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CHARACTERISTICS OF CIRCADIAN RHYTHM IN SIX VARIABLES OF MORNING ACTIVE AND EVENING ACTIVE HEALTHY HUMAN SUBJECTS

S. GUPTA AND A. K. PATI

School of Life Sciences,
Pt. Ravishankar Shukla University,
Raipur - 492 010

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Abstract : Ten apparently healthy human subjects, inhabitants of dry and hot tropical climate, volunteered for the study. They were instructed to self-measure, several variables, such as oral temperature, heart rate, subjective fatigue, subjective drowsiness, finger counting speed and random number adding speed, daily, preferably at an interval of 4h, for 10-15 days. However, they were instructed to have *ad libitum* sleep and not to truncate sleep for the sake of self-measurements. The subjects were categorized into two groups, viz, morning active and evening active and their circadian time structure in the above mentioned variables was studied. The results clearly show a higher order of internal synchronization among all the variables. Comparison of acrophase locations between morning active and evening active individuals reveals that the peaks in oral temperature, heart rate and random number adding speed occurs earlier in morning active individuals (P<0.002, P<0.005, P<0.001, respectively). The circadian profiles obtained in this study may serve as guide lines in the study of human health and diseases.

Key words : chronotype circadian rthyhm morning active evening active amplitude

INTRODUCTION

It has been well established that rhythms in various physiological, immunological, psychological and behavioural end points, under natural conditions, exist and exhibit periodicity of about 24 h (1, 2, 3, 4, 5, 6). Most of the studies performed in human circadian rhythms have been restricted to the temperate regions of the world. Climatic conditions have been reported to interfere in the process of rhythm synchronization or desynchronization. However, it is not well documented as to, howfar, climatic conditions specially of tropical regions could influence the pattern of circadian rhythms.

The present study included subjects those represent human population living in hot and dry tropical climatic conditions of Raipur 19°50' to 21°53' N and 81°25' to 83°38' E. Two types of chronotypes, such as morning active individuals and evening active individuals were identified in the local population with the help of specially designed questionnaire (7,8,9) and their circadian time structure was studied.

METHODS

Subjects : Ten apparently healthy subjects inhabitant of Raipur (19°50' to 21°53' N and 81°25' to 83°38' E) volunteered for the study. Various biometric and physiologic characterstics of the subjects are shown in Table I. During the course of this study, the subjects followed their normal routine and were instructed to maintain daily diary. No restriction was imposed upon their sleep schedules. They were allowed to sleep *ad libitum* during the customary timings.

Data collection : The subjects were instructed to self-measure several variables, such as oral temperature (OT), heart rate (HR), subjective fatigue (SF), subjective drowsiness (SDR) (10), finger counting

TABLE I : Biometric and physiologic characteristics of 10 diurnally active healthy human subjects.

Subject Code	*Sex*	*Age (y)*	*Weight (kg)*	*Height (cm)*	*BSA (m^2)*	*Chronotype*
C # 01 (AK)	M	22	50	165	1.53	MA
C # 02 (RR)	M	25	53	165	1.57	EA
C # 03 (PS)	F	23	52	162.5	1.54	MA
C # 04 (IVG)	F	23	50	160	1.5	MA
C # 05 (AD)	F	25	43	156.2	1.38	MA
C # 06 (DS)	F	22	58	170.0	1.67	EA
C # 07 (NM)	M	24	50	157.5	1.48	MA
C # 08 (SAC)	M	22	50	162.5	1.52	EA
C # 09 (MST)	M	23	64	170.0	1.74	EA
C # 10 (SJ)	F	25	42	147.5	1.31	MA

Body surface area (BSA/m^2) was obtained by Du Bois's formula. MA stands for morning active and EA for evening active.

speed (FCS) and random number addition speed (RNAS) (11), every 4 h, except during sleep, on daily basis at least for 10-15 days. Prior to the beginning of data collection, each subject was given data sheets, stop watch and clinical thermometer and was trained to use those properly. The subjects were also provided with questionnaires to obtain information on their day to day sleep quality and quantity. During the actual experimental periods, random checks were undertaken to ensure that the various self-measured variables are properly recorded. In case of any discrepancy, the whole 24-h data of that particular subject was rejected.

Statistical analysis : The self-measured variables were analysed for documenting a circadian rhythm (period = 24 h) with the help of cosinor rhythmometry (12, 13). Various end points, viz., mesor (rhythm-adjusted mean), amplitude (half the difference between the highest and the lowest value) were computed. A power spectrum method (14) was used to detect prominent period in the time series for each subject and for each variable. It has now been clearly demonstrated that missing data during sleep do not alter circadian rhythm parameters, viz., period, mesor, amplitude, and acrophase (15). Apart from the above special statistical techniques, t-test was also used to compare averages.

Morningness vs Eveningness : Morningness or eveningness in each subject was detected on the basis of a specially designed questionnaire. This was a shortened and simplified version of the questionnaire used by Ostberg (7). Such identifications were further confirmed by the results of the cosinor rhythmometry with a special reference to phases of individual's circadian rhythm in oral temperature (7,8,9).

RESULTS

In the present study, out of 10 subjects, 6 were found to be morning active and the remaining four were evening active.

Circadian periods : Prominent circadian periods for rhythms in all the six variables in each subject are presented in Table II. When the results of spectral analysis were pooled, irrespective of variable and personality of the subjects (Fig. 1), the frequency of 24 h period was 70%. In addition, 23% time series had period at the first circadian harmonics (i.e., period = 12 h). The remaining 7% time series neither exhibited exactly 24 h period nor 12 h period (Fig. 1). Fig. 1

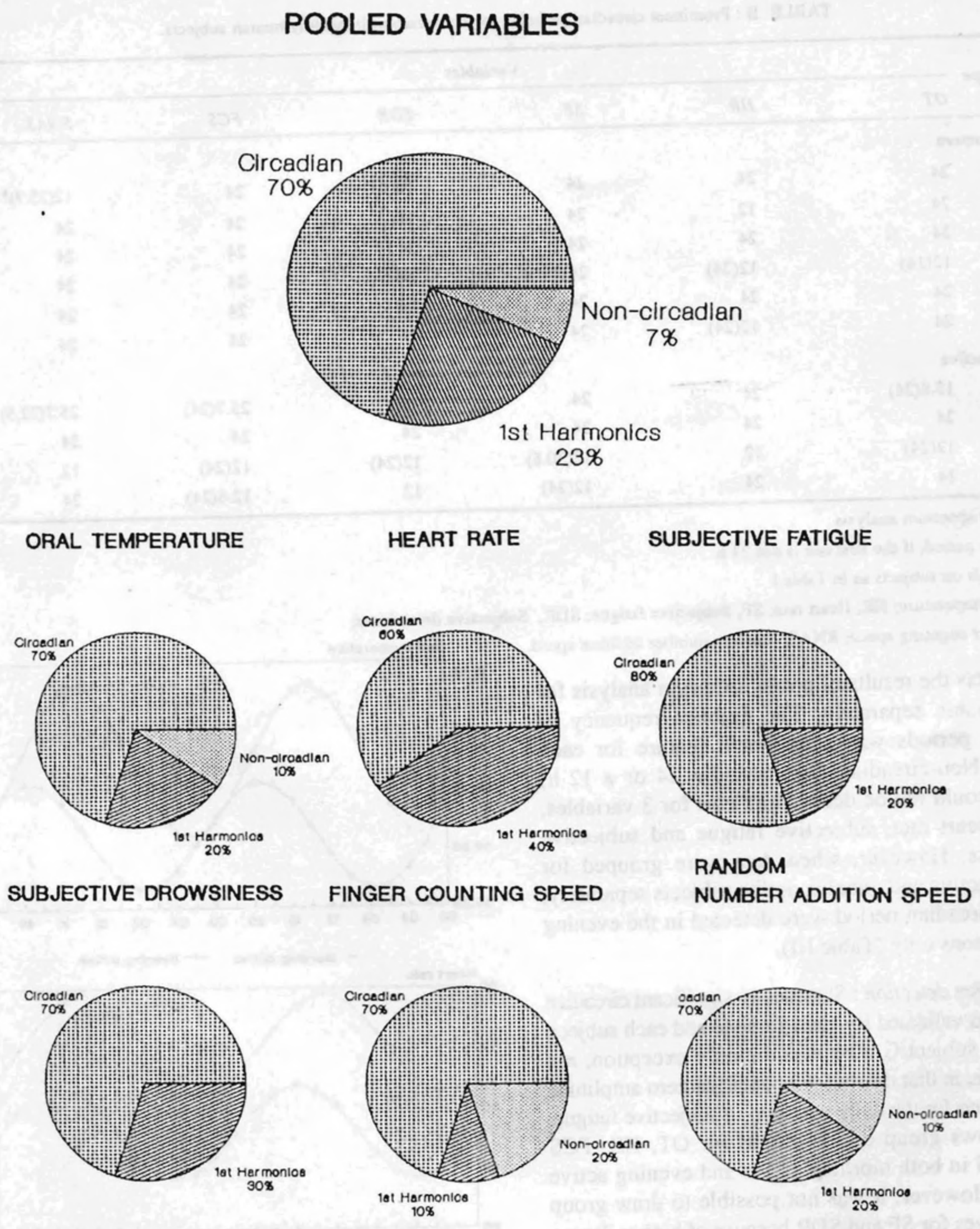

Fig. 1 : Individual and group frequency of circadian ($\tau = 24$ h), ultradian ($\tau = 12$ h, i.e., exactly of the first circadian **harmonics**) **and non-circadian** ($\tau = 24$ h and/or $\tau \neq 12$ h) rhythms in 6 variables. The data from all the 10 healthy human subjects have been pooled. The frequency of circadian periods was the dominant feature (see also Table II) and non-circadian rhythms were absent in heart rate, subjective fatigue and drowsiness.

TABLE II : Prominant circadian periods* for six variables in healthy human subjects.

Chronotype	Variables					
	OT	*HR*	*SF*	*SDR*	*FCS*	*RNAS*
Morning active						
C # 01	24	24	24	12(24)	24	12(25.7)[a]
C # 03	24	12	24	24	24	24
C # 04	24	24	24	24	24	24
C # 05	12(24)	12(24)	24	24	24	24
C # 07	24	24	24	24	24	24
C # 10	24	12(24)	24	24	24	24
Evening active						
C # 02	13.8(24)	24	24	24	25.7(24)	25.7(22.5)
C # 06	24	24	24	24	24	24
C # 08	12(24)	12	12(30.8)	12(24)	12(24)	12
C # 09	24	24	12(24)	12	12.6(24)	24

*By power spectrum analysis

[a]Prominant period, if the first one is not 24 h

Other details on subjects as in Table I

OT, Oral temperature; HR, Heart rate; SF, Subjective fatigue; SDR, Subjective drowsiness; FCS, Finger counting speed; RNAS, Random number addition speed.

also depicts the results of power spectrum analysis for each variable separately. The highest frequency of circadian periods was a dominent feature for each variable. Non-circadian (i.e., period ≠ 24 or ≠ 12 h) rhythms could not be detected at least for 3 variables, such as heart rate, subjective fatigue and subjective drowsiness. However, when data were grouped for morning active and evening active subjects separately, the non-circadian period were detected in the evening active persons only (Table III).

Rhythm detection : Statistically significant circadian rhythm was validated for each variable and each subject. However, subject C ≠ 08 was the only exception, not shown here, in that the null hypothesis of zero amplitude was accepted for the daily variation in subjective fatigue. Fig. 2 shows group cosine curves for OT, HR, FCS and RNAS in both morning active and evening active subjects. However, it was not possible to draw group cosine curves for SF and SDR because of higher degree of interindividual variation in these variables. However, for these two variables, individual cosine curves have been shown taking into cosideration two selected

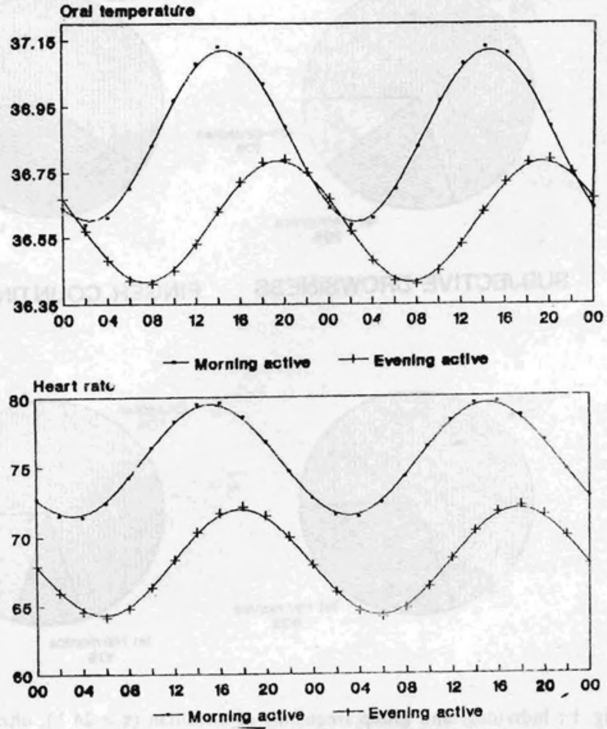

Fig. 2a : An illustrative example of circadian rhythm in oral temperature °C and heart rate (beats/min) of morning active and evening active subjects.

TABLE III : Showing the frequency of occurrence of circadian and non-circadian periods for six variables in human subjects.

	Morning active			*Evening active*		
	Circadian	*1st Harmonics*	*Non-circadian*	*Circadian*	*1st Harmonics*	*Non-circadian*
Variable						
OT	5	1	0	2	1	1
HR	3	3	0	3	1	0
SF	5	1	0	2	2	0
SDR	5	1	0	2	2	0
FCS	6	0	0	1	1	2
RNAS	5	1	0	2	1	1
TOTAL	29	7	0	12	8	4
PERCENT	81	19	0	50	33	17

Other details as in Table II.

individuals one from each morning active (C ≠ 03) and evening active (C ≠ 02) groups (Fig. 3).

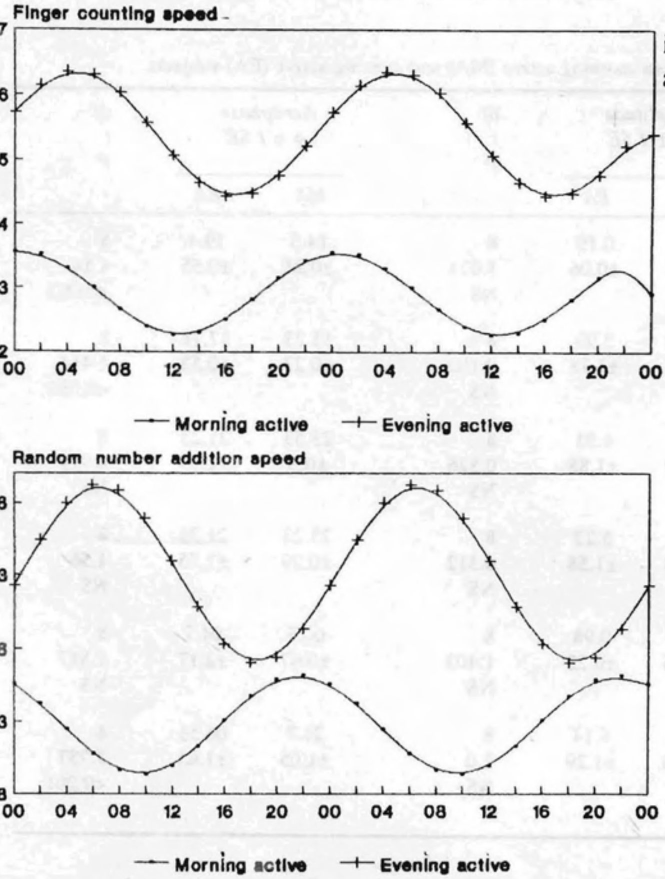

Fig. 2b : An illustrative example of circadian rhythm in finger counting speed (seconds) and random number adding speed (seconds) of morning active and evening active subjects.

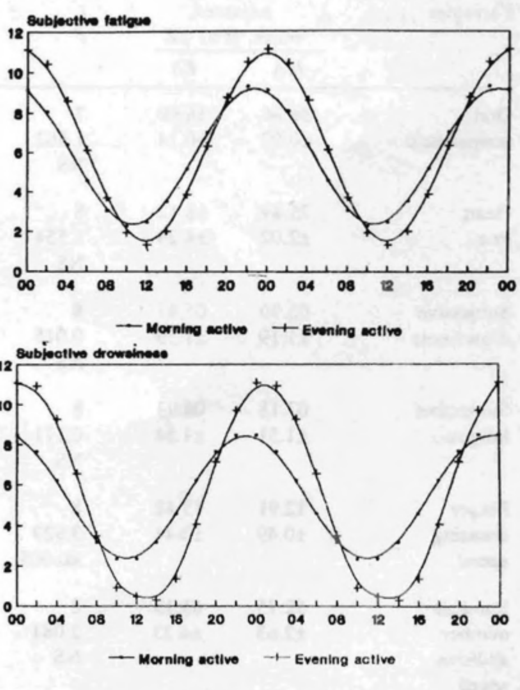

Fig. 3 : An illustrative example of circadian rhythm in subjective fatigue and subjective drowsiness of a morning active (C#02) and an evening active (C#03) subject.

Group averages were computed for mesors, amplitudes and acrophases for each variable under study with respect to morningness and eveningness. The data are presented in Table IV. When morning active and evening active groups were compared with each other, it was found that there was no statistically significant difference between both circadian mesors and amplitudes, excepting circadian mesor in the rhythm of finger counting speed (Table IV). However, when acrophase locations were compared it was noticed that peaks in oral temperature rhythm and heart rate rhythm occurred earlier in morning active individuals (Table IV; $P<0.002$ and $P<0.005$, respectively). Further, it was also revealed that the worst time for random number addition task in morning active persons was between 20.6-22.7 h. In contrast, the worst time for the same task in evening active persons was located in the morning between 4.9-8.2 h (Table IV).

DISCUSSION

Results of this study clearly demonstrate that variables under investigation show higher order of internal synchronization in terms of length of period and that the circadian period is the dominant feature for almost all the variables. The non-circadian component in the pooled data was negligible (only 7%). The detection of this component was restricted among evening active subjects. Could it be that evening active perosns suffer from internal desynchronization of rhythms? It is remarkble that in general the next prominant period was of 24 h, in all those vriables which showed non-circadian periods as the first prominent period. Occasional internal desynchronization among several circadian rhythms has also been demonstrated in apparently healthy, diurnally active persons (16,17). In this study, extent of internal desynchronization was of low magnitude.

TABLE IV : Comparison of rhythm parameters between morning active (MA) and evening active (EA) subjects.

Variables	*Rhythm adjusted mean, M±1 SE*		*df* *t* *P*	*Amplitude* *A ± 1 SE*		*df* *t* *P*	*Acrophase* *ϕ ± 1 SE*		*df* *t* *P*
	MA	*EA*		*MA*	*EA*		*MA*	*EA*	
Oral temperature	36.86 ±0.09	36.60 ±0.14	7 1.562 NS	0.27 ±0.05	0.19 ±0.06	8 1.024 NS	14.5 ±0.85	19.4 ±0.55	8 4.84 <0.002
Heart rate	75.49 ±2.02	68.12 ±4.29	8 1.554 NS	4.19 ±0.81	3.99 ±1.78	8 0.102 NS	15.23 ±0.23	17.78 ±0.53	8 4.414 <0.005
Subjective drowsiness	05.90 ±1.19	05.87 ±1.59	8 0.015 NS	6.14 ±1.39	4.91 ±1.88	8 0.526 NS	23.53 ±0.5	21.23 ±2.19	8 0.979 NS
Subjective fatigue	07.18 ±1.51	06.94 ±1.54	8 0.111 NS	4.64 ±0.98	5.22 ±1.58	8 0.312 NS	23.28 ±0.29	21.23 ±1.75	8 1.56 NS
Finger counting speed	12.91 ±0.49	15.42 ±0.41	8 3.929 <0.005	0.66 ±0.06	0.98 ±0.22	8 1.403 NS	00.5 ±0.67	04.7 ±2.17	8 1.847 NS
Random number addition speed	52.75 ±2.63	63.13 ±4.23	8 2.084 NS	3.42 ±0.43	6.14 ±1.29	8 2.0 NS	21.7 ±1.05	06.53 ±1.65	8 7.757 <0.001

df = degrees of freedom
t = t-value (Student's t-test)
P = probability level
NS = not significant

When the data were grouped on the basis of morningness and eveningness it was observed that later individuals have poor eye-hand coordination ability.

As regards phase position of several rhythms studied herein, statistically significant difference was witnessed in circadian rhythms of oral temperature (OT), heart rate (HR), and random number addition speed (RNAS) only. In both OT and HR rhythms, the circadian acrophases (peaks) occurred earlier in morning active individuals than that in evening active individuals.

The results of the present study coroborate the findings reported earlier in that the morning types show advancment and evening types delay in their circadian phase position in body temperature rhythm (6, 18, 19, 20, 21). The present results demonstrate phase advancement of HR rhythm in morning active individuals, in addition to OT rhythm reported earlier.

The other important finding of the present study relates to rhythm in random number addition speed. The morning active subjects were at their best between 8.6-10.7 h for this task, whereas evening active subjects had their best time between 16.9-20.2 h.

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