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0014-4754/84/111223-04$1.50 + 0.20/0

Sex differences in human circadian rhythms: Intrinsic periods and sleep fractions

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Summary. The period of freerunning circadian rhythms is significantly shorter and the fraction of sleep is significantly larger in human females than in males, as long as the rhythms run internally synchronized. The sex difference in the period could be a property either of the whole circadian system or of only one of the oscillators in a multi-oscillator system. The sex difference in the sleep fraction could be a fixed property of the sleep-wake rhythm or could depend on interactions in the multi-oscillator system. To investigate these questions, a sample of 33 long-term experiments, in which the rhythms ran internally synchronized in one section and internally desynchronized in another section, were analyzed. The periods of rhythms in rectal temperature were different in females and males during internal synchronization, but became identical during internal desynchronization. In contrast, sex differences in sleep-wake periods were more pronounced when the rhythms were desynchronized than when they were internally synchronized. This result provides evidence that the sex difference in periodicity is a property only of the sleep-wake rhythm; the intrinsic periods of temperature rhythms are identical in females and males, whereas those of sleep-wake rhythms are distinctly shorter in females than in males. In the state of internal synchronization, the joint period is a compromise between the intrinsic periods of the rhythms involved, and therefore it shows a small but significant sex difference. Moreover, the transition from internally synchronized to desynchronized rhythms is combined with a highly significant reduction in the sleep fraction, which is considerably greater in females than in males. These results suggest that the occurrence of internal desynchronization strongly affects the sleep-wake rhythm, and that the influence of rhythm disorders is considerably greater in females than in males.
Key words. Circadian rhythms; sex difference; sleep.

In a homogeneous sample of 27 long-term experiments in man with freerunning and internally synchronized circadian rhythms, the period was found to be significantly shorter in females than in males (on the average, by 28 min); in addition, the fraction of sleep (for definition, see below) was significantly larger in females than in males (on the average, by 18%)[7]. From these experiments the question arises whether the sex difference in the periods was due to differences in the whole circadian system, or only in individual components whithin the multi-oscillator system, as represented in the rhythms of sleep-wake and deep body temperature[3]. According to the multi-oscillator concept, the system accepts, within the range of mutual entrainment, a compromise period between the commonly differing intrinsic periods of the components. Consequently, a sex difference in the intrinsic periods of one component only is also reflected, though to a smaller extent, in the compromise period of the internally synchronized running system. However, the sex difference in the sleep fraction can be assigned only to that component within the multi-oscillator system that controls the sleep-wake alternation. This means that sleep-wake rhythms show differences between females and males, in any case. It is well known that the sleep fraction in internally synchronized rhythms is generally greater than in internally desynchronized rhythms[6]. Here, the question arises whether the interaction between the rhythm components within the multi-oscillator system (which is different inside and outside the range of mutual entrainment) influences the sex difference of human circadian rhythms.

Both questions can be answered only by investigating internally desynchronized rhythms, where the different components of the multi-oscillator system run steadily separated in time, i.e., with their own specific intrinsic periods. To obtain individual controls, it is of importance to analyze those experiments in which the different rhythm components run internally synchronized and desynchronized in different sections of the same experiment. For the purpose of this paper, two rhythm components were considered: the sleep-wake rhythm and the rhythm of rectal temperature. This was shown to be appropriate in previous publications[3,4].

Material and methods

The present analysis is based on a sample of 33 human long-term experiments where the rhythms of sleep-wake and deep body temperature ran internally synchronized in one section and internally desynchronized in another part of the same experiment. This study includes rhythms of 12 female and 21 male volunteers. In 13 experiments, the state of internal desynchronization was characterized by sleep-wake rhythms with periods that were distinctly shorter than the periods of the body temperature rhythms ('desynchronization by shortening'); in 20 experiments, the sleep-wake period was distinctly longer than the temperature period ('desynchronization by lengthening').

The experiments lasted for at least 1 month each; they were performed in an underground isolation station, details of which have been described elsewhere[4]. The sleep-wake alternation has been measured in several ways: 1. the subjects indicated subjectively when they had 'day' and 'night'; 2. bed movements were recorded, allowing differentiation between sleep and lying in bed awake (e.g., reading); 3. in about 10% of all subjects sleep was monitored polygraphically (EEG, EOG, EMG). Only the last method allows unambiguous determination of sleep. However, comparison of the results obtained by recording bed movements with those from EEG-recording showed that they almost coincided, with a consistent difference of only a few minutes. Therefore, for the sake of uniformity, the generally available bed movement results were always used for determination of the sleep-wake rhythm. It has to be borne in mind, however, that the 'true sleep' period was actually consistently a few minutes shorter than the period indicated in this paper. Body temperature was measured continuously by means of a rectal probe. The probe was connected with recording equipment outside the experimental unit by a wire which was long enough to allow the subjects free movement in the experimental unit.

The period analyses used in this paper were based on 2 different methods. A) The linear regression was computed from corresponding events in successive cycles; these events are onset of wake or sleep, and extremum values in the rhythm of rectal temperature. B) Particularly for the data on rectal temperature, periodogram analyses were computed, using not only 2 pieces of data per cycle but all data obtained within the time series. Both types of analysis led to period estimates which coincided within a few minutes in all cases. Also both types of analysis were computed separately for the sections with internally synchronized and those with desynchronized rhythms. The sleep fractions were calculated, in a 1st step, for every one of the sleep-wake cycles; in a 2nd step, as averages over a whole experimental section.

Results and discussion

Typical examples of the experimental results are shown in figure 1. On the left, the course of an experiment is shown where internal desynchronization by shortening occurred spontaneously after 18 days. On the right, the course of an experiment is shown where internal desynchronization by lengthening occurred spontaneously after 14 days. For the 1st section of each experiment, all rhythms ran internally synchronized; during the 2nd section, the rhythms ran internally desynchronized. In both examples, the shift towards a 'new' periodicity of the sleep-wake rhythm was accompanied by a slight change of the temperature period in the opposite direction. And in both experiments, the sleep fraction became smaller in the 2nd section. The other 31 experiments of the sample showed, in principle, similar courses; only the time of the spontaneous change-over to internal desynchronization varied from experiment to experiment. In all experiments included in the sample each section lasted for at least 1 week.

The relevant results of all experiments are presented in table 1. This table shows for all individual subjects peri-

Table 1. Periods and sleep fractions for individual subjects before and after the spontaneous occurrence of internal desynchronization

Direction, sex	n	Internal synchronization		Internal desynchronization		
		$\tau_{RT=SW}$ (h)	Sleep (%)	τ_{RT} (h)	τ_{SW} (h)	Sleep (%)
Shortening, ♀♀		24.2	41.9	24.7	16.6	33.8
		24.3	36.6	24.6	18.9	31.7
		24.4	31.4	24.9	15.7	28.2
		24.5	43.2	24.8	18.2	30.8
		24.6	30.8	24.9	18.0	24.4
		24.7	39.7	25.0	17.2	37.0
		25.0	43.0	25.2	17.4	37.7
	7	24.53 ± 0.27	38.09 ± 5.28	24.87 ± 0.20	17.43 ± 1.07	31.94 ± 4.73
Shortening ♂♂		24.3	32.5	25.0	18.8	29.0
		24.5	29.0	24.8	18.6	25.8
		24.5	38.4	24.9	18.0	36.6
		24.6	41.5	24.9	18.1	38.2
		24.6	34.5	24.9	16.8	27.8
		24.9	28.0	25.1	18.4	26.3
	6	24.57 ± 0.20	33.98 ± 5.27	24.93 ± 0.10	18.12 ± 0.71	30.62 ± 5.40
Lengthening ♀♀		24.9	38.0	24.5	31.3	36.0
		24.9	39.1	24.6	31.4	28.9
		25.1	42.5	24.7	40.7	28.0
		25.8	35.9	25.1	33.3	21.8
		26.6	35.3	25.2	36.5	32.6
	5	25.46 ± 0.74	38.16 ± 2.87	24.82 ± 0.31	34.64 ± 3.99	29.46 ± 5.33
Lengthening ♂♂		24.8	25.7	24.8	32.2	15.6
		24.9	26.4	24.7	33.3	18.0
		25.0	27.4	24.4	33.6	26.9
		25.1	28.2	24.6	33.6	25.4
		25.2	34.5	25.0	33.4	29.0
		25.2	25.0	24.8	36.0	26.7
		25.3	28.6	24.9	33.4	27.0
		25.3	33.7	25.0	30.9	32.9
		25.4	37.5	24.8	30.9	36.2
		25.8	39.3	24.9	36.9	39.5
		25.8	31.7	25.1	32.8	34.7
		25.9	20.5	24.8	38.8	19.4
		25.9	29.7	24.7	36.0	27.5
		26.0	33.0	24.8	32.8	29.6
		26.5	32.9	25.3	34.3	31.5
	15	25.47 ± 0.48	30.27 ± 5.03	24.84 ± 0.21	33.93 ± 2.18	27.99 ± 6.67
Shortening	13	24.55 ± 0.23	36.19 ± 5.48	24.90 ± 0.16	17.75 ± 0.95	31.33 ± 4.88
Lengthening	20	25.47 ± 0.54	32.25 ± 5.72	24.84 ± 0.23	34.11 ± 2.63	28.36 ± 6.26
♀♀	12	24.92 ± 0.68	38.12 ± 4.27	24.85 ± 0.24	24.60 ± 9.22	30.91 ± 4.92
♂♂	21	25.21 ± 0.59	31.33 ± 5.26	24.87 ± 0.19	29.41 ± 7.55	28.74 ± 6.32
Total	33	25.11 ± 0.63	33.80 ± 5.88	24.86 ± 0.21	27.66 ± 8.38	29.53 ± 5.86

Symbols: τ_{RT} = period of the freerunning rhythm of rectal temperature, τ_{SW} = period of the freerunning rhythm of sleep-wake.

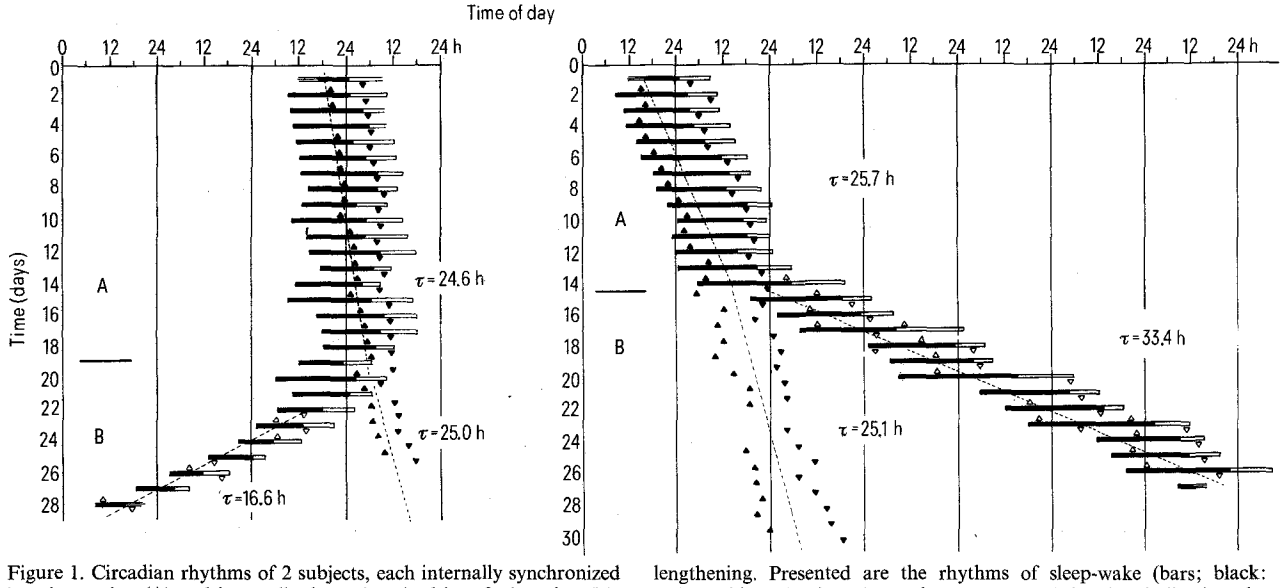

Figure 1. Circadian rhythms of 2 subjects, each internally synchronized in a 1st section (A) and internally desynchronized in a 2nd section (B), with spontaneous transitions. The left example shows desynchronization by shortening and the right example shows desynchronization by lengthening. Presented are the rhythms of sleep-wake (bars; black: wake; white: sleep) and rectal temperature (triangles, indicating the temporal positions of the extremum values).

ods and sleep fractions, before and after the spontaneous occurrence of internal desynchronization. Before the separation, by definition, the periods of the rhythms of sleep-wake and rectal temperature coincided, while they were different after the separation and are, therefore, given separately. Moreover, the data are presented separately for the 2 sexes and the 2 directions of internal desynchronization. Means and standard deviations are given from the 4 groups, but also for the whole pool of the 2 sexes and for the 2 directions and, finally, for all experiments together.

Figure 2 illustrates the results concerning the sleep-wake rhythms. It presents, separately for every subject, sleep fractions versus sleep-wake periods (on a logarithmic scale); data from females and males are given with full and dashed lines respectively; means and standard errors of the 4 groups are also indicated. This figure shows that the sleep fractions are smaller during the state of internal desynchronization as compared with the synchronized condition, independent of the direction of internal desynchronization (the only exceptions derive from males with desynchronization by lengthening). Figure 2 also shows that, during the state of internal synchronization, the periods of those rhythms which will later desynchronize by lengthening are generally longer than the periods of those rhythms which will later desynchronize by shortening.

The (unequal) numbers of females and males included in the study (cf. table 1 and fig. 2) are the results of the fact that numbers were not equal in the sample of volunteers participating in the experiments involving temporal isolation; consequently, the portions of experiments showing internal desynchronization, in general, cannot be differentiated with regard to sex[4]. The direction of internal desynchronization, if it occurs, does however appear to be sex-dependent, although this dependency does not attain statistical significance (Fisher exact probability test: $p = 0.075$). Nevertheless, the phenomenon is similar to that found in a larger but less homogeneous sample where the sex-difference was significant[4]. 7 out of the 12 females (= 58%) who showed internal desynchronization, showed desynchronization by shortening, whereas only 6/21 males (= 29%) showed this distinct shortening of the sleep-wake rhythm. Conversely, only 5/12 females (42%) but 15/21 males (= 71%) showed internal desynchronization by lengthening.

The changes in the relevant rhythm parameters with the transition from internal synchronization to desynchronization are given in table 2, together with the statistical analyses and their respective levels of significance. It is not meaningful to include changes in the period of the sleep-wake rhythm, because these changes define the transition mentioned. Therefore, table 2 includes changes in the period of the temperature rhythms; because these changes are almost always in directions opposite to those in the sleep-wake periods (only in 1 subject did the temperature period remain unchanged, and in no subject did it change in the same direction as the sleep-wake period), the absolute amounts of these changes are indicated. The application of the non-parametric Wilcoxon test did not improve the results of the statistical analyses; therefore, only results of the parametric t-test are given. Table 2 also includes changes in the sleep fraction. Only in one sub-group (males with desynchronization by lengthening) have a few exceptions from the otherwise general 'rule' of reduction in the sleep fraction been found (cf. Table 1). In this sub-group also, the application of the Wilcoxon test did not

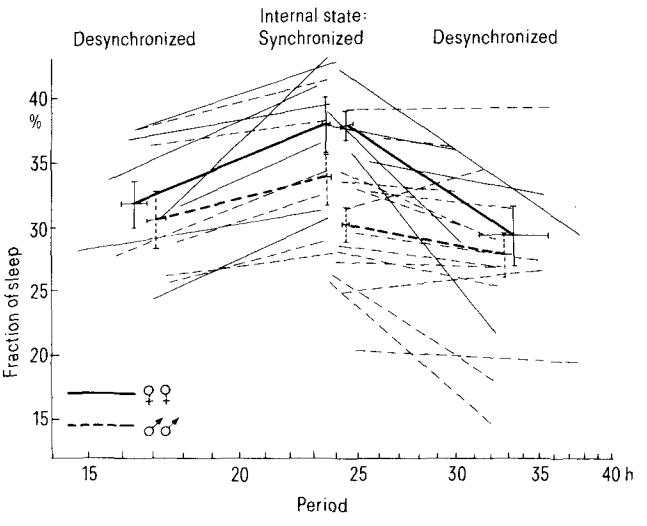

Figure 2. Fraction of sleep (ordinate) versus period of sleep-wake rhythms (abscissa; with logarithmic scale), obtained from subjects who showed internally synchronized rhythms in one section and internally desynchronized rhythms in another section of the same experiment; the lines combine results from the 2 sections each. Results from the 2 sexes are drawn with different signations. Means from the 4 sub-groups (2 directions of desynchronization and 2 sexes) are drawn with thick lines: SE for these sub-groups are indicated for both rhythm states.

Table 2. Changes in rhythm parameters with the transition between internal synchronization and desynchronization

Direction of desynchronization	Sex	n	Absolute change in the temperature period Mean SD (h)	t-test, t =	Error probability p <	Change in the sleep fraction Mean SD (%)	t-test, t =	Error probability p <
Shortening	♀♀	7	0.34 ± 0.11	8.000	10^{-3}	6.14 ± 3.31	4.908	0.01
Shortening	♂♂	6	0.36 ± 0.17	5.129	10^{-2}	3.37 ± 1.81	4.561	0.01
Lengthening	♀♀	5	0.64 ± 0.45	3.176	10^{-2}	8.70 ± 6.04	3.221	0.02
Lengthening	♂♂	15	0.63 ± 0.40	6.087	10^{-3}	2.28 ± 3.48	2.537	0.02
Shortening	Both	13	0.35 ± 0.14	9.170	10^{-6}	4.86 ± 2.99	5.861	10^{-3}
Lengthening	Both	20	0.63 ± 0.40	7.047	10^{-6}	3.89 ± 4.97	3.500	10^{-3}
Both	♀♀	12	0.47 ± 0.32	5.007	10^{-3}	7.21 ± 4.58	5.451	10^{-3}
Both	♂♂	21	0.56 ± 0.37	6.909	10^{-6}	2.59 ± 3.09	3.841	10^{-3}
Both	Both	33	0.52 ± 0.35	8.582	10^{-6}	4.27 ± 4.27	5.745	10^{-6}

lead to deviating error probabilities; therefore, again only results of the t-test are indicated. Table 2 shows that the observed changes are statistically significant at high levels; even all sub-groups the changes are still significant at fair levels. Only in the sub-groups with desynchronization by lengthening was the significance in the reduction of the sleep fraction rather low; this appeared to be due, in the female group, to the small number of subjects, and in the male group to the exceptions which we left in this sub-group.

The results shown in table 2 indicate that the occurrence of internal desynchronization is defined not only by a distinct change in the sleep-wake period but also by an opposite change in the period of the rectal temperature rhythm. Although the latter change was not so spectacular as the change in the sleep-wake period, it was still also highly significant. With regard to the consistency of the changes, it is obvious that the occurrence of desynchronization by lengthening does not only lead to more pronounced changes than does the occurrence of desynchronization by shortening, but the changes are also more variable. On the other hand, if females were compared to males there were not only no systematic differences in the amount of the changes, but also no difference in the variability of the changes. Table 2 also indicates that the occurrence of internal desynchronization is, with high probability, accompanied by a reduction in the average fraction of sleep. In evaluating this latter result, it must be considered that the intraindividual variability of the sleep fraction is much greater in the sections with internal desynchronization than in those with internal synchronization, as can be seen in figure 1.

With these computations, we have now a basis for a closer look at the sex differences between the 2 rhythm parameters. Table 3 summarizes differences between females and males (including statistical analyses) in the periods of the 2 rhythms investigated and in the sleep fractions. The values during the sections with internal synchronization and desynchronization are relevant; so are the variations of these values with the change in rhythm state (again, the absolute amounts of the changes form the bases of the analyses). Table 3 also includes, as an interesting side-results, analyses concerning the differences between the directions of internal desynchronization. From all data we extracted not only mean differences and standard deviations of these data, but also results of the parametric t-test and the non-parametric u-test; significances were calculated only for the (in most cases more conservative) u-test.

The combined periods during the state of internal synchronization show a significant sex difference when considered collectively, comparable to the sex difference in a previously analyzed sample of subjects with internally synchronized rhythms[7]. When considering the results of the cases with the 2 directions of the later desynchronization separately, no more sex differences were observed, for reasons to be discussed later. This indicates that the sex difference is due only to the sex difference in the fractions of rhythms to desynchronize in opposite directions. After the switch to internal desynchronization, the same is true of the separated periods of the sleep-wake rhythms. They also show a sex difference when considered collectively but not when considered separately for the 2 sub-groups. This, however, is not true for the periods of the separated temperature rhythms; neither all subjects when considered collectively, nor the sub-groups divided according to the direction of previous desynchronization show any sex difference; i.e., the separated temperature periods of females and males are identical within the accuracy of the

Table 3. Differences in several rhythm parameters between the different sub-groups with statistical analyses

Sub-groups to be compared	n_1/n_2	$\tau_{RT=SW}$ (syn) (h)	τ_{RT} (desyn) (h)	$\Delta\,\tau_{RT}$ (h)	τ_{SW} (desyn) (h)	$\Delta\,\tau_{SW}$ (h)	SF (syn) (%)	SF (desyn) (%)	Δ SF (%)
♀♀ vs ♂♂									
Shortening	7/6	0.04 ± 0.23	0.06 ± 0.16	0.02 ± 0.13	0.69 ± 0.88	−0.65 ± 0.91	−4.11 ± 5.05	−1.32 ± 4.83	−2.77 ± 2.62
		t = 0.287	t = 0.688	t = 0.296	t = 1.342	t = 1.223	t = 1.397	t = 0.472	t = 1.825
		u = 0.357	u = 0.714	u = 0.071	u = 1.214	u = 1.000	u = 1.429	u = 0.571	u = 1.500
		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Lengthening	5/15	0.01 ± 0.53	0.02 ± 0.23	−0.01 ± 0.40	−0.71 ± 2.62	−0.73 ± 2.49	−7.89 ± 4.51	−1.47 ± 6.23	−6.42 ± 4.07
		t = 0.047	t = 0.514	t = 0.031	t = 0.514	t = 0.550	t = 3.291	t = 0.444	t = 2.971
		u = 0.528	u = 0.000	u = 0.044	u = 0.000	u = 0.218	u = 2.750	u = 0.480	u = 2.313
		n.s.	n.s.	n.s.	n.s.	n.s.	p < 0.005	n.s.	p < 0.02
Total	12/21	0.29 ± 0.61	0.02 ± 0.21	0.09 ± 0.35	4.81 ± 8.05	−0.09 ± 2.22	−6.79 ± 4.85	−2.17 ± 5.77	−4.62 ± 3.63
		t = 1.315	t = 0.220	t = 0.707	t = 1.625	t = 0.105	t = 3.803	t = 1.021	t = 3.460
		u = 1.665	u = 0.318	u = 0.580	u = 1.722	u = 0.393	u = 3.219	u = 1.048	u = 2.900
		p < 0.05	n.s.	n.s.	p < 0.05	n.s.	p < 0.001	n.s.	p < 0.002
Shortening vs lengthening									
♀♀	7/5	0.93 ± 0.49	−0.05 ± 0.24	0.30 ± 0.28	17.21 ± 2.53	2.08 ± 2.46	0.07 ± 4.27	−2.48 ± 4.75	2.56 ± 4.39
		t = 3.116	t = 0.352	t = 1.702	t = 11.08	t = 1.378	t = 0.028	t = 0.851	t = 0.949
		u = 2.517	u = 0.482	u = 1.543	u = 2.842	u = 0.650	u = 0.406	u = 0.893	u = 0.487
		p < 0.01	n.s.	n.s.	p < 0.005	n.s.	n.s.	n.s.	n.s.
♂♂	6/15	0.90 ± 0.41	−0.09 ± 0.18	0.27 ± 0.35	15.81 ± 1.86	2.00 ± 1.75	−3.71 ± 4.97	−2.63 ± 6.20	−1.09 ± 3.05
		t = 4.397	t = 1.014	t = 1.545	t = 17.19	t = 2.305	t = 1.503	t = 0.854	t = 0.720
		u = 3.387	u = 1.323	u = 1.401	u = 3.503	u = 2.413	u = 1.362	u = 0.584	u = 1.557
		p < 0.001	n.s.	n.s.	p < 0.001	p < 0.01	n.s.	n.s.	n.s.
Total	13/20	0.92 ± 0.49	−0.06 ± 0.20	0.28 ± 0.32	16.36 ± 2.11	1.84 ± 2.03	−3.94 ± 5.54	−2.97 ± 5.67	−0.97 ± 4.28
		t = 5.858	t = 0.696	t = 2.412	t = 21.40	t = 2.495	t = 1.969	t = 1.446	t = 0.635
		u = 4.532	u = 0.903	u = 2.174	u = 4.790	u = 2.395	u = 1.787	u = 1.646	u = 1.603
		$p < 10^{-5}$	n.s.	p < 0.02	$p < 10^{-6}$	p < 0.01	p < 0.05	p < 0.05	n.s.

period determination. As a consequence, the changes of the periods of both rhythms – body temperature and sleep-wake – do not show any sex difference with changing state of the rhythm.

If the influence of the direction of desynchronization is considered, the result is similar: There is a highly significant difference in the temperature periods when coinciding with the sleep-wake periods in the state of internal synchronization; in either sex, this difference is significant statistically. In turn, there are identical periods in the separated temperature rhythms after the occurrence of internal desynchronization; the difference in the sleep-wake periods after the separation is trivial because it defines the difference in the direction of desynchronization. It is a rather secondary result that the absolute amounts in the changes of the sleep-wake periods are larger with desynchronization by lengthening than by shortening (when considering the logarithms of the sleep-wake periods, this difference disappears).

The interdependence between temperature periods and sex, and direction of desynchronization, respectively, are illustrated in figure 3. It presents the different distributions of the periods of freerunning temperature rhythms, in the upper part for females and in the lower part (with inverted ordinates) for males, with different indices for the opposite directions of desynchronization. From each of the 4 sub-samples, the period distributions before (uppermost and lowest curves) and after the spontaneous occurrence of internal desynchronization (center curves) are presented. For clarity, the hypothetical normal distributions for all samples are drawn; the distributions of the actual data do not show systematic deviations from the calculated ones. The temperature rhythms, while internally synchronized to the sleep-wake rhythms (upper and lowest curves), show clearly separated distributions with regard to the direction of the later desynchronization, in females and in males. With regard to sex, these distributions show equal peak periods and equal widths but different weights; in females, the (always smaller) distribution indicates that desynchronization by shortening has preponderance, and in males the (always broader) distribution indicates that desynchronization by lengthening preponderates. The combination of the two distributions would lead to combined distributions where 'center of gravity' would clearly be shifted to the left of the 25-h line in females, and to the right in males. For comparison, we have included in dotted lines the period distributions from a previously analyzed sample of subjects whose rhythms had remained internally synchronized over the whole experimental period[7]; the relative weights of these distributions are estimated according to the relative weights of the incidence of the different rhythm forms within the total sample of our experiments[4]. The comparison between the results from the present experiments (exhibiting internal desynchronization) and from the previous ones (remaining internally synchronized) suggests that the distributions originate from the same sample of female and male subjects.

After the shift to internal desynchronization, the distributions of the periods of the temperature rhythm coincide in all sub-samples, regardless of their origin. Moreover, the distributions of temperature periods become generally narrower after internal desynchronization. Furthermore, if one connects the centers of gravity of the distributions before and after the occurrence of internal desynchronization (as done in fig. 3), the slopes of these connecting arrows are identical for both sexes and only slightly different for the 2 desynchronization directions (i.e., somewhat steeper with desynchronization by shortening). The distributions of the sleep-wake periods in the state of internal desynchronization are not included in figure 3; they would be located far outside the abscissa scale, and the total width of the distribution, at least with desynchronization by lengthening, would exceed the total abscissa scale.

Table 3 includes, in addition to the periods, differences in the sleep fractions. In internally synchronized rhythms, the sleep fractions are greater by 21.7% in fe-

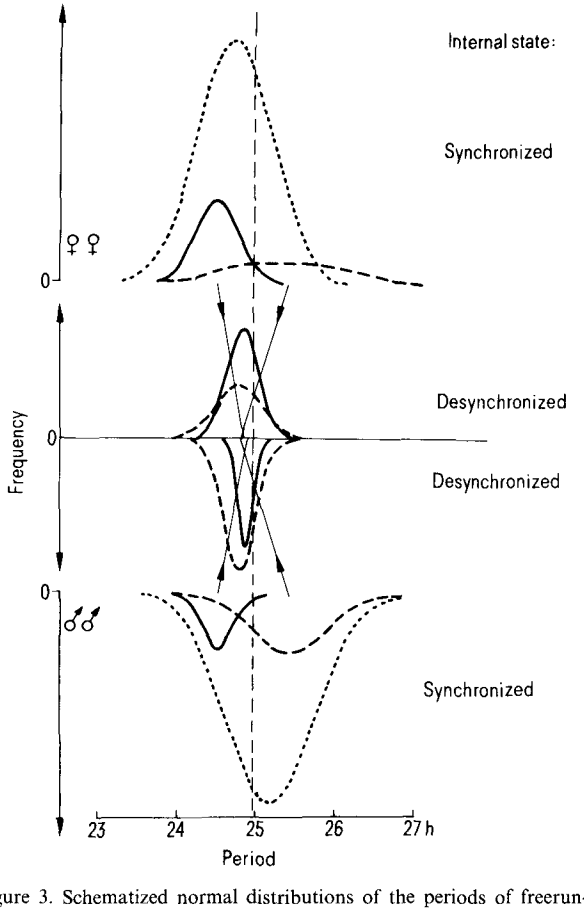

Figure 3. Schematized normal distributions of the periods of freerunning rhythms in rectal temperature (based on means and SD of the measured data) from a sample of 33 subjects. The data are presented, separately for 12 female (upper part) and 21 male subjects (lower part; with inverted ordinates), before (uppermost and lowermost diagrams) and after (middle diagrams) the spontaneous occurrence of internal desynchronization. The distributions from the different directions of desynchronization (according to the changes in sleep-wake) are indicated by differently drawn lines. The arrows indicate the changes in the mean periods accompanying the occurrence of internal desynchronization. Dotted lines: distributions of periods from samples with rhythms that remain internally synchronized in the long run as found in our earlier work[7], for comparison. —, Desynchronizing by shortening; ——, desynchronizing by lengthening; · · · · ·, remaining internally synchronized.

males than in males; this difference is fairly statistically significant. This sex difference is very similar to the one found in another sample of subjects with maintained internally synchronized rhythms[7]. During the synchronized condition, the sex difference in the sleep fraction is clearly larger in subjects with rhythms which will later desynchronize by lengthening than in those who will desynchronize by shortening; in the latter sub-sample it is not even significant. In the sections of the same experiments with internally desynchronized rhythms, the sleep fractions are, in fact, also slightly larger in females than in males (in the whole sample and in the sub-samples divided according to the direction of desynchronization), but not to a significant extent. This indicates that the change in the sleep fractions with changing state of the rhythm is considerably (nearly 3 times) larger in females than it is in males. Similarly to the sex differences in the sleep fractions themselves, the sex difference in the changes of the sleep fraction is more pronounced in rhythms with desynchronization by lengthening than in those with desynchronization by shortening. The relations between sleep fractions and state of the rhythm had been illustrated already in figure 2.

Conclusions

With regard to the periods, the present analyses clearly showed that internally synchronized rhythms, and the separated sleep-wake rhythms in internally desynchronized rhythms, are dependent on the sex of the subject. On the other hand, the separated periods in body temperature in internally desynchronized rhythms, are independent of the sex of the subject. According to the multi-oscillator concept (that had been shown to be successful in various respects), the period of an internally synchronized rhythm is a compromise between the intrinsic periods of the oscillators involved, while in the internally desynchronized condition the (specific) intrinsic periods of the different oscillators become clearly overt – at least in the average situation. Two different oscillators are represented by the overt deep body temperature rhythm, on the one hand, and the sleep-wake rhythm, on the other[3,4]. Since the 'temperature oscillator' ('type-I oscillator') is much stronger than the 'sleep-wake oscillator' ('type-II oscillator'), the former has a much greater weight in the establishment of the compromise; consequently, the equilibrium period is asymmetrically placed between the two intrinsic periods (i.e., closer to the temperature period[3]). Furthermore, with the shift from internal synchronization to desynchronization, the remaining mutual interaction between the 2 oscillators becomes insufficient for mutual entrainment; the release may be due to an exceptional disturbance in the course of random fluctuations, either in the amplitude of the rhythms (as a determinant of the coupling strength), or in the interval between the intrinsic periods of the 2 rhythms (or correspondingly, the mutual phase relationship). Due to the remaining interaction in the state of internal desynchronization, the rhythms show the phenomenon of 'relative coordination' as is obvious in the scalloping patterns of the phases of successive cycles within both rhythms.

The intrinsic periods of type–I oscillators are independent of sex, and independent of the tendency of the rhythm to desynchronize later. According to the present study, the mean $\pm$ SD of the human type-I periods ($n = 33$) is 24.86 ± 0.21 h. It is exclusively the type-II oscillator, represented by the sleep-wake rhythm, that is obviously sex-dependent. Since the measured periods in internally synchronized rhythms are compromises between the (stronger) sex-independent type-I oscillators and the (weaker) sex-dependent type-II oscillators, the differences between the intrinsic sleep-wake periods of females and males must be considerably larger than the measured sex difference in internally synchronizes rhythms. And since the interindividual variability of the periods of type-II oscillators is considerably greater than that of the type-I oscillators, the compromise periods in internally synchronized rhythms must show a greater variability (i.e., a broader distribution) than the periods of separated type-I oscillators in the state of internal desynchronization.

These interpretations are schematically illustrated in figure 4. It shows the hypothetical distributions of the intrinsic periods of the 2 oscillator types (with logarithmic abscissa scale). The distributions of type-I, or temperature, oscillators coincide for both sexes; the common distribution is very narrow and, correspondingly, tall (the frequency at the peak is a multiple of the figure size, as indicated by the percentage values). For type-II, or sleep-wake, oscillators, there are 2 separate broad distributions for the 2 sexes. The distribution of female sleep-wake oscillators has a mean period shorter than that of the (common) distribution of temperature periods, and the distribution of the male sleep-wake oscillators has a mean period clearly longer than the mean temperature period; the distribution for females is slightly broader than that for males. The arrows indicate the range of mutual entrainment, i.e., the ranges where the intrinsic periods of the sleep-wake and temperature rhythms are close enough to become synchronized. Although the total period range of mutual entrainment (i.e., the range covered by the arrows) is identical in females and males according to the identical temperature periods, the center of gravity of the sleep-wake periods within this range is shifted towards shorter periods in females as compared to males. Correspondingly, the mean compromise periods of internally synchronized rhythms are, on the average, shorter in females than in males. The ranges outside the arrows indicate sleep-wake periods of internally desynchronized rhythms; since the sections of the 2 distributions within these ranges have similar courses each but with different levels, the mean periods of internally desynchronized rhythms in either direction should be similar in both sexes though they should have different weights in the 2 different directions. In general, the limitations of the different period ranges mentioned are determined by the periods of the (stronger) temperature oscillator which are identical in females and males. Consequently, the limitations coincide in females and males, regardless of the different weights of the periods ranges in the 2 sexes. It is for this reason that the periods in the different rhythm states appear to be 'gated', independent of sex.

The observed difference in the periods of internally synchronized rhythms that will desynchronize later by shortening and lengthening respectively, makes it possible to predict the direction of the later desynchronization. This obvious difference also allows rejection of the frequently expressed opinion that desynchronization by shortening is not fundamentally different from desynchronization by lengthening, but is possibly based on wrongly estimated naps; this erroneous opinion may be based on the observation that the sleep-wake periods in both types of desynchronization are, on the average, close to the integral relationship of 1:2. Moreover, the present sub-samples of periods of internally synchronized rhythms when divided according to the direction of later desynchronization, deviate significantly from the previously analyzed sample of periods from rhythms that stay internally synchronized over the whole experiment[7]. Such differences make it possible to predict whether or not a freerunning, internally synchronized rhythm will desynchronize in its later course: if the period is close to 24.9 h, it will stay internally synchronized, but if the period deviates from this value by about 0.5 h or more, it will desynchronize internally with high probability later-on. In particular: if the freerunning period is shorter than about 24.5 h, the rhythm will probably desynchronize by shortening; and if the period is longer than about 25.5 h, the rhythm will probably desynchronize by lengthening.

In summary, the present results show the following: the intrinsic periods of female sleep-wake rhythms are shorter than those of male rhythms, while the intrinsic periods of the body temperature rhythms coincide in both sexes. Consequently, the temperature rhythm relative to the sleep-wake rhythm should be relatively later in phase in females as compared to males (provided that the amplitudes, which also determine the internal phase relationship, are comparable). In the natural 24-h day where both rhythms are externally and internally synchronized, the phase of the combined circadian rhythms relative to the zeitgeber, i.e., to local time, should be earlier in females than in males; i.e., females should tend more than males to be 'morning types'. The results mentioned also suggest that the intrinsic periods are closer to 24 h in females than in males. Consequently, the probability of becoming desynchronized from the 24-h day is greater in males than in females. In other words, men need stronger external zeitgebers than women to guarantee synchronization to the 24-h day. The sex differences in the intrinsic periods of circadian sleep-wake rhythms, therefore, may be the source of the sex-dependent incidence of specific rhythm disorders, and of diseases accompanying these rhythm disorders. If such disorders occur, those with shortened rhythms, or phase advances in the case of remaining external synchronization, should be more frequent in females than in males.

Another aspect of the present results deserves attention. The mean intrinsic period of the sleep-wake rhythm deviates clearly from that of the temperature rhythm in either sex, in opposite directions but by similar amounts (cf. fig. 4). Possibly, these internal deviations have the same adaptive value as the (general) deviation of the periods of freerunning rhythms from the earth's rotation[6]: if 2 mutually synchronizing rhythms have identical intrinsic periods, i.e., if the system is in an indifferent equilibrium with respect to the phases, their mutual phase relationship is less stable than with slightly differing intrinsic periods. In the case of external synchronization this means that the intrinsic periods should not coincide with the zeitgeber period to guarantee a stable external phase relationship. Correspondingly, in the case of mutually synchronizing rhythms this means that the intrinsic periods of the 2 rhythms should deviate from each other to guarantee a stable internal phase relationship. Such a deviation has, in fact, been observed.

The other rhythm parameter discussed in this paper, i.e. the fraction of sleep, is, by definition, a property of the sleep-wake rhythm. However, it is not independent of the temperature rhythm, as had been shown by the significantly positive interindividual correlation between sleep fraction and amplitude of the temperature rhythm[5]. The existence of such a correlation is confirmed also in the present sample. The amplitude of the temperature rhythm had previously been shown to be generally smaller in internally desynchronized than in synchronized rhythms[4], and the sleep fraction was also shown to be generally smaller in internally desynchronized than in synchronized rhythms[6]. The present study not only confirms this result, but also enlarges on it concerning the sex difference.

In the first instance, in internally synchronized rhythms the fraction of sleep is larger in females than in males (on the average by 21.7%); the direction of later desynchronization is only of marginal influence. This sex difference is very similar to that found previously in rhythms that remain internally synchronized in the long run[7]. According to this result (together with the sex difference in the period), the average sleep episode is longer in females than in males by 1.60 h, and the average wake episode is shorter in females than in males by 1.89 h; particularly the latter estimate may have implications with regard to a differential 'feeling of time' in both sexes.

The primary result of this study concerning the sleep

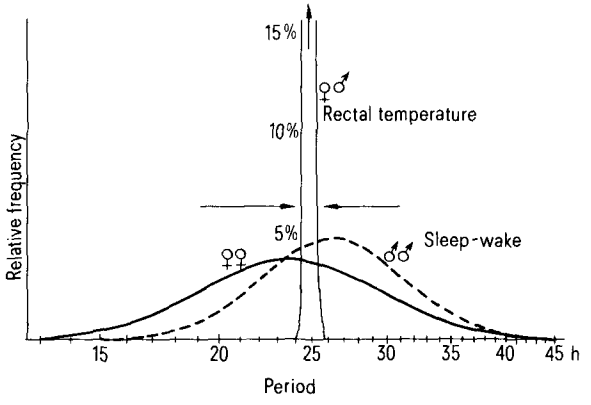

Figure 4. Schematized normal distributions of intrinsic periods of freerunning rhythms, as deduced from the measured data. Presented are separated distributions of sleep-wake periods for females and males, and the combined distribution of temperature periods (identical for females and males).

fraction is the sex difference in the reduction of this fraction with the transition from internal synchronization to desynchronization (the reduction is nearly independent of the direction of desynchronization); on the average, this reduction is nearly 3 times greater in females than in males. As a consequence, the sex difference in the sleep fractions in internally desynchronized rhythms nearly disappears. In the total sample and in the 2 sub-samples separated according to the direction of desynchronization, the sleep fraction is still larger in females than in males, but no longer to a significant extent. Unfortunately, therefore, it cannot be decided whether or not a relevant sex difference exists in the sleep fraction of internally desynchronized rhythms, although there is a trend in the same direction as with internally synchronized rhythms. It can be stated, however, with certainty that freerunning female rhythms react, with respect to the sleep fraction, more strongly to the occurrence of rhythm disorders than do male rhythms, independent of the direction of the rhythm disorder. This sex difference in the change of the sleep fraction may reflect a higher sensitiveness of females than of males to rhythm disorders, in general.

As mentioned above, all results concerning internally synchronized rhythms obtained in the present study are in agreement with results of a previous study including another sample of subjects, with rhythms that remained internally synchronized in the long run[7]. The present results concerning sleep fractions are also in agreement with results of another previous study dealing with seasonality[8]. That study was based on a much larger but non-homogeneous sample of subjects, with internally synchronized and desynchronized rhythms; it described a seasonal variation in the sleep fraction (smaller fractions in spring and larger fractions in autumn) but always a larger sleep fraction in females than in males. Results concerning sex differences in freerunning human rhythms, unfortunately, are not known from other groups of authors. Corresponding results from animal experiments are also rare. Aschoff[1] reported a slight but not significant sex difference in the period of chaffinches (*Fringilla coelebs* L.): the freerunning period was, in different intensities of constant illumination, slightly longer in females than in males. The only other species where sex differences in freerunning rhythms have been reported, seems to be the hamster (*Mesocricetus auratus* L.); Davis et al.[2] found slightly shorter periods and slightly more sleep in females than in males; hence, the direction of the sex differences in these mammals were the same as in man; unfortunately, however, these animal results were not significant statistically, at an acceptable level.

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0014-4754/84/111226-09$1.50 + 0.20/0

Immune-mediated glomerulonephritis induced by mercuric chloride in mice[1]

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Summary. The BALB/c mouse developed mesangial deposits of immune constituents and light microscopical changes characteristic of immune complex glomerulonephritis after 8 weeks' treatment with mercuric chloride given by s.c. injection. There were no signs of linear of granular immune deposits along the glomerular capillary basement membrane after 2 or 8 weeks. The antigen could not be identified. No antibodies to nuclear or renal structures were found. Using a histochemical method (silver amplification) mercury was detected by light and electron microscopy in tubular and glomerular structures. Mercury was present in secondary lysosomes of the mesangial cells after eight weeks of mercury poisoning.
Key words. Mercuric chloride; BALB/c mice; immunofluorescence; silver amplification.

Introduction

The kidney is the main target organ after exposure to inorganic mercuric salts[2], which are generally believed to damage the proximal tubular epithelial cells in both man[25,27] and rodents[16,21]. Brown Norway (BN) rats given mercurial substances orally, parenterally, or by inhalation develop a biphasic autoimmune disease[4,11,12] which is often self-limiting if the animal survives the initial phase[3,10]. Antibodies to the glomerular basement mem-