1. Mean rectal temperature (°C) curves for seven M-types (bold line) and seven E-types (thin line), recorded during constant routines. The mean times at which a fitted combined 24 h fundamental and 12 h harmonic sinusoidal curve reached its minimum were 0438 and 0645 h, respectively.

Ambulatory recording of the rectal temperature rhythm is likely to be confounded by the masking effect of sleep [11]. Therefore, the constant routine (CR) protocol was used to reveal the phase of the endogenous circadian oscillator. The CR protocol as introduced by Mills et al. [12] and subsequently modified by Czeisler et al. [4], minimizes the confounding effects of exogenous environmental (light-dark) and behavioral (sleep-wake) stimuli, and thus exposes the unmasked phase of the circadian oscillator. Following the 2 week log period, the subjects were recorded in a 24 h constant routine procedure, preceded by 2 h of laboratory adaptation. Briefly, this procedure consisted of subjects being maintained awake in a near supine position in bed, in constant ambient temperature (21°C) and constant illumination (100 lx). Subjects were occupied with activities such as reading, watching video movies, and casual conversation. Their daily nutritional and liquid intake was divided into hourly equi-caloric quantities. Rectal temperature was automatically recorded every 2 min; subjective alertness was assessed by the use of a 20-item checklist [16], which was filled out by the subject every half-hour. Both the CR rectal temperature values and the CR subjective alertness scores were fitted with a combined 24 h fundamental and 12 h harmonic sinusoidal curve.

The $t_{min}$ (CR rectal temperature) occurred on average at 0438 and 0645 h, for the M-types and the E-types respectively, and differed significantly (see Fig. 1). The 2.1 h difference between the means is substantial, considering that their respective 95% confidence intervals (for M-types 0354–0524 h; for E-types 0554 –0736 h) do not overlap.

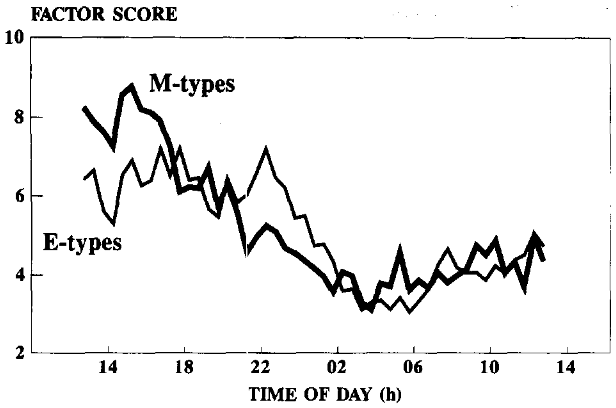

Fig. 2. Mean subjective alertness factor scores for seven M-types (bold line) and seven E-types (thin line), recorded during constant routine. The mean times at which a fitted combined 24 h fundamental and 12 h harmonic sinusoidal curve reached its maximum were 1525 and 1942 h, respectively.

In a 'blind' procedure, using a median split of the $t_{\min}$ (CR rectal temperature) values, the subjects were categorized as 'early phase' or 'late phase'. The Fisher exact probability test confirmed that the 'early phase' and 'late phase' individuals could be reliably identified as members of the original M-type and E-type groups ($P < 0.001$). The between-group difference for $t_{\max}$ (CR alertness) was also significant, being about twice that for rectal temperature (see Fig. 2 and Table 1).

The circadian phase difference between M-types and E-types, observed under constant routine conditions, far exceeds the inter-individual variability that may be expected on the basis of previous studies [5,6,13]. Apparently, the selection criteria employed in the present study were successful in exposing the boundaries of inter-individual phase variability. Underlying this inter-individual variability may be differences in the entrainment of the circadian oscillator, originating from differences in habit formation and/or conditioning. Differences in the 24 h activity pattern, as observed in the present study, may entail differences in exposure to phase-resetting stimuli such as the light-dark cycle, and subsequently give rise to differences in the phase of entrainment of the circadian oscillator. Moreover, the results of a recent study [1] in rats indicate that environmental cues, through associative learning, can come to mimic the effect of light on the circadian oscillator and thereby bring about entrainment of circadian rhythms. Alternatively, differences in the intrinsic period $\tau$ of the circadian oscillator may account for the phase differences during entrainment. Although still awaiting supporting evidence, this would imply that M-types are characterized by relatively short periods, and E-types by relatively long periods [7,20].

The correspondence between the M/E classifications of the subjects on the basis of their CR rectal temperature and ambulatory oral temperature data suggests that, given appropriate data acquisition and data analysis techniques, oral temperature measurements can provide a practical means whereby individuals can be 'phase typed'. Apparently, masking influences only partially account for the M-type versus E-type difference measured under ambulatory, natural conditions. This can have important clinical implications, now that there is a growing recognition of the involvement of circadian rhythmicity in the development and treatment of a wide variety of diseases [2].

We thank Hans Duindam for his expert technical assistance, and Humphrey Teerlink and Jolande Treep for their valuable help in data acquisition. This work was supported by grants 575-65-068 and 575-10.020 from the Foundation for Behavioral Sciences (SGW), Netherlands Organization for Scientific Research (NWO).

[1] Amir, S. and Stewart, J., Resetting of the circadian clock by a conditioned stimulus, Nature, 379 (1996) 542–545.
[2] Arendt, J., Minors, D.S. and Waterhouse, J.M., Biological Rhythms in Clinical Practice, Wright, London, 1989.
[3] Brown, E.N. and Czeisler, C.A., The statistical analysis of circadian phase and amplitude in constant-routine core temperature data, J. Biol. Rhythms, 7 (1992) 177–202.
[4] Czeisler, C.A., Brown, E.N., Ronda, J.M., Kronauer, R.E., Richardson, G.S. and Freitag, W.O., A clinical method to assess the endogenous circadian phase (ECP) of the deep circadian oscillator in man, Sleep Res., 14 (1985) 295.
[5] Czeisler, C.A., Johnson, M.P., Duffy, J.F., Brown, E.N., Ronda, J.S. and Kronauer, R.E., Exposure to bright light and darkness to treat physiologic maladaptation to night work, N. Engl. J. Med., 322 (1990) 1253–1259.
[6] Dawson, D., Lushington, K., Lack, L., Campbell, S. and Matthews, C., The variability in circadian phase and amplitude estimates derived from sequential constant routines, Chronobiol. Int., 9 (1992) 362–370.
[7] Folkard, S. and Monk, T.H., Individual differences in the circadian response to a weekly rotating shift system. In A. Reinberg, N. Vieux and P. Andlauer (Eds.), Night and Shift Work: Biological and Social Aspects, Pergamon Press, Oxford, 1981, pp. 367–374.
[8] Horne, J.A. and Östberg, O., A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms, Int. J. Chronobiol., 4 (1976) 97–110.
[9] Kerkhof, G.A., A Dutch questionnaire for the selection of morning and evening types (in Dutch), Ned. Tijds. Psychol., 39 (1984) 281–294.
[10] Lomb, N.R., Least-squares frequency analysis of unequally spaced data, Astrophys. Space Sci., 39 (1976) 447–462.
[11] Mills, J.N., Minors, D.S. and Waterhouse, J.M., The effect of sleep upon human circadian rhythms, Chronobiologia, 5 (1978) 14–27.
[12] Mills, J.N., Minors, D.S. and Waterhouse, J.M., Adaptation to abrupt time shifts of the oscillator(s) controlling human circadian rhythms, J. Physiol. (London), 285 (1978) 455–470.
[13] Minors, D.S. and Waterhouse, J.M., Does 'anchor sleep' entrain circadian rhythms? Evidence from constant routine studies, J. Physiol. (London), 345 (1983) 451–467.
[14] Monk, T.H. and Folkard, S., Individual differences in shiftwork adjustment. In S. Folkard and T.H. Monk (Eds.), Hours of Work, Wiley, Chichester, 1985, pp. 227–237.
[15] Roehrs, T. and Roth, T., Chronic insomnias associated with circadian rhythm disorders. In M.H. Kryger, T. Roth and W.C. Dement (Eds.), Principles and Practice of Sleep Medicine, 2nd edn., W.B. Saunders, Philadelphia, 1994, pp. 477–481.

[16] Thayer, R.E., Factor analytic and reliability studies in the activation-deactivation adjective check list, Psychol. Rep., 42 (1978) 747–756.

[17] Tryon, W.W., Activity Measurement in Psychology and Medicine, Plenum Press, New York, 1991.

[18] Van den Hoofdakker, R.H., Chronobiological theories of nonseasonal affective disorders and their implications for treatment, J. Biol. Rhythms, 9 (1994) 157–183.

[19] Wever, R.A., Internal interactions within the human circadian system: the masking effect, Experientia, 41 (1985) 332–342.

[20] Wever, R.A., The Circadian System of Man, Springer-Verlag, New York, 1979..