Short-duration judgment in young Indian subjects under 30 h constant wakefulness

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The present study aimed to investigate probability of a possible endogenous circadian rhythm in human cognitive attribute to estimate short intervals. Apparently healthy young males and females were selected for our study. Eight subjects prospectively produced the short-time intervals 10 s and 60 s at 2 hourly intervals in 30 h constant routine (CR) study conducted in spring (CR-1). The study was repeated again in autumn (CR-2) in the remaining eight subjects. The established circadian markers, namely serum cortisol, salivary melatonin levels and tympanic temperature were also measured either in CR-1 or CR-2. Oral temperature was measured simultaneously. Circadian rhythms were validated in serum cortisol, salivary melatonin, oral, and tympanic temperatures. Circadian rhythm in 60 s estimates was observed in a few subjects and in all males at group level in CR-1. The cognitive attribute to perceive short intervals vary as function of season. The results provide evidence in support of interaction among the interval, circadian and circannual timing systems in human.

Keywords: Constant routine, Cortisol, Endogenous circadian rhythm, Melatonin, Prospective paradigm, Time estimates, Tympanic temperature

In human, most of the physiological and behavioural processes exhibit circadian rhythms. Presence of externally and internally synchronized circadian rhythms is a sign of health in humans under daily changing ambient milieu. In addition to circadian timing system, humans possess interval timing system. The later is popularly called biological stopwatch¹⁻³. The interval timing is related to the mental tracking of durations in the range of seconds to minutes⁴,⁵. It is a cognitive trait that helps in tracking short-lasting events in the instant environment and in producing commensurate response timely. The importance of interval timers has been recognized in wild animals. They use it frequently in foraging and also as a safety tool to survive attack from its predators⁶,⁷. In our day-to-day activities, we can notice its implication in decision making activities, such as catching a cricket ball in the playground, timely shuffling of bread on top of the pan in the kitchen, taking a decision to move left or right on confronting a car on the road, to name a few. The time sense of an individual also determines the mood of that individual waiting for his/her turn in a long queue in shop. The suprachiasmatic nucleus (SCN) in the brain is well known as the central circadian pacemaker. The brain also has ensemble of specialized structures that carry out interval timing. The main regions involved in interval timing in humans are the prefrontal cortex, basal ganglia, thalamus and the cerebellum along with the neurotransmitter dopamine⁶,⁸⁻¹⁰.

The prospective paradigm is mostly used to gauze the passage of time. In this paradigm the subjects have prior knowledge that they have to judge the duration of ongoing events. The second mode of judgment is the retrospective judgment. In this paradigm an individual is unaware of the task to estimate the duration of any event and after the completion of the event the individual is asked about the elapsed duration of the event¹¹,¹². The prospective estimation of time is based on the attention paid during estimation, whereas the retrospective judgment mostly relies on the memory. Production method is one of the popular methods that rely on the production of the verbally asked target interval using some operation, for example pressing the button of a stopwatch twice to indicate start and stop of the target interval¹³⁻¹⁷.

The association of cognitive variables with body temperature or sleep-wake cycle has been reported
earlier. Increasing body temperature has been reported to speed up the estimation rate, thus shorter estimations become overt with elevated body temperature. Pati and Gupta reported better judgment of short-intervals at higher body temperature. Gender difference in interval timing has also been observed in many studies. Among environmental factors, the effect of light on short-time estimation has been well studied. The psychological and performance variables, like alertness, mood, and concentration exhibit circadian rhythm. The time of the day has been observed to influence the short-time estimation and performance variables, such as alertness and mood. The isolation from the periodic changing environmental factors like day-night is regarded as the best way to evaluate the underlying endogenous rhythm. Mills et al. proposed a protocol, namely the “constant routine protocol” that is proved to be a reliable protocol to gauze circadian variation in any physiological, biochemical or behavioral variables. The purpose of the constant routine protocol is to minimize the masking effects of the ambient environmental conditions and behavioral processes. To achieve this ambient room temperature and light conditions are kept constant with minimum variations. In addition, sleep-wake cycle is made constant by allowing wakefulness of more than 24 h with reduced physical activities, so that the endogenous circadian rhythm could be expressed. Constant routine protocol has also been used to ascertain endogenous nature of rhythms in cognitive and performance variables, such as mood, alertness, vigilance task and their probable interaction with the sleep-wake cycle and body temperature rhythm.

The interval 60 s was habitually used to denote the duration of any event such as “one minute please” or “just a minute”. We observed earlier that the young subjects judge 60 s interval near to accuracy followed by 10 s. Therefore, to evaluate the possible endogenous rhythm in the judgment of the best short-time intervals (60 s and 10 s) in young Indians we designed the constant routine studies. The effects of seasons on circadian rhythm in interval timing have not yet been studied. Therefore, CR studies were planned in two different seasons of the year, spring and autumn. Simultaneously, circadian variability in oral temperature and circadian markers, such as serum cortisol, salivary melatonin and tympanic temperature was also examined in 30 h CR.

Materials and Methods

Subjects—Sixteen apparently healthy young subjects participated in two constant routine (CR) studies, namely CR-1 and CR-2. Different demographic and anthropometric data, such as age (y), height (cm), and weight (kg) were recorded. The body mass index (BMI in kg/m²) was calculated. In CR-1, eight subjects (mean age: 23.1 ± 0.58 y; median age: 23.5 y; mean BMI = 22.17 ± 1.47 SE) consisting of four males (mean age: 23.3 ± 0.85 y; median age: 23.5 y; mean BMI = 20.58 ± 0.99 SE) and four females (mean age: 23.0 ± 0.91 y; median age: 25.0 y; BMI = 23.76 ± 2.73 SE) participated. In CR-2, also eight subjects (mean age: 24.6 ± 0.78 y; median age: 23.5 y; BMI = 23.60 ± 1.86 SE), having four males (mean age: 25.3 ± 0.25 y; median age: 25.0 y; BMI = 23.76 ± 2.98 SE) and four females (mean age: 24.00 ± 1.58 y; median age: 24.5 y; BMI=23.9±2.7 SE) participated. Five subjects were common in both studies. We considered the subjects of CR-1 and CR-2 independent especially because the studies were conducted in two different seasons. The subjects were regular university students and were not engaged in any shift work. They voluntarily participated (were not paid for their participation). They did not self-report any neurodegenerative disease or psychological disorders or any sleep problem. All female subjects self reported regular menstrual cycle. One male subject (M#3) in CR-1 was an occasional smoker. Participants filled up informed consent form. The whole work involved non-invasive techniques, except the procedure involving collection venous blood for determination of serum cortisol. The protocol of both the CR studies was approved by the Institutional Ethics Committee of Pt. Ravishankar Shukla University, Raipur, India.

Protocol and procedure—The first constant routine study was conducted in spring season (April 13th-14th) and the second constant routine experiment was performed in autumn season (in October 10th-11th). The two studies were conducted with a gap of more than one year and in two different seasons as per
traditional classification of seasons in India. The study was conducted in Raipur, Southeastern India [latitude: 21° 30′ N; longitude: 82° 0′ E].

Prior to the beginning of the study a brief demo was given to the subjects regarding purpose of the study and the procedure to be followed by them throughout the session. Subjects were instructed to maintain their regular sleep-wake cycle, five days before the beginning of the study. They practiced for at least three times to estimate/produce the 10-s and 60-s intervals using digital stopwatch. During production of the intervals, each subject pressed the button of the stopwatch twice; the first indicating start of the estimation and the second indicating the end of the estimation of the target interval. The display of the watch was on the experimenter’s side. During estimation, the subjects were advised not to use counting strategy. They were also not permitted to keep with them wrist watch or any other time-keeping devices. All participants filled Horne & Östberg morningness-eveningness questionnaire (MEQ) for the assessment of chronotype[46]. In CR-1, out of eight subjects two were intermediate type and six were morning type. In CR-2, four subjects were intermediate chronotype and the remaining four were morning type.

The whole experiment was conducted in a sound attenuated, well-ventilated, and time piece free room. During the studies, each participant was tested separately and the result was not declared till the end of the experiment. During the study session, subjects sat comfortably in semi recumbent position. Subjects stayed awake continuously for 30 h in a room under partial social isolation with the minimum physical/locomotor activity. Room temperature (in CR-1: mean±SE = 32.3±1.59 °C; CR-2, = 31.9±1.99 °C) and light intensity (30 lux at subject’s eye level) were nearly constant. Similar and Isocaloric diet (200–300 kcal) was provided at 2-hourly intervals to each subject till termination of the study. Non – sparkling water was provided as per subjects demand. Light music and video were allowed half an hour prior to the beginning of each session.

The participants enter the experimental room at 09:00 hrs on the first day. They sat quietly in semi recumbent position. The study started at 10:00 hr. In each session, each subject produced 10- and 60-s intervals using production method with a digital stopwatch (Sunway, S2 – 1380). Each interval was produced thrice. The oral temperature (°F) was measured sublingually with digital thermometer (Digi Flexi) in both constant routine studies. In addition the tympanic temperature (°C) through ear thermometer (CT 830, CITIZEN) was also recorded in CR-2. The ear thermometer using its infrared sensor measures the temperature of the area around hypothalamus, the center for temperature control, thus regarded equivalent to core body temperature (CBT). In CR-1, blood (5 mL) was sampled through an intravenous catheter in the subject’s forearm to measure serum cortisol level (µg/dL) (Direct Immunoenzymatic assay, Equipar Diagnostici, Saronno, Varese, Italy). In CR-2, saliva sample was collected to measure the melatonin level (pg/mL) in saliva (Direct Saliva Melatonin ELISA, Bühlmann, Laboratories AG, Switzerland). All measurements were carried out at 2-hourly intervals for 30 h. Isocaloric food was provided after completion of each session. The study was terminated at 16:00 of day two after 30 h. Only one subject reported slight intolerance to CR.

Statistical Analysis—The data were recorded in MS Excel worksheet. For 10-s and 60-s estimates the average of two nearest estimates were worked out and mean theta estimate ‘θ’ (subjective estimation/ actual duration[41-43], such that θ = 1; perfect estimation, θ >1; longer estimation and θ<1; shorter estimation) was calculated. The circadian rhythms in different variables were analyzed using Cosinor method. The rhythm parameters estimated are Mesor (M, rhythm-adjusted mean), the amplitude (A, half of the difference between minimum and maximum in fitted cosine function), and the acrophase (∅, time of maximum in fitted cosine function). Student’s t-test was employed using SPSS (ver.16.0) for Windows. Two-way ANOVA was also employed using CoStat (CoHort Software; ver.4.02, ©). Duncan’s Multiple-Range test was applied as the Posthoc test for comparison of means.

Results

Circadian Rhythm—Circadian markers: Circadian rhythms in serum cortisol and salivary melatonin were documented in all females, all males and in all subjects at group level (Fig. 1A and B; Table 1). The peaks of cortisol and melatonin rhythms were located in the late and early morning hours, respectively (Fig. 1A and B; Table 1).

60-and 10-s production: A significant circadian rhythm was validated in production of 60-s interval in three subjects including two males and one female in
CR-1 (Fig. 2A-C) and one female in CR-2 (F#8; N=16, \( P<0.05, M\pm SE=0.27\pm0.01, A=0.05 \) [0.003, 0.103], \( \varnothing=06.34 \) [1.69, 10.99]). The significant circadian variation in 60-s production was also observed in males at group level in CR-1 (Fig. 2D; Table 2). The acrophase of 60-s estimation was observed in evening or night (M#3, F#2 and all males in group; Fig. 2A, C and D) or morning hours (in M#4; Fig. 2B and in F#8). A statistically significant circadian rhythm in 10-s production, with a peak in the morning, was observed in one female subject only (F#1: N=15, \( P=0.04, M\pm SE=0.88\pm0.02, A=0.09 \) [0.01, 0.17], \( \varnothing=09.19 \) [04.32, 14.06]) in CR-1. Circadian rhythm in 10-s production could not be validated at individual level in CR-2 and in group level in both CR-1 and 2 (Table 2).

Body temperature: Circadian rhythm in oral temperature was validated in all females, all males and all subjects at group level in both CR-1 and CR-2 (Fig. 3A and B; Table 2). All subjects exhibited circadian rhythm in ear temperature in CR-2 (Fig. 4; Table 1). The peaks of both oral and ear temperature rhythms were located between afternoon and evening hours.

Effects of seasons, time, and gender on short-time production: Although the ambient environments during both constant routine studies were almost similar, results from ANOVA revealed the effects of season (spring vs. autumn) on the production of both 60- and 10-s intervals (Table 3). From \( t \)-test it was confirmed that both 60- and 10 s were estimated better in spring than autumn season (Fig. 5). The interval 10 s was produced perfectly and 60 s was produced shorter in spring season. The interval 60 s was judged longer in spring than autumn season and vice versa for production of 10-s intervals. The interval was under produced in autumn season. Effect of seasons on oral temperature was not observed (Table 3). Effect of time on pooled subjects was observed only in oral temperature (Table 3).

Effect of gender on 60-s and 10-s judgment was not significant in both CR (\( P>0.05 \)). Gender as a factor influenced oral temperature in both constant routines (CR-1: \( F_{1.96}=18.571, P<0.001; \) CR-2: \( F_{1.98}=22.129, P<0.001 \)). The mean oral temperature in females was marginally elevated than that in males.

### Table 1—Cosinor summary: characteristics of rhythm parameters of circadian markers, such as serum cortisol (µg/dL), salivary melatonin (pg/mL) and tympanic temperature (°C) in apparently healthy human subjects at group level under 30-h constant routine in CR-1 (spring season) and CR-2 (autumn season)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Data point</th>
<th>Rhythm detection</th>
<th>Rhythm adjusted (mean ( \pm ) SE)</th>
<th>Amplitude, A (95% CL)</th>
<th>Acrophase, ( \varnothing ) in h (95% CL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum cortisol (CR-1)</td>
<td>Male</td>
<td>45</td>
<td>&lt;0.001</td>
<td>7.12 ( \pm ) 0.42</td>
<td>2.75 (1.24, 4.26)</td>
<td>08.29 (6.06, 10.52)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>41</td>
<td>&lt;0.001</td>
<td>7.82 ( \pm ) 0.52</td>
<td>3.30 (1.42, 5.18)</td>
<td>09.28 (6.85, 11.71)</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>86</td>
<td>&lt;0.001</td>
<td>7.44 ( \pm ) 0.33</td>
<td>2.97 (1.79, 4.15)</td>
<td>08.79 (7.22, 10.36)</td>
</tr>
<tr>
<td>Salivary melatonin (CR-2)</td>
<td>Male</td>
<td>64</td>
<td>&lt;0.001</td>
<td>3.09 ( \pm ) 0.32</td>
<td>3.66 (2.56, 4.76)</td>
<td>03.12 (1.95, 4.29)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>64</td>
<td>&lt;0.001</td>
<td>2.08 ( \pm ) 0.25</td>
<td>2.06 (1.20, 2.92)</td>
<td>05.05 (1.79, 5.05)</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>128</td>
<td>&lt;0.001</td>
<td>2.58 ( \pm ) 0.21</td>
<td>2.86 (2.15, 3.57)</td>
<td>03.23 (2.26, 4.20)</td>
</tr>
<tr>
<td>Tympatic temperature (CR-2)</td>
<td>Male</td>
<td>64</td>
<td>&lt;0.001</td>
<td>35.8 ( \pm ) 0.07</td>
<td>0.76 (0.53, 0.99)</td>
<td>15.80 (14.62, 16.98)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>64</td>
<td>&lt;0.001</td>
<td>36.2 ( \pm ) 0.05</td>
<td>0.62 (0.44, 0.80)</td>
<td>14.56 (13.42, 15.70)</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>128</td>
<td>&lt;0.001</td>
<td>36.0 ( \pm ) 0.05</td>
<td>0.68 (0.52, 0.84)</td>
<td>15.25 (14.36, 16.14)</td>
</tr>
</tbody>
</table>

Fig. 1—Cosine-fitted curves of (A) serum cortisol (in CR-1) and (B) salivary melatonin (in CR-2) in apparently healthy males, females and all at group level; refer Table 1 for Cosinor summary.
Fig. 2—Cosine-fitted curves of 60-s production of (A, B) two male subjects (M#3, M#4), (C) a female subject (F#2) and (D) males, females and all at group level in CR-1. Cosinor summary: M#3: N = 16, P < 0.05, M ± 1SE = 0.94 ± 0.03, A = 0.113 (0.003, 0.12), ∅ = 22.06 (16.75, 27.37), M#4: N = 16, P < 0.05, M ± 1SE = 0.95 ± 0.02, A = 0.11 (0.02, 0.20), ∅ = 04.04 (0.25, 7.83), F# 2: N=16, P<0.05, M ± 1SE = 0.53 ± 0.02, A = 0.09 (0.01, 0.17), ∅ = 20.10 (15.92, 0.28); for Cosinor summary of males, females and all at group level in CR-1 see Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Data points</th>
<th>Rhythm detection</th>
<th>Rhythm adjusted (mean ± SE)</th>
<th>Amplitude, A (95% CL)</th>
<th>Acrophase, ∅ in h (95% CL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 s (CR-1)</td>
<td>Male</td>
<td>64</td>
<td>&lt;0.05</td>
<td>0.93 ± 0.02</td>
<td>0.09 (0.01, 0.17)</td>
<td>00.10 (4.80, 19.40)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>63</td>
<td>0.63</td>
<td>0.88 ± 0.03</td>
<td>0.04</td>
<td>21.41</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>127</td>
<td>0.08</td>
<td>0.90 ± 0.02</td>
<td>0.06</td>
<td>23.24</td>
</tr>
<tr>
<td>60 s (CR-2)</td>
<td>Male</td>
<td>64</td>
<td>0.62</td>
<td>0.80 ± 0.03</td>
<td>0.04</td>
<td>00.46</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>64</td>
<td>0.94</td>
<td>0.72 ± 0.04</td>
<td>0.02</td>
<td>07.97</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>128</td>
<td>0.88</td>
<td>0.76 ± 0.02</td>
<td>0.02</td>
<td>02.82</td>
</tr>
<tr>
<td>10 s (CR-1)</td>
<td>Male</td>
<td>64</td>
<td>0.75</td>
<td>0.97 ± 0.08</td>
<td>0.09</td>
<td>07.96</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>63</td>
<td>0.72</td>
<td>1.04 ± 0.03</td>
<td>0.03</td>
<td>20.96</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>127</td>
<td>0.89</td>
<td>1.01 ± 0.04</td>
<td>0.03</td>
<td>07.41</td>
</tr>
<tr>
<td>10 s (CR-2)</td>
<td>Male</td>
<td>64</td>
<td>0.61</td>
<td>1.19 ± 0.04</td>
<td>0.06</td>
<td>05.19</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>64</td>
<td>0.89</td>
<td>1.08 ± 0.04</td>
<td>0.03</td>
<td>16.90</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>128</td>
<td>0.90</td>
<td>1.14 ± 0.03</td>
<td>0.02</td>
<td>05.40</td>
</tr>
<tr>
<td>Oral temperature (CR-1)</td>
<td>Male</td>
<td>64</td>
<td>&lt;0.001</td>
<td>97.8 ± 0.06</td>
<td>0.56 (0.34, 0.78)</td>
<td>17.22 (15.79, 18.65)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>64</td>
<td>&lt;0.001</td>
<td>98.1 ± 0.06</td>
<td>0.55 (0.36, 0.74)</td>
<td>15.30 (13.94, 16.66)</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>128</td>
<td>&lt;0.001</td>
<td>97.9 ± 0.05</td>
<td>0.54 (0.38, 0.70)</td>
<td>16.27 (15.17, 17.37)</td>
</tr>
<tr>
<td>Oral temperature (CR-2)</td>
<td>Male</td>
<td>64</td>
<td>&lt;0.001</td>
<td>97.7 ± 0.07</td>
<td>0.68 (0.44, 0.82)</td>
<td>15.63 (14.24, 17.02)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>64</td>
<td>&lt;0.001</td>
<td>98.1 ± 0.06</td>
<td>0.50 (0.30, 0.70)</td>
<td>15.48 (13.96, 17.00)</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>128</td>
<td>&lt;0.001</td>
<td>97.9 ± 0.05</td>
<td>0.59 (0.42, 0.76)</td>
<td>15.57 (14.47, 16.67)</td>
</tr>
</tbody>
</table>
in both CR-1 (female: 98.2 °F ± 0.07, mean ± 1SE; male: 97.8 °F ± 0.08) and CR-2 (female: 98.2 °F ± 0.07; male: 97.8 °F ± 0.09). The effect of interaction of factors either season x time or gender x time was not significant.

The effect of interval was statistically significant on short-time estimation in both CR-1 and CR-2 conducted during spring and autumn seasons (Table 4). The mean theta production of 10 s (1.07 ± 0.02) was found to be longer than the 60 s (0.82 ± 0.02). However, the judgment of both intervals was near to accuracy. The effect of season was not significant on pooled data of both 10- and 60-s intervals. The effect of interaction of factors interval x season was found to be significant (Table 4).

Discussion

Circadian rhythm analysis—The constant routine protocol is regarded as one of the best preferred techniques to evaluate circadian rhythm in any variables. The core body temperature (CBT) is established as a robust physiological circadian maker. The melatonin and cortisol both are the biochemical circadian markers that strongly exhibit 24-h period.

In the present constant routine studies conducted in spring and autumn, the ambient temperature range was almost similar and light intensity was constant. We obtained significant circadian rhythms in serum

![Fig. 3](image)

**Fig. 3**—Cosine-fitted curves show variability in oral temperature of males, females and all at group level in (A) CR-1 and (B) CR-2. For Cosinor summary see Table 2

![Fig. 4](image)

**Fig. 4**—Cosine-fitted curves exhibit variability in ear temperature rhythm in males, females, and all at group level in CR-2; refer Table 1 for Cosinor summary

![Fig. 5](image)

**Fig. 5**—Mean theta production of 60 s and 10 s in all subjects in spring (CR-1) and autumn (CR-2) season. t-test summary: 60 s: *t*<sub>253</sub> = 4.39, *P* < 0.001; 10 s: *t*<sub>253</sub> = 2.61, *P* < 0.01

| Table 3—Effects of the factors ‘season’ (spring vs. autumn) and ‘time of the day’ on different variables studied in apparently healthy subjects under 30-h constant routine (n=16) |
|---------------------------------|-----------------|-----------------|-----------------|
| Variable                        | Factor          | *F*-value       | Degree of freedom | *p*              |
| 60-s production                 | Season (S)      | 18.508          | 1, 223           | <0.001           |
|                                 | Time (T)        | 0.641           | 15, 223          | 0.84             |
|                                 | T x S           | 0.498           | 15, 223          | 0.94             |
| 10-s production                 | Season (S)      | 6.16            | 1, 223           | <0.05            |
|                                 | Time (T)        | 0.190           | 15, 223          | 0.9997           |
|                                 | T x S           | 0.219           | 15, 223          | 0.9992           |
| Oral temperature                | Season (S)      | 12.367          | 1, 224           | 0.633            |
|                                 | Time (T)        | 0.229           | 15, 224          | <0.001           |
|                                 | T x S           | 0.815           | 15, 224          | 0.661            |
cortisol (in CR-1) and salivary melatonin (in CR-2), that corroborate with the previous reports. This also confirms that the conditions of our CR studies were perfect for the elucidation of circadian rhythms in any variable. The peaks of serum cortisol and salivary melatonin appeared as expected, i.e., in the late and early morning hours, respectively.

Statistical validation of circadian rhythms in oral temperature and ear temperature under CR protocol suggests that like CBT, oral temperature could also be considered as reliable physiological circadian marker. The peaks of rhythms in both oral and tympanic temperatures were witnessed in the afternoon hours and corroborate with the findings of earlier studies.

The circadian variation in 10-s estimation has been reported under various constant routine studies with different hours of sustained wakefulness. Pati and Gupta reported circadian rhythm in 10-s interval estimation in normal subjects. The interval 10-s was found to be estimated longer in the morning hours than evening. The 10-s interval production has also been reported to increase with advancing evening. These authors alleged that interval timing in 10-s perception is under the influence of the circadian pacemaker. In the present study, significant 10-s circadian variation was there in one subject only with the peak estimation in the morning hours. The present observations indicating validation of circadian rhythm in 60-s interval productions in three subjects and all males at group level in CR-1 and one subject in CR-2 imply that the circadian timing system also influence the intervals longer than 10 s. The production of 60-s duration increased with progressing evening. The acrophase of rhythm in 60-s production appeared in the evening or at night (in one male and one female subject and all male) or early morning (in one subject in each CR-1 and CR-2). It signifies that interval timer interact with circadian clock for 60-s interval. A significant circadian rhythm (τ>24 h) in estimation of 120-s was also documented under complete environmental and social isolation. The subject in the study cited above was isolated inside a subterranean cave for a period of about two months. The findings of the study provide additional credence to the present results. It has been shown that some variables are more susceptible to masking than others. The body temperature and melatonin are the least affected variables. Short-interval estimation could be the cognitive variable that appears to belong to this class (more susceptible) of variable. Pöppel and Giedke purported “......there is also an influence of one or several endogenous oscillations which secondarily change the diurnal variation of TP ("internal masking"). It has also been suggested that circadian fluctuation in short-time estimation partly depends on variations in body temperature and sleep-wake cycle and the circadian variation in other variables could modulate the short-time estimation. Pöppel also alleged that “When subjects have to stay awake during night, the rhythm of body temperature is only slightly altered, whereas a variation of TP is nearly absent. Furthermore, the extreme values during a 24 hour period do not coincide.” So, we could also suspect that the short-time estimation could be the cognitive property in human that could be sensitive to both internal and external masking agents.

### Table 4—Effects of the factors ‘interval’ (60 s and 10 s) and ‘season’ (spring and autumn) on short-time estimates studied in apparently healthy subjects under 30-h constant routine (n = 16)

<table>
<thead>
<tr>
<th>Factor</th>
<th>F-value</th>
<th>Degree of freedom</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interval (I)</td>
<td>72.867</td>
<td>1, 506</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Season (S)</td>
<td>0.014</td>
<td>1, 506</td>
<td>0.906</td>
</tr>
<tr>
<td>I x S</td>
<td>20.670</td>
<td>1, 506</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Effects of seasons, time and gender on short-time estimation & oral temperature and of interval on short-time estimation—During the two constant routines performed in spring and autumn, in spite of having similar experimental conditions, some variables were influenced by the effect of season. It was apparent in production of both 60- and 10-s intervals. The interval 60 s was produced shorter than 10 s. In spring season the 10 s was produced perfectly and 60 s was produced near to accuracy. Contrary to this the mean 60-s production was over produced and 10 s was under produced in autumn season. This finding suggests that the cognitive attribute to perceive short-intervals vary as function of season. We also observed mean body temperature higher in autumn (98.02 °F) than spring (97.99 °F), although it was not statistically significant (t_{127}= 0.534, p = 0.59). The longer judgment of 60 s in autumn than spring season could be explained through the concept that short-interval estimation is inversely associated with body temperature.

The duration of intervals also modulates the subjective perception. The interaction between interval and seasons as factors shows that they are

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PANKAJ B. PANDE et al.: JUDGMENT OF SHORT INTERVALS UNDER 30 H CONSTANT WAKEFULNESS 565
associated and affect the subjective time perception. Seasonal rhythm in rectal temperature was observed in a study with regular sleep-wake in control ambient condition. We didn’t get any seasonal effect on oral temperature because the studies in both seasons were conducted in almost similar ambient and behavioral conditions during 30-h constant routine.

The effects of gender on time estimation are equivocal. A gender difference on time estimates has been substantiated in normal living condition. However, no gender difference was found on the observed time estimates in both constant routine studies. This is convincing with earlier findings that alleged absence of gender effect on interval estimation. The higher body temperature in females than males substantiated in this study are comparable with previously reported findings. As per McGann et al., the higher body temperature in young females could be attributed to menstrual physiology.

### Conclusion

The short-time perception under two constant routine conditions that were conducted in spring and autumn seasons in two young cohorts provides evidence of interaction among three timing systems, viz., the interval timing system, the circadian and the circannual timing systems. The variation across the day in cognitive attribute to judge 60-s interval, the frequently used interval in humans, seems to have endogenous basis. Further, oral temperature also appears to be under the control of circadian oscillator and can be favorably compared with the known circadian markers, such as rhythms in melatonin, cortisol and tympanic temperature. Thus, the oral temperature rhythm can be safely used as a circadian marker. The study of variability in 10-s and 60-s intervals should be carried out in CR taking large number of subjects. This could reveal endogenous origin of these cognitive rhythms with confidence. Consolidation of this hypothesis could be achieved only when CR studies are conducted more frequently (probably every month!) involving large number of subjects. The subject of time perception is extremely complex; nonetheless the present investigation provides meaningful evidence in support of both endogenous circadian and circannual bases of rhythms in short-interval estimates. The circadian and circannual involvement in time estimates may have tremendous implications in the management of attention-demanding and time-perception-related cognitive tasks in personal, academic, industrial and security maintenance spheres.

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### Conflict of interest

The authors declare no conflict of interest.

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