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Intrinsic circadian period of adolescent humans measured in conditions of forced desynchrony

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Abstract

Circadian timing was assessed with forced desynchrony (FD) in 10 healthy adolescents (five boys, five girls; mean age 13.7 years). Following 10 days of entrainment to a fixed light-dark (LD) schedule at home, participants were studied under dim light (<20 lux) in the laboratory. A 28-h schedule (FD) was imposed for 12 × 28-h cycles. Saliva was collected at 30- or 60-min intervals throughout; core temperature was measured in constant routines (CR) before and after FD. Intrinsic circadian period was estimated by linear regression using temperature minimum from CRs and dim-light salivary melatonin onsets and offsets from FD. Average intrinsic circadian period for core temperature ($n = 7$) was 24.30 ± 0.20, for melatonin onset was 24.33 ± 0.21, and for melatonin offset was 24.35 ± 0.21. Intrinsic circadian period in every adolescent was greater than 24 h.

Keywords: Adolescent; Circadian period; Temperature; Melatonin; Sleep; Forced desynchrony

A consistent change in sleep behavior accompanying adolescent development is a delay of bedtime and rising time. This pattern has been reported in numerous studies from a variety of countries [9,10,16,19]. A delay in circadian phase preference (morningness/eveningness) has also been identified in adolescents [12]. One study found a relationship between morningness/eveningness and self-reported pubertal status in girls ages 11 and 12 years [4]. More recently, we have found that the timing of melatonin secretion in young adolescents is correlated with pubertal status assessed by clinical examination of secondary sexual characteristics [3]. Based upon classical circadian rhythms theory [1], a possible explanation for these observations is that an adolescent circadian phase delay is favored by a long period of the intrinsic circadian oscillator.

A major impediment in testing this hypothesis is to measure the phenomenon. Techniques for assessing intrinsic circadian period in rodents through long-term isolation in conditions of constant darkness or by temporal and social isolation as done in adult humans are not suitable for evaluating young humans. Assessment of circadian period has not been feasible in adolescent humans, therefore, until the technique of forced desynchrony (FD) came to prominent and routine use in adult humans [6]. FD requires placing individuals on a schedule that is beyond the circadian range of entrainment by enforcing such day lengths as 20, 28 or 30 h. This technique has been shown by others to provide an accurate measure of intrinsic circadian period in adults [7]. We report here a successful adaptation of this methodology to assess the adolescent circadian timing system in a sample of 10 adolescents.

Five boys and five girls (one boy age 10.9 years, others aged 13.2–15.2 years; mean = 13.7 years) were studied. Pubertal status was determined using Tanner staging [17] performed by consensus among three independent examiners. Data are presented for pubic hair growth, which is the more reliable of the Tanner stage measures [17]. The distribution of Tanner stages in this sample included only one early (Tanner stage 2) and one mid (Tanner stage 3) pubertal child and four each at the late pubertal Tanner stages 4 and 5. (Until additional subjects are studied, we are unable to

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assess whether changes in intrinsic circadian period occur in relation to pubertal development.)

As shown in Fig. 1, participants were first entrained to a fixed sleeping and waking and light-dark (LD) schedule (lights on at 0800 h, lights off at 2200 h) while living at home for 10 days. They were instructed to be active during waking hours and to wear black-out eyeshades and attempt to sleep in the scheduled dark hours. Compliance was checked by daily telephone calls, sleep logs, and continuous wrist actigraphy. Participants came to the laboratory in groups of three or four on day 11 and were instrumented for sleep recordings and rectal temperature monitoring (1-min intervals, Minilogger; Minimitter, Sun River, OR, USA). They slept on the entrainment schedule for another night in the laboratory.

Initial measures of circadian phase were obtained during a 36-hour constant routine (CR) [5] procedure in which participants in individual rooms sat in a semi-recumbent (45°) posture and had small meals, performance testing, and multiple sleep latency testing at 2-h intervals. Throughout the laboratory phase of the study, participants were in dim light of 15–20 lux during all waking hours and less than 1 lux during the sleep phase. After the initial CR, participants were given a recovery sleep episode lasting 11.67 h. A 28-h FD was then imposed, with lights on for 16.33 h and lights dimmed for 11.67 h over the course of 12, 28-h cycles (14 sidereal days), keeping the LD schedule at the 14:10 ratio of the baseline entrainment. During waking hours participants were continuously attended by a staff member, except during testing. The youngsters were placed on a daily schedule that included breakfast, luncheon, supper, and an evening snack, all of which they partook as a group with laboratory staff. Other scheduled group activities included 'afternoon' craft making and 'evening' video watching. Participants took performance tests and the MSLT at 2-h intervals in individual rooms throughout scheduled waking hours in the lab. On the fourth and tenth FD cycles, participants stayed in their individual rooms through the light phase under CR procedures described above. Sleep was recorded for all scheduled sleep episodes; core temperature was recorded only during the CRs. At the end of the twelfth FD cycle, the study ended with a 36-h CR followed by a 12-h recovery night. Thus, the full in-lab schedule was 20 sidereal cycles.

Saliva samples (2 ml) were collected during all CR and FD waking episodes at 30-min or 1-h intervals for determination of melatonin. Samples were frozen within 4 h and subsequently sent for radioimmunoassay (Alpco, Windham, NH, USA). Coefficient of variation within samples ranged from 6.9 to 8.3% for a value of 2.1 pg/ml, 5.0–5.6% at 7.6 pg/ml, and 6.9–9.6% at 16 pg/ml; between sample c.v. was 1.2–13.1% at 2.1 pg/ml, 7.5–9.8% at 7.6 pg/ml, and 10.1–12.7% at 16 pg/ml. Rectal temperature was sampled only on the CRs and bracketing nights. Measurement of melatonin from saliva constrained the assessment of this measure to the waking hours. Scheduled waking on certain cycles overlapped the onset phase of melatonin secretion, on others the offset phase; rarely were both onset and offset available on the same cycle. By using melatonin onset and offset phases, we had measures made in multiple cycles providing added accuracy for our estimates of intrinsic period. We chose the multiple measurement strategy to ensure that we had adequate estimates of intrinsic circadian period in these first studies of adolescents. Melatonin onset and offset phases were calculated with a threshold method (4 pg/ml) for every available cycle. Because salivary melatonin reflects approximately 40% of the plasma value [8], a 4 pg/ml threshold was chosen to parallel the level used to assess threshold from plasma melatonin [13]. The times of onset and offset were computed by interpolation from values rising above or falling below, respectively, the 4 pg/ml threshold. Minimum temperature phase was determined from CRs using the method of Brown and Czeisler [2].

The intrinsic circadian period for each participant was estimated using each of these three phase markers. The first estimate was derived from temperature minimum phase of the two CRs occurring approximately 18 circadian cycles apart. The other estimates were computed by linear

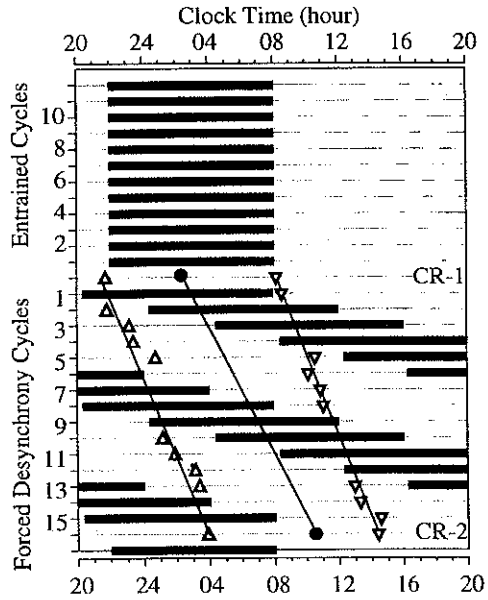

Fig. 1. Schedule of wake and sleep showing linear regression of data points on cycles that were used to estimate circadian period during FD in one 13.9-year-old girl. Scheduled dark/sleep phases are indicated by black bars; scheduled waking episodes by thin lines. Cycles indicated as entrained are those during which the LD was fixed at 24 h; cycles indicated as FD are those for which participants were in the laboratory with LD at 28 h, with the exception of a 36-h constant routine at start (CR-1) and finish (CR-2). Phase of salivary melatonin onset is indicated by upward pointing triangles and of salivary melatonin offset by downward pointing triangles. The core temperature phase minima from CR are indicated by dark circles. In this girl, circadian period was estimated at 24.51 h from temperature minimum phase estimates, 24.41 from linear regression through melatonin onset phases, and 24.39 from linear regression through melatonin offset phases.

regression from melatonin onset and offset phases, with approximately 10 values available across 18 cycles for each participant. Fig. 1 illustrates these data in one 13.9-year-old girl. The cycle-to-cycle variability in individuals may reflect transient effects due to relative coordination to the LD schedule [18].

The average estimate of intrinsic circadian period computed with core temperature (available on only seven subjects due to technical difficulties) was 24.30 ± 0.20 h (minimum 24.12 h; maximum 24.68 h); for melatonin onset was 24.33 ± 0.21 h (minimum 24.08; maximum 24.60); for melatonin offset was 24.35 ± 0.21 h (minimum 24.12; maximum 24.64). Correlation coefficients indicated strong coherence among the three estimates of intrinsic period: melatonin onset versus melatonin offset, $r = 0.995$; melatonin onset versus core temperature, $r = 0.969$; melatonin offset versus core temperature, $r = 0.962$. No sex differences were found in this small sample.

These data indicate a comparable or perhaps somewhat longer intrinsic circadian period in adolescents than in adults depending upon the comparison group. For example, the most comparable assessment in adults, using a 28-h FD with 2:1 LD in dim lighting conditions, estimated circadian period in young adults with a mean of 24.1–24.2 (longest 24.4) h [7]. Estimated period in our sample was longer on average, and three adolescents exceeded the maximum period computed for that sample of young adults. Other adult samples have included significant differences in the FD period and/or in lighting conditions, making comparisons more problematic. Thus, Waterhouse et al. [18] using 27- or 30-h FD with 150–500 lux for waking found a mean period of about 24.4 h. Similarly, Hiddinga et al. [11] measured average period at 24.30 ± 0.36 h in 12 young adult male subjects placed on a 20-h FD in dim (10 lux) light and darkness.

Our data indicate that intrinsic circadian period can be measured in adolescents using the FD paradigm. Whether adolescence is associated with a lengthening of intrinsic period is not apparent at this time, but our data do not rule out this possibility. If intrinsic period is slow in certain adolescents, then oscillator theory posits that such slow rhythms will lag the entraining oscillator [1]. Hence, individuals with long intrinsic circadian periods are more liable to phase delays, as reported in many adolescents. Comparisons with adults run under identical protocol conditions that account for possible effects of LD differences and social cues among the various protocols are required to achieve a definitive examination of this issue. Precedents for a change in intrinsic circadian period are found in reports of shorter period lengths as a function of advanced age in rodents [15]. We are unaware of animal studies of circadian period directed at the pubertal developmental stage. Alternative explanations for the delayed sleep phase in adolescents include increased sensitivity to evening light stimuli or greater amplitude of the evening (delaying) portion of the phase response curve to light [14].

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