Photic Entrainment in Hamsters: Effects of Simulated Twilights and Nest Box Availability

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Abstract Entrainment of wheel-running activity rhythms was compared in hamsters exposed to daily light-dark (LD) cycles with abrupt transitions between 0 and 10 lux or with artificial twilights simulating summer solstice conditions at 41°N latitude but truncated at 10 lux. The photoperiod in LD-rectangular was set at 16.24 h, equating the total light (in lux • min) emitted under the two schedules. The LD cycles were maintained for 35 days and were followed by 14 days of constant darkness (DD). Half the animals in each condition had access to a dark nest box connected to the outer compartment by a tunnel, the remaining animals being confined to a single compartment. Body temperature and locomotor activity inside the nest boxes were recorded by telemetry. Movements between the nest box and the outer compartment were monitored and the data were used to calculate light exposure at different times of the day. In all groups, the phase angle difference between wheel-running onset and dusk was more positive than that between activity offset and dawn. Hamsters with access to nest boxes, however, had later onsets, earlier offsets, and shorter activity durations (αs) than those without. These effects could be accounted for by the difference in light exposure between the nest and no-nest animals, particularly light exposure in the morning. The inclusion of twilights also resulted in later onsets and shorter αs, but the differences were relatively small and were only observed in the nest animals. The day-to-day variability in activity onset was negatively correlated with onset time and was smaller in the twilight/nest animals than in the other three groups. Most animals showed an expansion of α during the first few days of DD, resulting from a rapid advance of activity onsets relative to offsets. The period of the rhythms, determined from the first five activity onsets in DD, was negatively correlated with the balance of evening and morning light exposure. These results are discussed in the context of nonparametric entrainment of compound pacemakers.

Key words circadian rhythms, entrainment, light-dark cycles, twilight, wheel-running, body temperature, motor activity, hamsters

As adaptations to a diurnally periodic environment, circadian rhythms are most effective when entrained to external daily cycles: only then do the rhythms assume exact 24-h periodicity, matching that of the external environment, and a stable phase relative to the entraining cycle. For most organisms, the

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JOURNAL OF BIOLOGICAL RHYTHMS, Vol. 11 No. 3, September 1996 216-233

primary entraining agent is the daily illumination cycle, which provides the most reliable index of local time (Pittendrigh, 1981).

The natural illumination cycle includes graded changes in light intensity, spanning an approximate 8-log unit illuminance range from starlight levels to midday maxima, which take place largely during the dawn and dusk twilight hours. Such a prominent feature of the illumination cycle is likely to play a significant role in shaping the temporal organization of animal behavior and physiology. Indeed, dawn and dusk are crucial times in the daily ecology of most animals: many start and/or end their daily activity during twilights, and several species are active almost exclusively at these times of day. Twilights are times when the activity of diurnal animals can overlap that of nocturnal species, with important consequences for both predators and prey. Dawn and dusk also define the limits of visual sensitivity of animals with pure cone or pure rod retinas, and they are times when duplex retinas switch between photopic and scotopic vision. Finally, dawn and dusk are critical for the entrainment of circadian rhythms, because it is at these times of day that exposure to light causes the corrective daily phase shifts necessary for maintaining circadian entrainment.

Activity onsets and offsets of many animals are confined to narrow, species-specific, illuminance ranges within the twilight intervals (e.g., Kavanau, 1969; Kavanau and Ramos, 1970, 1975; Kavanau and Peters, 1976; Mech et al., 1966; Pariente, 1975; Daan and Aschoff, 1975). Such close temporal relationships may result from direct stimulation or inhibition of activity at particular light intensities, or through entrainment of underlying pacemakers. Both mechanisms are probably involved in most cases, but in differing proportions depending on the species (Daan and Aschoff, 1975). The two may also interact, given recent demonstrations of feedback effects of locomotor activity on circadian pacemakers (Mrosovsky et al., 1989; Turek, 1989).

The duration of twilight shows a bimodal annual variation, with peaks at the summer and winter solstices. The fact that similar seasonal patterns are seen in circadian phase angle measures of several species—midpoint of daily activity in hamsters and tree shrews (Daan and Aschoff, 1975), activity onset in kangaroo rats (Kenagy, 1976), and lake chub (Kavaliers and Ross, 1981)—suggests that twilights may play a role in circadian entrainment. Such a role has been proposed, based on a mathematical model of circadian oscillations that predicts that the phase angle difference between an entrained activity rhythm and the entraining light cycle will become more positive (or less negative) the longer the duration of twilight (Wever, 1965). This prediction was confirmed in laboratory studies in diurnal birds and in the nocturnal hamster (Aschoff and Wever, 1965; Wever, 1967). Tests of this prediction were also performed under natural illumination, by comparing phase angle differences at two different latitudes around the equinoxes, when photoperiod duration is approximately equal but twilight duration is longer at the higher latitude (Daan and Aschoff, 1975). Under these conditions, however, the predicted increase in the phase angle difference at the higher latitude was not found in any of the three species of birds studied, whereas in hamsters, this effect was observed at the spring but not at the fall equinox.

The model also predicts an increase in the strength of the LD zeitgeber with increasing twilight durations (Wever, 1965), but the evidence on this point is very limited. It consists of preliminary data by Kavanau (1962a, 1962b) showing synchronization of behavioral rhythms in a deer mouse, *Peromyscus maniculatus*, by 16-h LD cycles with artificial twilights. Because a 16-h period lies well outside the normal range of mammalian entrainment by rectangular LD cycles, these results suggest that twilight transitions may increase the strength of the LD zeitgeber. However, the data do not rule out the possibility that the observed synchronization reflected direct, or masking, effects of light on behavior rather than entrainment of a circadian pacemaker.

Perhaps the most convincing evidence that twilights influence circadian timing was obtained in the lake chub, *Couesius plumbeus* (Kavaliers and Ross, 1981). This diurnal fish shows clear seasonal variations in free-running period following exposure to LD cycles with seasonally adjusted photoperiods, but only if the LD cycles include twilight transitions. Inclusion of twilights also changes the seasonal patterns in phase angle of entrainment and in daily activity duration, suggesting that the changes in free-running period may represent aftereffects of entrainment to twilight cycles.

Many nocturnal species spend most of the daylight hours in light-excluding shelters, emerging at dusk and regaining their burrows at dawn. Such animals are only exposed to daylight for brief durations, and entrainment would therefore result primarily from discrete daily phase shifts, as observed under one- or two-pulse skeleton photoperiods (Pittendrigh and Daan, 1976b). The fact that their exposure to light is largely self-selected also makes the behavior of nocturnal-fossorial animals an integral component of the entrainment process.

A clear demonstration of this process was described in flying squirrels, *Glaucomys volans* (DeCoursey, 1986, 1989). Animals kept in LD and provided with dark nest boxes showed entrained patterns of wheel-running activity, but instead of taking place at the same time each day, activity onsets showed a characteristic zigzag pattern, occurring a little earlier each day for several days then showing a single abrupt phase delay. Close monitoring of the animals revealed that they saw very little light for several consecutive days, during which time their activity rhythm free ran with a period less than 24 h. Eventually, the light sampling behavior exhibited by these animals before the start of their daily activity period exposed them to light for a few minutes, causing an instantaneous phase delay.

Such experiments (see also Rusak, 1975; Lynch et al., 1985; Korenman et al., 1988; Pratt and Goldman, 1986) are an improvement over traditional entrainment studies with inescapable light sources, most of which have used nocturnal fossorial rodents, but, with one exception (Experiment III in Korenman et al., 1988), they still involved exposure to highly unnatural rectangular LD cycles. The inclusion of twilights may well result in a different pattern of entrainment. Thus, instead of being exposed to either darkness or full intensity light, the animals would, upon emerging from their burrow in the evening, encounter a range of possible intensities: the earlier the exit, the higher the light intensity. Because a more premature exit requires a larger corrective phase shift, this suggests that one effect of twilight transitions may be to tailor the amount of light received to the entrainment requirements of the animal.

Preliminary studies by Terman et al. (1990, 1991) examined entrainment and light exposure patterns in albino rats housed in dual-compartment chambers, with daily illumination cycles, including naturalistic twilight transitions, in the outer compartment. The results include the following observations: (1) albino rats expose themselves to surprisingly low light intensities (about 1 lux or less) during late dusk and/or early dawn; (2) total daily light exposure at dawn and dusk shows a systematic dependence on photoperiod; (3) the balance of dawn/dusk exposure to light varies as a function of the individual animal's free-running period, and of the period of the illumination cycle, in a manner consistent with the phase response curve (PRC).

The present study was designed to compare entrainment and daily light exposure patterns in hamsters with or without access to nest boxes, under daily LD cycles with either abrupt or gradual transitions, the latter simulating natural twilights at the summer solstice at 41°N latitude. More specifically, we examined the effects of twilights and of nest box availability on the phase angle of entrainment of the hamster's wheel-running activity rhythm and on daily activity duration, as well as the aftereffects of these conditions on free-running period and activity duration in DD. Activity inside the nest box was also recorded by telemetry, along with body temperature; inclusion of these measures was prompted by the observations of Korenman et al. (1988) that hamsters show a peak in nest box activity immediately preceding the nocturnal increase in activity in the outer compartment, and those of Terman et al. (1990) showing differential effects of lighting condition on the duration of feeding and drinking behavior in the two compartments. In all conditions, maximum light intensity was set at 10 lux, as nocturnal rodents tend to avoid exposure to higher light intensities when given the opportunity to do so (Korenman et al., 1988; Terman et al., 1990, 1991). This relatively low illuminance level was also expected to minimize masking effects of light on locomotor activity.

MATERIALS AND METHODS

Animals and Maintenance

Adult male Syrian hamsters (*Mesocricetus auratus*, Charles River LVG:lak, 90-100 g), were individually housed in open-topped compartments equipped with running wheels (17 cm diameter). Four compartments (24 × 24 × 30 cm) were enclosed in each of two ventilated wooden isolation chambers, the interiors of which were painted white. Two of the compartments in each chamber were connected to light-tight nest boxes (16.5 × 22 × 16 cm) by cylindrical PVC tunnels (6 cm i.d. [inner diameter]). The compartments and nest boxes were made of black plexiglas and had wire mesh floors. Removable lids allowed access to the nest boxes for cleaning and replenishing food supplies. The tunnels were 60 cm long and had two 90° turns. Three infrared LEDs and detectors were placed across the tunnel, as indicated in Figure 1. The inner and outer beams were positioned 6 cm from either end of the tunnel.

Lighting was provided through a 56 × 56 cm opening in the top of the chamber by a single incandescent light bulb (GE 40 watt standard) held in the center of

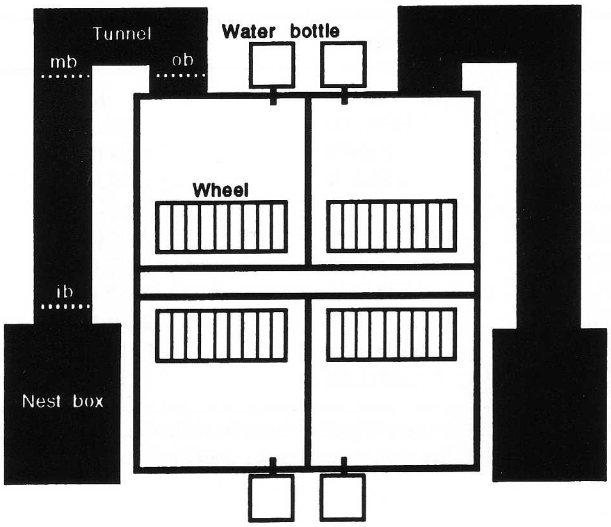

Figure 1. Diagram showing four open-topped compartments equipped with running-wheels. Two of the compartments are each connected to a nest box by a tunnel. Three infrared photobeams, placed across the inner (ib), middle (mb), and outer (ob) sections of the tunnel, provided information about the location of the animal throughout the day.

a tent-like reflector (Wafer, Plume Ltd.). A white translucent lexan sheet covering the chamber opening served as a diffusing screen.

Temperature in the chambers was kept at 23-26°C. Continuous white noise in the experimental room helped mask extraneous sounds. Food (PMI Feeds rodent diet 5001) and water were continuously available, the latter only in the outer compartment. Litter was changed weekly in the outer compartments and three times a week in the nest boxes. At those times, the interior of the nest boxes was also cleaned and the hamsters were provided with fresh paper towels as nesting material. Frequent cleaning was necessitated by the fact that the animals urinated and defecated almost exclusively in the nest boxes. During LD conditions, cleaning was performed during the light portion of the cycle, whereas during constant darkness (DD), cleaning was performed with the aid of an infrared light source and viewer (FJW Optical Systems).

Light Delivery System

Lighting in the chambers was controlled by a twilight simulation system (Naturalistic Illuminator, Medic-Light, Inc.) equipped with MacLite software (Research Foundation for Mental Hygiene, Inc.). The software includes a predictive algorithm that specifies the expected momentary illuminance on the earth's surface across the 24-h day on any day of the year and at any latitude. Following lamp calibration, the system adjusts the voltage level delivered to the lamp to produce simulated naturalistic illumination patterns. Calibration consisted of dividing the total voltage range into 256 steps and entering the illuminance (in photopic lux) measured at each step. A fifth order polynomial function is then fitted to the empirical illuminance readings and used to provide the best match between predicted and actual light intensity levels. Illuminance was measured with a Tektronics J16 photometer and a cosine-corrected J6511 illuminance probe placed on the floor of the outer compartments.

Two LD cycles were compared. The first (LD-twilight) included gradual dawn and dusk transitions simulating twilight patterns at the summer solstice (June 21) at a latitude of 41°N, but the LD cycle was truncated at 10 lux. Given these parameters, the transition between 0.001 and 10 lux at dusk and dawn required 86 min, light intensity remained at 10 lux for 16.05 h, and night (<0.001 lux) lasted 5.08 h. The criterion illuminance level of 0.001 lux used to distinguish night from twilight lies near the lower limit of astronomical twilight (6.5×10^{-4} lux, Thorington, 1980); the level is arbitrary, but was chosen because it was safely below the threshold for light-induced phase shifts in Syrian hamsters, which, for brief pulses of green light, falls somewhere between 10^{10} and 10^{11} photons cm^{-2} s^{-1}, or between 0.023 and 0.23 lux (DeCoursey, 1989; Nelson and Takahashi, 1991a, 1991b; Boulos, 1995). In principle, light intensity fell to starlight levels (3×10^{-4} lux) at night, although these levels were below the sensitivity of the photometer. The second LD cycle (LD-rectangular) included abrupt transitions between 0 and 10 lux, with light and dark portions lasting 16.24 h and 7.76 h, respectively. The total amount of light (in lux • min) emitted per day was identical in the two LD cycles. In LD-twilight, 10 lux was reached at 23:32 and ended at 15:35, whereas in LD-rectangular, 10 lux began approximately 6 min earlier and ended 6 min later. The timing of dusk and dawn was chosen to minimize the phase difference between the experimental LD cycles and those used at the breeder's.

Procedure

The hamsters were placed in the isolation chambers upon arrival in the laboratory. A total of 24 hamsters were exposed to LD-twilight, and 16 hamsters to LD-rectangular. Half the animals in each condition had access to nest boxes and were implanted intraperi-

Table 1. Parameters (mean ± s.d.) of entrained and free-running rhythms of wheel-running activity in the four groups of hamsters.

	RN	*RnN*	*TN*	*TnN*
n	12	8	12	8
Onset	14:57 (46)	14:23 (30)	15:43 (12)	14:03 (65)
Onset s.d.	0.23 (0.07)	0.30 (0.10)	0.17 (0.09)	0.39 (0.13)
Offset	22:37 (23)	23:33 (36)	22:28 (31)	23:40 (28)
Midpoint	18:47 (22)	18:58 (24)	19:05 (19)	18:51 (35)
α_{LD}	7.66 (0.95)	9.18 (0.75)	6.74 (0.45)	9.62 (1.21)
τ_{on-1}	23.71 (0.15)	23.71 (0.37)	23.78 (0.18)	23.85 (0.18)
τ_{off-1}	24.02 (0.15)	23.93 (0.21)	23.96 (0.21)	23.97 (0.17)
τ_{on-2}	23.88 (0.11)	23.94 (0.15)	23.97 (0.10)	24.06 (0.16)
τ_{off-2}	23.94 (0.20)	24.00 (0.15)	23.95 (0.21)	24.08 (0.18)
α_{DD}	9.18 (1.43)	11.10 (0.98)	9.08 (1.30)	11.44 (1.69)

NOTE: Means for onset, offset, and midpoint are times of day and standard deviations are in minutes. All other values are in hours. Free-running periods were measured from the first five activity onsets (τ_{on-1}) and offsets (τ_{off-1}) and the last five activity onsets (τ_{on-2}) and offsets (τ_{off-2}) in DD.

toneally with Mini-Mitter transmitters (Model VM-FH, Mini-Mitter Co., Sunriver, OR) to allow telemetric recording of body temperature and locomotor activity in the nest boxes, through receivers placed under the nest boxes. Surgery was performed under sodium pentobarbital anesthesia (80 mg/kg i.p.) 2 days after arrival. The animals were kept under LD for 35 days, followed by 14 days of DD.

Data Collection and Analysis

Wheel running, nest box activity, and body temperature were recorded at 5-min intervals on a personal computer equipped with Dataquest III hardware and software (Mini-Mitter Co., Sunriver, OR). The times of breaking and unbreaking of the photobeams in the tunnels were recorded to the nearest second on a separate computer. The data were transferred to a Macintosh computer for graphical display and analysis using Circadia software (Behavioral Cybernetics, Cambridge, MA).

Wheel-running activity onsets were defined by a threshold number of wheel revolutions (usually 100) preceded by a specified interval of below-threshold activity (usually 6 h). A much lower threshold (usually 5-20 revolutions) followed by 6 h below threshold was used to determine activity offsets. The duration of daily activity (α) is given by the difference between daily offset and onset times.

Daily light exposure was determined from the beam breakage data of 8 hamsters, 4 in LD-twilight and 4 in LD-rectangular. An animal was considered to be in the outer compartment from the first crossing of the outer beam preceded by crossing of the middle beam until a crossing of the outer beam followed by crossing of the middle beam. This represents a slight overestimate of the actual time spent outside, because on several occasions a hamster would cross the outer beam repeatedly without crossing the middle beam, going back and forth between the outer compartment and the tunnel entrance. In such cases, the direction in which the hamster was moving, and therefore its exact location, could not be determined accurately.

Upon leaving the nest box and crossing the tunnel, the hamsters often stopped at the tunnel entrance, in the path of the outer beam, and remained there for several seconds. On some occasions, they then emerged into the outer compartment, whereas on others, they returned toward the nest box without actually leaving the tunnel, as indicated by the occurrence of a single breakage of the outer beam preceded and followed by a crossing of the middle beam. In both cases, the time spent in the path of the outer beam was considered light sampling behavior, but the two categories of light sampling were quantified separately.

Differences between the four conditions were tested by two-way ANOVA with the factors cage (nest vs. no-nest) and light (rectangular vs. twilight). When a significant cage × light interaction was obtained, the individual group means were compared by means of *t* tests. The level of significance was set at $p = 0.05$.

RESULTS

Wheel-Running, Nest Activity, and Body Temperature in LD

Figure 2 shows the wheel-running records of 4 representative animals, 1 from each of the four experimental groups: rectangular/no-nest (RnN, $n = 8$), rectangular/nest (RN, $n = 8$), twilight/no-nest (TnN,

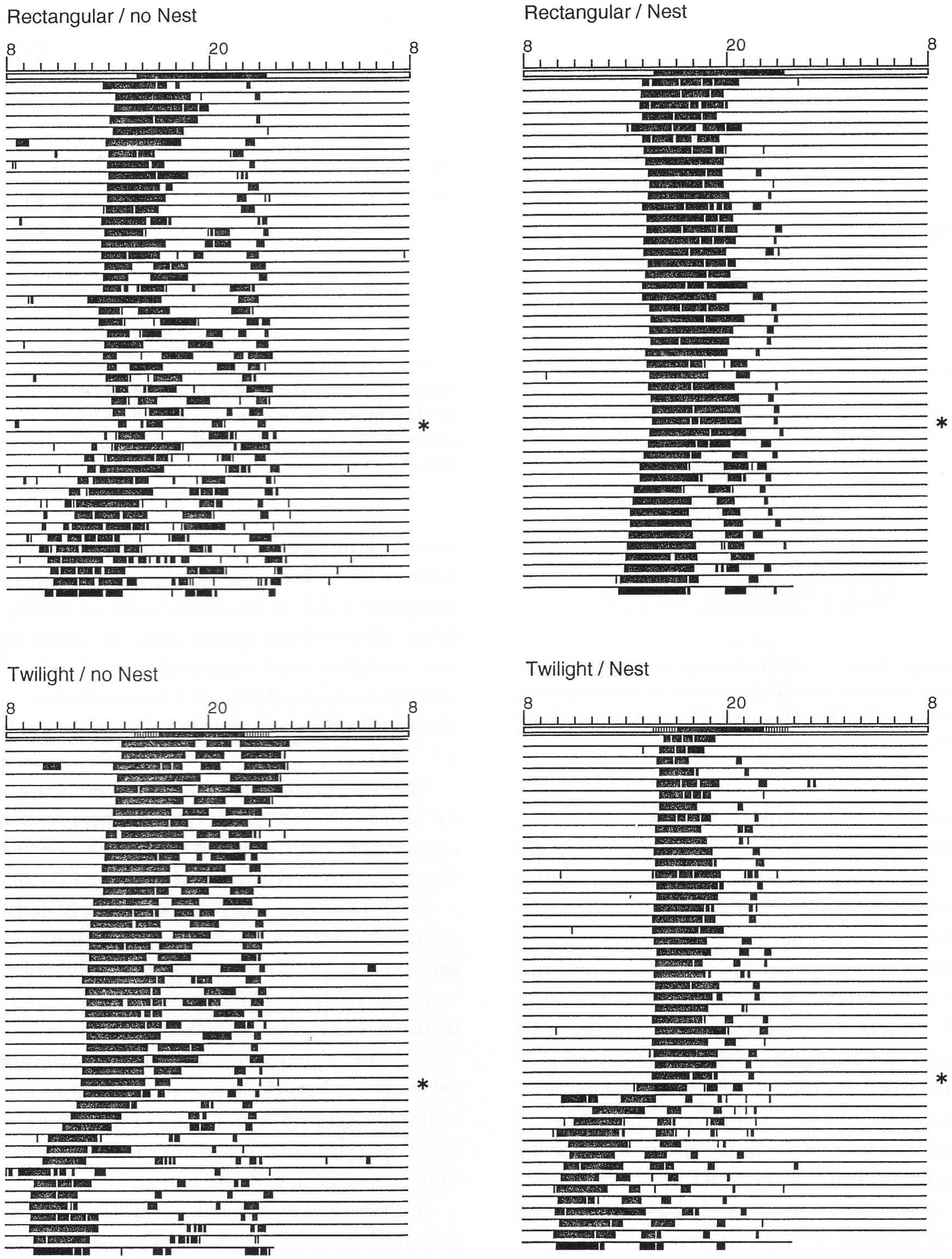

Figure 2. Wheel-running activity records of four hamsters initially exposed to LD-rectangular or LD-twilight, with or without access to nest boxes. Vertical marks indicate 5-min intervals with five or more wheel revolutions (the threshold level used to define activity offsets). Included in the records are the last 30 days of LD followed by 14 days of DD, the latter starting on the day indicated by an asterisk. The LD cycle is shown at the top of each record, with vertical stripes representing twilight transitions (0.001-10 lux). Occasional bouts of wheel running during an animal's subjective day were elicited by cage cleaning.

n = 12), and twilight/nest (TN, n = 12). The times of wheel-running activity onsets, offsets, and midpoints, averaged over the last 5 LD cycles, are shown for all animals in Figure 3, and the group means (± s.d.) are listed in Table 1.

Activity onsets were significantly earlier in the no-

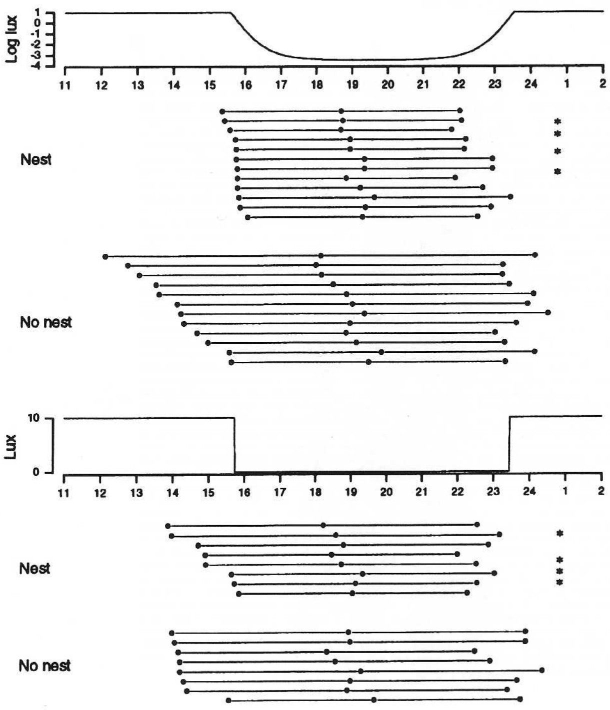

Figure 3. Average times of wheel-running activity onset, midpoint, and offset for individual animals in each of the four groups, shown in relation to the LD cycles. Asterisks indicate the 8 hamsters for which tunnel photobeam data were available.

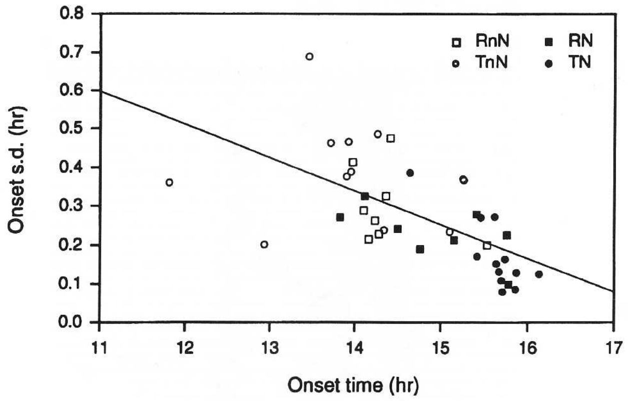

Figure 4. Standard deviation of activity onset on the last 28 days of LD plotted as a function of onset time for all hamsters. Also shown is the linear regression line through the data ($y = -0.086x + 1.55$, $r^2 = 0.384$).

nest relative to the nest groups, $F(1, 36) = 22.83$, $p < 0.0001$. There was no significant main effect of light condition. There was, however, a significant light × cage interaction, $F(1, 36) = 5.38$, $p < 0.05$, with earlier onsets in the RN than in the TN group, but no significant difference between the RnN and TnN groups. There were clear differences in within-group variability as well. Onsets in the TN group were the least variable and were clustered around the beginning of dusk, with mean onset time occurring at a light intensity of 2.5 lux. In contrast, onsets in the TnN group were the most variable, spanning a 4-h range; mean onset for the group preceded the beginning of dusk by 92 min. Onsets in the RN and RnN groups preceded lights-off by an average of 44 min and 78 min, respectively.

Several animals also showed gradual changes in wheel-running activity onset during the 35 days of entrainment to the LD cycles. The most pronounced changes were seen in the no-nest animals and consisted of a gradual advance in activity onset, by as much as 1-2 h, followed in some cases by a gradual delay. This was confirmed by comparing standard deviations of onset times for individual animals over the last 28 days of entrainment (Table 1): the mean standard deviation was smallest in the TN group, followed in order by groups RN, RnN, and TnN. Thus the group order of intraindividual variability of onset times mirrored the group order of onset times. The two variables were negatively correlated at the individual level as well (Fig. 4). The correlation, calculated across all conditions, was highly significant ($r = -0.62$, $p = 0.0001$).

Between-group differences in wheel-running activity offset were less pronounced. There was, however, a significant main effect of cage, with earlier offsets in the nest than in the no-nest groups, $F(1, 36) = 43.95$, $p < 0.0001$, but no significant effect of light and no significant interaction. Mean activity offset for the TN group corresponded to a light intensity of 0.003 lux, with individual offsets occurring at intensities of 0.0006-3.69 lux. In contrast, half of the animals in the TnN group ended their daily wheel running after light intensity had reached 10 lux, with offsets in the remaining 6 hamsters occurring at 0.092-3.13 lux. Activity offsets preceded lights-on in all RN hamsters, but in only 3 of the 8 RnN hamsters.

Activity midpoints did not differ significantly between the four conditions. There was, however, a significant main effect of cage on α, $F(1, 36) = 58.15$, $p < 0.0001$, and a significant light × cage interaction, $F(1, 36) = 5.59$, $p < 0.05$; α was shortest in the TN group, followed in order by the RN group and by the two no-nest groups. Despite the pronounced differences in α, there were no differences between the groups in the total number of wheel revolutions per day, and no significant correlation between the number of revolutions per day and α.

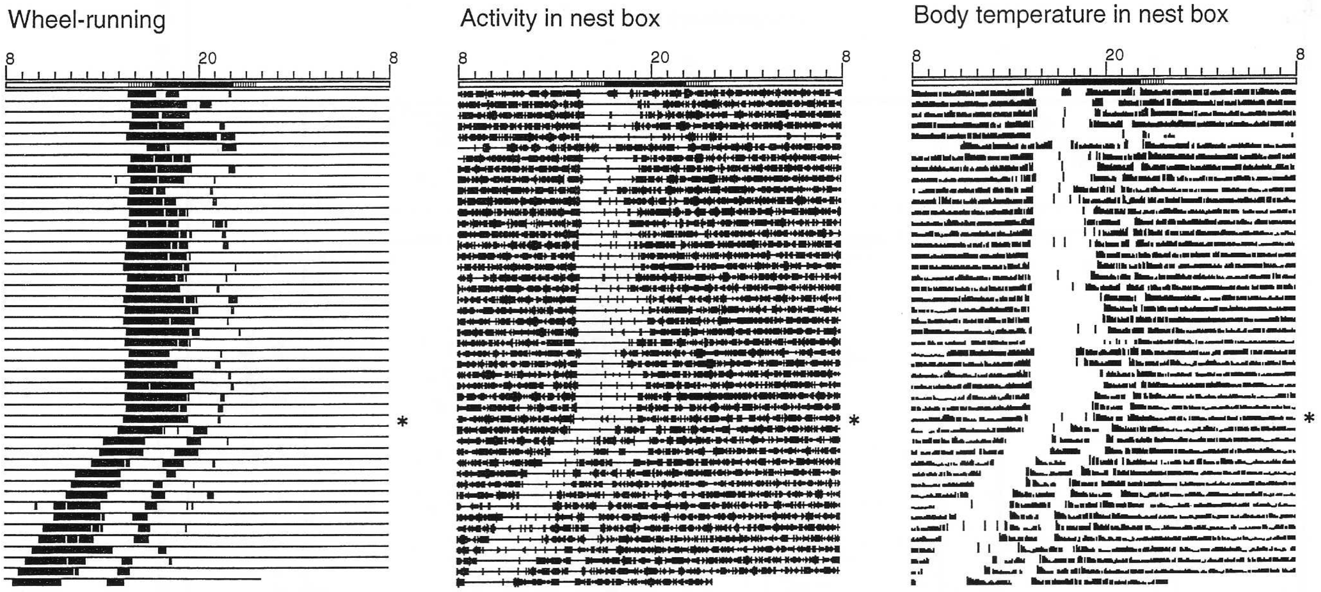

Figure 5. Records of wheel-running activity and of activity and body temperature inside the nest box for a hamster in LD-twilight. The wheel-running record shows 5-min intervals with ≥5 revolutions; nest box activity is indicated by vertical marks of three different heights, representing 1-4, 5-50, and >50 counts; the vertical scale in the body temperature record ranges from 35.80 to 38.90°C.

Wheel running, nest activity, and body temperature records of a hamster in LD-twilight are shown in Figure 5. As seen in the records, the only times the hamster was outside the nest box for an extended period were during bouts of wheel-running activity, primarily in the first half of the night. Furthermore, when wheel-running activity was divided into two or three major bouts, the intervals between the bouts were spent primarily in the nest box. This was typical of animals in both LD-twilight and LD-rectangular conditions.

Figure 6 shows daily waveforms of wheel running, nest activity, body temperature, and time spent in the outer compartment (time outside) for one hamster in LD-twilight (Fig. 6A) and one in LD-rectangular (Fig. 6B). The daily waveforms are averages across 8 and 6 days, respectively. In both LD cycles, body temperature started to increase 1-2 h before the daily onset of wheel-running activity, while the animals were still spending most of their time in the nest box. In most cases, however, nest box activity showed little or no increase during this time. The next 3-6 h were spent primarily in the outer compartment. In the 8 hamsters for which photobeam data were available, emergence in the evening, defined as the time when the animals spent at least 30 sec/min in the outer compartment, preceded the onset of wheel-running activity by an average of 16 min (range = 7-33 min). A final and relatively small bout of wheel-running activity generally took place at the beginning of the dawn transition in LD-twilight and just before lights-on in LD-rectangular.

Figure 7 shows the time spent in the outer compartment and light sampling behavior on 5 consecutive days for one hamster in LD-twilight (Fig. 7A) and one in LD-rectangular (Fig. 7B). The figure illustrates the following points: in LD-twilight, the animals were continuously exposed to light during the dusk transition but only intermittently during dawn. Very few, brief emergences into the outer compartment took place during the day. Most emergences were preceded by brief periods of light sampling (triangles). In addition, there were several instances of light sampling that were not followed by exits into the outer compartment (circles), occurring primarily during and shortly after the dawn twilight and in the period immediately preceding dusk. In LD-rectangular, the hamsters spent the last 1-2 h of the daily light period in the outer

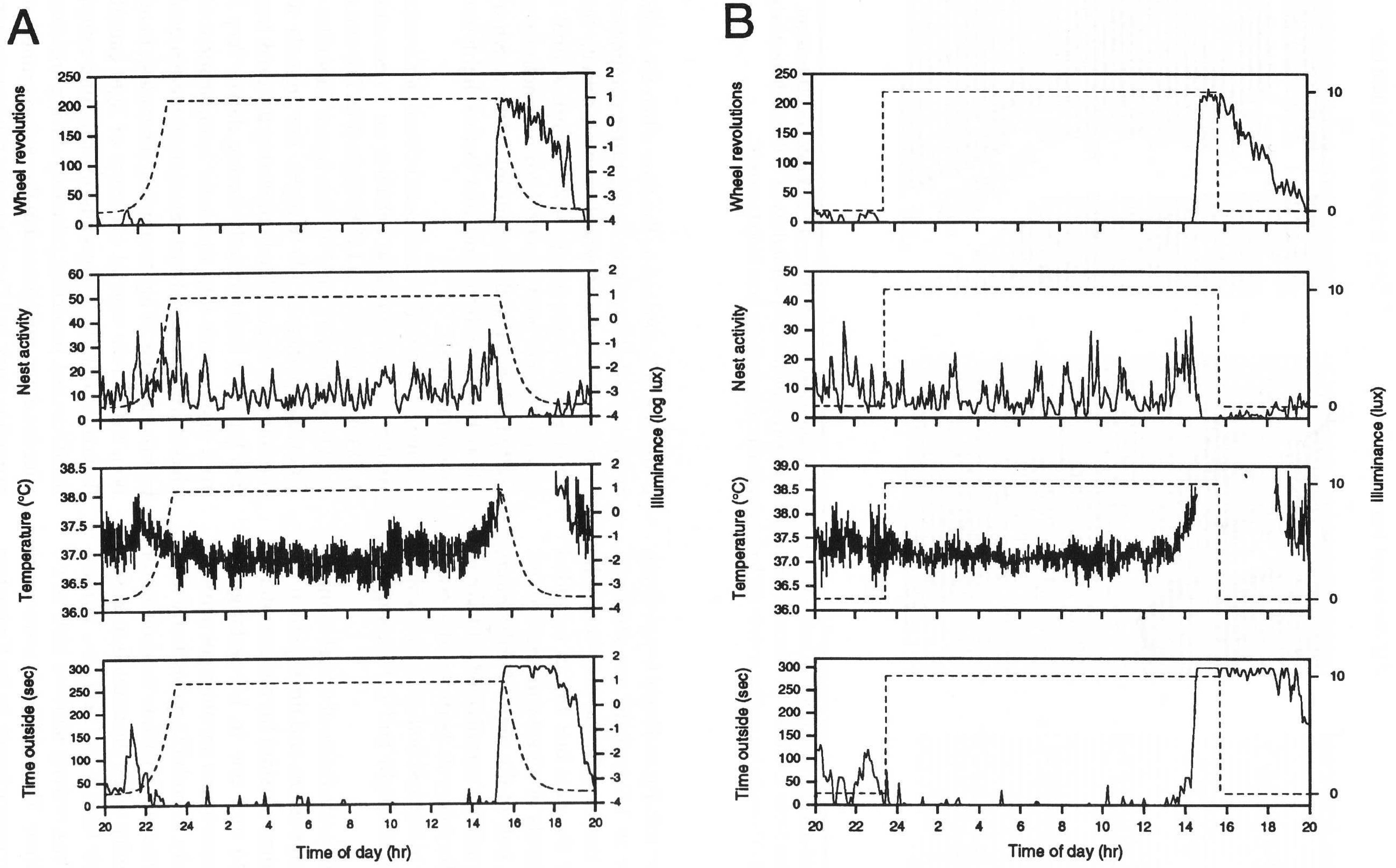

Figure 6. Average daily waveforms of wheel-running activity, of activity and body temperature (± s.e.m.) in the nest box, and of time spent in the outer compartment for a hamster in LD-twilight (A) and one in LD-rectangular (B). The data are plotted in 5-min intervals.

compartment, but were exposed to very little light in the morning. Compared to LD-twilight, emergences during the rest of the day were equally brief but tended to occur more frequently, and light sampling behavior was distributed more uniformly across the late portion of the night and throughout much of the day.

Wheel-Running Rhythms in DD

During DD, wheel-running onsets and offsets free ran in all cases from the phase held during entrainment (Fig. 2). This indicates that the differences between the groups observed during LD represent effects of the two environmental factors on the circadian pacemaker, rather than masking effects on locomotor activity. Free-running periods were calculated by fitting regression lines to the first and last five activity onsets (τ_{on}) and offsets (τ_{off}) of the DD condition (Table 1). Initially, τ_{on} was very short (overall mean = 23.77 ± 0.25 h) and did not differ significantly between the four groups. By the end of DD, τ_{on} had lengthened by an average of 0.2 h (paired t test, $p < 0.0001$) and was slightly but significantly longer in the no-nest than in the nest groups, $\tau_{on} = 24.03 \pm 0.17$ h and 23.92 ± 0.14 h, respectively, $F(1, 36) = 5.40$, $p < 0.05$. In contrast, τ_{off} was close to 24 h both initially (overall mean = 23.97 ± 0.18 h) and at the end of DD (overall mean = 24.00 ± 0.19 h), with no significant differences between the groups at either time and no significant difference between the values measured at the beginning and end of DD.

The fact that τ_{on} was initially shorter than τ_{off} indicates that α increased over the 2 weeks in DD; on average, α was 1.94 h longer on the last 5 days of DD relative to the last 5 days in LD (paired t test, $p = 0.0001$). The increase ranged from 1.51 h in the RN group to 2.34 h in the TN group, but the differences between groups were not statistically significant. Thus the difference in α between the nest and no-nest conditions observed during entrainment was maintained at the end of DD, nest: $\alpha = 9.1 \pm 1.3$ h, no-nest: $\alpha = 11.3 \pm 1.4$ h, $F(1, 36) = 22.36$, $p = 0.0001$.

Light Exposure

Mean daily light exposure values for the four groups of hamsters, calculated in lux • min, are summarized in Table 2. The data represent 5-day averages for 4 hamsters in each of two nest groups (photobeam data were not collected from the remaining hamsters), and for all animals in the two no-nest groups. For nest animals, light exposure was obtained by multiplying the time spent in the outer compartment in each 1-min interval by the mean illuminance during that interval. Light sampling was not included in these calculations. For the no-nest animals, the amount of light received at a given time of day was considered equal to the amount of light available at that time.

Table 2 also shows mean light exposure in the evening, in the morning, and during the rest of the day, as well as the difference between evening and morning light exposure ($L_{E\text{-}M}$). Light exposure determinations for evening, morning, and day were intended as estimates of the amount of light falling respectively during the delay, advance, and unresponsive segments of the PRC of each individual animal.

In the hamster, PRCs for brief light pulses (10-60 min) show a delay segment extending roughly from CT 11 to CT 16 and an advance segment from CT 16 to CT 1 (DeCoursey, 1964; Daan and Pittendrigh, 1976a; Ellis et al., 1982; Takahashi et al., 1984; Boulos et al., 1992). An alternative procedure for describing the PRC, however, was recently proposed by Meijer and De Vries (1995); it consists of plotting the size and direction of light-induced phase shifts in relation to α, taking into account both the onset and offset of activity, rather than by CT, which is derived from activity onset alone. Elliott and Pittendrigh (reported in Pittendrigh, 1981; Pittendrigh et al., 1984) have shown convincingly that decreases in α are accompanied by parallel reductions in the range of the PRC (the duration of the light-responsive region), during both steady-state and transient conditions (Fig. 5 in Pittendrigh et al., 1984; Fig. 9C in Pittendrigh, 1981; see also Meijer and De Vries, 1995; Elliott and Tamarkin, 1994). A similar relationship between α and the range of the PRC has also been observed in rats (Honma et al., 1985).

In the present analysis, morning, representing the phase advance segment of the PRC, was therefore defined as the interval starting at lights-on or at the beginning of dawn and ending 1 h after wheel-running activity offset, and evening, representing the phase delay segment, as the interval starting 1 h before activity onset and ending at lights-off or at the end of dusk.

Total light exposure in the nest animals represented only a small fraction of available light, averaging 2.9% in LD-twilight and 7.3% in LD-rectangular. Most of the daily exposure occurred in the evening, and very little of it in the morning; indeed, 3 of the 4 RN animals saw no morning light at all on any of the 5 days analyzed. Light exposure in the no-nest animals was much greater during all three periods of the day, with only

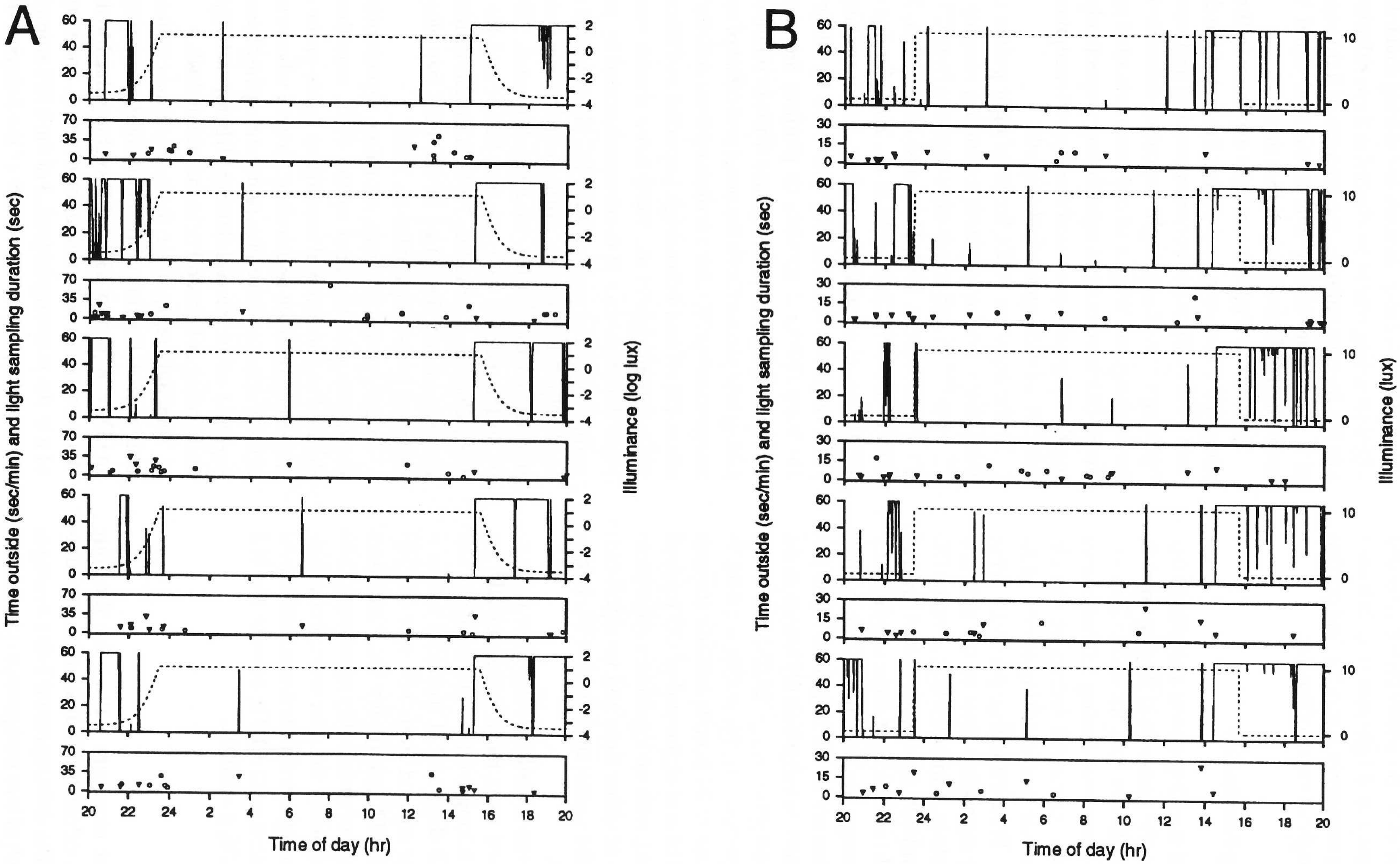

Figure 7. Time spent in the outer compartment (in 1-min intervals) and light sampling behavior (duration of individual light sampling episodes) on 5 days for a hamster in LD-twilight (A) and one in LD-rectangular (B). Light samplings followed by emergence into the outer compartment are represented by triangles and those followed by a return toward the nest box by circles. Emergences associated with cage cleaning have been edited from the records.

Table 2. Light exposure (mean and range, in lux • min) in the four groups of hamsters during different periods of the 24-h day.

	RN	*RnN*	*TN*	*TnN*
n	4	8	4	12
Evening	623 (155, 1128)	1381 (660, 1608)	216 (172, 272)	1586 (634, 2734)
Morning	3.7 (0, 15)	668 (36, 1158)	3.1 (0.02, 10)	744 (366, 1246)
Day	63 (46, 83)	7691 (7106, 8282)	54 (18, 104)	7410 (5980, 8570)
Total	711 (239, 1220)	9740 —	280 (224, 378)	9740 —
Total %	7.3 (2.5, 12.5)	100 —	2.9 (2.3, 3.9)	100 —
$L_{E\text{-}M}$	619 (155, 1128)	713 (–138, 1464)	213 (161, 272)	841 (–352,1868)

NOTE: Also shown is total daily exposure, total exposure as a percentage of available light, and the difference between evening and morning light exposure ($L_{E\text{-}M}$). Light exposure for the RN and TN groups was determined from the tunnel photobeam data, which were only recorded in 4 animals from each group.

small and statistically nonsignificant differences between the RnN and TnN groups. In all groups, the mean balance of evening and morning light exposure ($L_{E\text{-}M}$) was positive, although each of the two no-nest groups included one hamster with a negative balance.

The relationship between light exposure in LD and free-running period in DD was examined by calculating correlation coefficients between $L_{E\text{-}M}$ and τ, using initial and final values of τ_{on} and τ_{off}. For this analysis, the data from the two no-nest groups were combined, as these did not differ significantly in any of the variables measured. A significant negative correlation was obtained between $L_{E\text{-}M}$ and initial τ_{on} ($r = –0.51, p = 0.02$; Fig. 8) but not between $L_{E\text{-}M}$ and initial τ_{off} ($r = –0.06$). There were also no significant correlations between $L_{E\text{-}M}$ and either final τ_{on} ($r = –0.31$) or final τ_{off} ($r = –0.34$). Negative correlations between $L_{E\text{-}M}$ and initial τ_{on} were obtained in the two nest groups (RN: $r = –0.87$, TN: $r = –0.56$), but with only 4 hamsters in each group, these were not statistically reliable.

Morning light exposure was also calculated in terms of CT, assuming a conventional PRC (evening light was the same with both procedures). By this definition, morning ended at CT 1, or 13 h after activity onset. Morning light exposure was greater in all groups when morning was defined by CT; the average balance of evening and morning light remained positive in the nest groups but became negative in the no-nest groups (data not shown). Correlation coefficients between $L_{E\text{-}M}$ and τ were uniformly lower when morning was defined by CT than when it was defined by α, and none were significant.

DISCUSSION

Timing of Activity and Temperature Rhythms in LD

The inclusion of simulated twilights in the daily LD cycle and access to dark nest boxes affected several parameters of the hamsters' entrained wheel-running activity rhythms. Significant main effects included later activity onsets, earlier offsets, and shorter αs in the nest compared to the no-nest condition, whereas interactive effects of the two environmental factors were seen in activity onset and in α. In addition, both inter- and intraindividual variability in activity onsets were smallest in the TN group and greatest in the TnN group. There were, however, no significant differences between the groups in the time of activity midpoint or in the daily number of wheel revolutions. These results are discussed below, in relation to the light exposure patterns observed in the different groups and the characteristics of the animals' rhythms in DD.

In all hamsters in the TN and RN groups, body temperature started to increase 1-2 h before the onset of wheel-running activity, while the animals were still inside the nest box. This initial rise in temperature was apparently not generated by an increase in locomotor activity, as most animals showed little or no increase in nest box activity during that time. In studies in which hamsters are not provided with nest boxes (Golombek et al., 1993; Refinetti, 1994), changes in body temperature and locomotor activity occur in close temporal association, and endogenous and activity-induced increases in temperature are therefore less distinct.

The absence of a clear increase in nest box activity preceding the start of activity in the outer compartment contrasts with the results of Korenman et al. (1988). In that study, the hamsters were exposed to both rectangular and natural LD cycles, with long photoperiods comparable to those used here, but the nest boxes were considerably larger. The small size of the nest boxes in the present study may not have allowed much of an increase in activity. Instead, the hamsters crossed the tunnel with increasing frequency during that time; such activity would not have been picked up by the receivers placed under the nest boxes, although it may have contributed to the rise in body temperature. The animals also spent between 7 and 33 min in the outer compartment before the onset of wheel running, presumably engaged in other forms of motor activity. This is consistent with data obtained by Aschoff et al. (1973), showing an increase in general activity in a spring-suspended cage starting about 10-40 min before wheel-running onset.

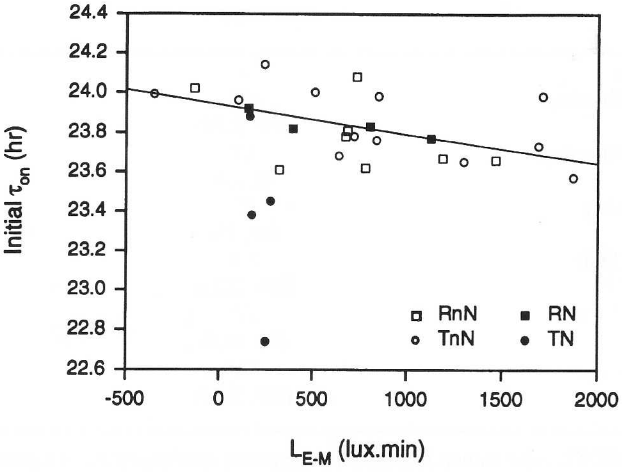

Figure 8. Free-running period determined from the first five activity onsets in DD (initial τ_{on}) plotted as a function of the balance of evening and morning light exposure (L_{E-M}) in LD for all hamsters in the two no-nest groups and for 4 hamsters in each of the two nest groups. The linear regression line was fitted through the data from the no-nest animals only ($y = -0.00015x + 23.94$, $r^2 = 0.259$).

Activity Rhythms in DD

Most animals showed a rapid expansion, or decompression, of α during the first few days of DD. The rate of expansion was similar in the four groups, despite the fact that α in LD was shorter in the nest than in the no-nest groups. As a result, α during the last 5 days of DD remained about 2 h shorter in the nest groups than in the no-nest groups. This suggests that there may have been a ceiling effect on the rate of α expansion and that, given sufficient time in DD, α would have eventually expanded to the same extent in all animals.

The expansion of α was accomplished by a rapid advance of activity onsets relative to activity offsets. The mean τ, measured from activity onsets, was initially 23.77 h—considerably shorter than the species average of 24.1 h (Pittendrigh and Daan, 1976a; Aschoff, 1979)—with no significant differences between the four groups. By the end of the 2 weeks in DD, τ_{on} had lengthened in all groups: the mean was 24.03 h in the no-nest animals, approaching the species average and, at 23.92 h, was slightly but significantly shorter in the nest animals. In contrast, activity offsets free ran with a period close to 24 h throughout the DD condition.

The behavior of the activity rhythms in DD resembles that reported by other investigators in some respects but differs in others. A compression of α during entrainment to long photoperiods is commonly observed in nocturnal rodents but, in hamsters and in *Peromyscus leucopus*, it persists for some time in DD, although a rapid decompression is occasionally observed in the latter species (cf. Fig. 12 in Pittendrigh and Daan, 1976a). A shortening of τ as an aftereffect of entrainment to LD 18:6 has also been observed in *P. leucopus* and in *Mus musculus*, but not in hamsters (Pittendrigh and Daan, 1976a). In the present study, although the values of α and τ observed in DD may be considered in a general sense aftereffects of entrainment to long photoperiods, the rapidity with which these parameters changed qualifies them more as transients. We have no explanation for these differences, except to note that the photoperiods studied by Pittendrigh and Daan, LD 1:23 and LD 18:6 in the case of hamsters, were different from that used here (LD 16:8, approximately), and the light intensity (100-200 lux) was substantially higher. As discussed below, however, the distinction between transients and aftereffects may not be critical for interpreting the present results.

Light Sampling and Light Exposure

A number of nocturnal animals that normally spend the day in light-excluding shelters have been observed to come up to the entrance of the shelter and to remain there for various durations, often returning to the interior before emerging fully for the start of their main outdoor activity period (e.g., Saint Girons, 1966; DeCoursey and DeCoursey, 1964; DeCoursey, 1989). Such behavior may inform the animal of the presence of conspecifics or predators, but it often serves a light sampling function as well. Indeed, in several fossorial rodents and in bats, activity onset at dusk coincides with a relatively fixed (but usually season-dependent) light intensity level, occurring earlier on overcast than on clear days (DeCoursey, 1960; Saint Girons, 1966); this can only happen if activity onset is regularly preceded by light sampling behavior. In the present study, light sampling was observed in all 8 hamsters for which photobeam data were available; this behavior may have contributed to the greater stability of activity onsets in the TN group, as these occurred at a relatively constant light intensity during the dusk transition.

As expected, the animals with access to nest boxes spent little time in the outer compartment during the day and were exposed to only a portion of the available light. In all cases, the animals were consistently exposed to light only in the evening, starting shortly before the daily onset of wheel-running activity; light exposure in the morning was much less prolonged and more sporadic. The total daily light exposure ranged between 2.3% and 3.9% of available light in the TN group and between 2.5% and 12.5% in the RN group. These values are somewhat higher than those obtained by DeCoursey (1989) in flying squirrels (0.3%-3.5%); the difference may be due, at least in part, to the longer photoperiods used in the present study (LD 16:8 vs. LD 9:15) and to the lower light intensity levels, but a species difference may also be involved.

In the TN group, activity onsets and offsets generally fell during the twilight transitions, with mean onset occurring at a higher light intensity than mean offset (2.5 lux and 0.003 lux, respectively). In the other three groups, the mean phase angle difference between activity onset and dusk or lights-off was also greater (more positive) than that between activity offset and dawn or lights-on. These results are consistent with the observations of Daan and Aschoff (1975), who recorded wheel-running activity in hamsters exposed to natural illumination throughout the year in Southern Germany (48°N latitude) and near the arctic circle (66°N latitude). At both latitudes, activity onsets occurred at higher light intensities than activity offsets, the phase angle difference between activity onset and sunset being greater than that between activity offset and sunrise.

During the rest of the day, the animals emerged into the outer compartment only infrequently and for brief durations, a result previously obtained in several nocturnal rodents studied in the laboratory (Rusak, 1975; Lynch et al., 1985; DeCoursey, 1986, 1989; Korenman et al., 1988; Pratt and Goldman, 1986; Terman et al., 1990, 1991). In the flying squirrel, drinking accounted for 71% of all daytime emergences (DeCoursey, 1989), but preliminary observations in hamsters indicated that transferring the water bottle from the outer compartment to the nest box had no effect on the emergence pattern (Pratt and Goldman, 1986). During the night, all hamsters made several trips to the nest box, as determined from photobeam data and/or telemetric recordings of nest box activity; both the frequency and duration of the trips increased as the night progressed. At least some of these nocturnal trips may have been associated with feeding, because all food was always stored in the nest box, and with urination and defecation, of which there was rarely any trace in the outer compartment.

Phase-Shifting Effects of Evening and Morning Light

The model of nonparametric entrainment accounts for entrainment of circadian rhythms by a net daily phase shift, resulting from periodic light exposure, which compensates for the difference between τ and the period of the entraining cycle; given τ and the PRC, the model predicts the behavior of circadian rhythms under a variety of lighting conditions (Pittendrigh and Daan, 1976a, 1976b, 1976c; Daan and Pittendrigh 1976a, 1976b). However, neither τ nor the PRC are fixed parameters, and both have been shown to be influenced by the entrainment process itself (Pittendrigh and Daan, 1976a; Daan and Pittendrigh, 1976a; Menon et al., 1988). The same is also true of α, and there are several indications that α, τ, and the PRC are interdependent (Pittendrigh and Daan, 1976c).

The plasticity and interdependence of the three parameters are accounted for by assuming that the pacemaker in nocturnal rodents consists of two mutually coupled oscillators (Pittendrigh, 1981; Pittendrigh and Daan, 1976c). One of these, the evening oscillator (E), controls the onset of activity and is coupled to dusk, whereas the other, the morning oscillator (M), controls the end of activity and is coupled to dawn. The phase relation between the two oscillators is flexible, within limits, and it determines α, τ, and the shape of the PRC. Thus the coupling of E and M to dusk and dawn, respectively, accounts for the changes in α in response to changes in photoperiod. The model also implies that a given pulse of light will fall on different phases of the two oscillators and may, therefore, cause unequal phase shifts; the transients that can follow such pulses reflect the motion of one or both oscillators as they regain their former phase relation. In some situations, the normal phase relation is regained only gradually, thus accounting for aftereffects on the three parameters of the compound pacemaker.

In the present study, the balance of evening and morning light exposure ($L_{E\text{-}M}$) affected the hamsters' rhythms in two ways. First, entrainment of the rhythms implies that $L_{E\text{-}M}$ caused a net daily phase shift equal to the difference between the period of the pacemaker and 24 h; the period of the pacemaker, however, could not be determined due to the occurrence of transients in DD. Second, the compression of α in LD and its subsequent expansion in DD indicate that $L_{E\text{-}M}$ also delayed the phase of E relative to that of M, thereby opposing the coupling interaction between the two oscillators. Upon termination of the LD cycle, this coupling interaction, now unopposed, would have resulted in a rapid advance of E relative to M, as reflected in the transient shortening of τ_{on}, until a more stable phase relation was reestablished between the two oscillators. Analysis of the relationship between light exposure patterns in LD and free-running periods in DD showed a significant negative correlation between $L_{E\text{-}M}$ and initial τ_{on}, but not between $L_{E\text{-}M}$ and final τ_{on}, initial τ_{off}, or final τ_{off}. These results suggest that, of the four measures of free-running period, initial τ_{on} is the one that best reflects both the period of the pacemaker during entrainment and the delay of E relative to M.

Although significant, the correlation between $L_{E\text{-}M}$ and initial τ_{on} in the no-nest animals accounted for only 26% of the variance. This is not surprising, given the number of simplifying assumptions implicit in this analysis: (1) the amount of light received in the no-nest condition was assumed equal to the amount of light available, when it is undoubtedly modulated by eyelid closure and postural variations; (2) we assumed that light exposure at any time within the delay (or advance) segment of the PRC caused delays (or advances) of the same magnitude; given the shape of the PRC, this is clearly unrealistic; (3) our use of lux • min as the unit of light exposure assumes a constant reciprocal relation between light intensity and duration; the hamster circadian system, however, has been shown to be relatively more sensitive to 5-min light pulses than to either shorter (1-3 sec) or longer (1 h) durations (Nelson and Takahashi, 1991a); (4) the changes in light intensity at dusk and dawn were produced by varying the voltage to an incandescent lamp, and were therefore unavoidably accompanied by shifts in spectral composition. Under these conditions, illuminance (in photopic lux) is somewhat inaccurate as a measure of effective light intensity, because the spectral sensitivity function of the hamster circadian system is more similar to the action spectrum for rhodopsin than to that for human photopic vision (Takahashi et al., 1984).

Effects of Twilights and Nest Box Availability

The largest differences in the times of activity onset and offset observed in this study were between the nest and no-nest conditions. These differences may be accounted for by the light exposure patterns of the animals in the evening and in the morning. Hamsters with access to nest boxes were exposed to less light at both times of the day, but the difference was greater in the morning, due, in part, to the fact that the nest animals spent less time in the outer compartment at that time of day. Thus the later onsets observed in the nest groups would have provided the means for decreasing light exposure in the evening and obtaining the necessary balance between the phase-delaying effects of evening light and the phase-advancing effects of morning light.

Hamsters in the nest groups, however, also showed earlier activity offsets than those in the no-nest groups. This seems paradoxical, because an earlier activity offset would result in an even smaller phase advance (assuming that the range of the PRC is proportional to α). A possible explanation is provided by the finding that the times of activity midpoint did not differ significantly between the different conditions. This indicates that the time of activity midpoint relative to the LD cycle was determined primarily by factors common to all four conditions, namely photoperiod duration and daytime light intensity. If so, then light exposure in the evening could only be decreased by a symmetrical compression of α about its fixed midpoint, such that any delay in activity onset is accompanied by an equal advance in activity offset. Because activity midpoint almost always preceded the middle of the night (there were only 3 exceptions out of a total of 40 animals), a symmetrical compression of α would decrease light exposure in the evening more than in the morning. Such a mechanism would therefore account for both the earlier offsets and the later onsets observed in the TN and RN groups.

The effects of twilight transitions were relatively small and were only observed in hamsters that had access to nest boxes: mean activity onset was significantly later and mean α significantly shorter in the TN than in the RN group, whereas the TnN and RnN groups did not differ in any of the measured parameters. These results differ from those obtained by Wever (1967), who found earlier activity onsets in hamsters (without nest boxes) under long (45 min) than under short (5.67 min) twilights. The contrasting results probably reflect the differences in the lighting conditions used in the two studies, which included differences in daytime and nighttime light intensity, in photoperiod duration, and in the rate of change of light intensity at dawn and dusk.

Pittendrigh and Daan (1976b) compared the activity rhythms of hamsters entrained to natural light cycles throughout the year at high latitude (original data from Daan and Aschoff, 1975), or to artificial, rectangular light cycles with different photoperiod durations (original data from Elliott, 1974). For photoperiods of 11 h or longer, the phase angle of entrainment was similar in the two LD conditions; this is consistent with the absence of significant differences between the activity onset times of the two no-nest groups in the present study. With decreasing photoperiod durations, however, activity onsets occurred progressively earlier under natural than under rectangular light cycles: under LD 6:18, for example, activity began more than 3 h earlier under the former than under the latter conditions (Fig. 17 in Pittendrigh and Daan, 1976b). The extent to which these differences are attributable to the presence of twilights in one condition and their absence in the other is of course uncertain, because the two light cycles differed in other respects as well. Nevertheless, the data suggest that the influence of twilights on the phase angle of entrainment in hamsters may be greater under short than under long photoperiods.

We have recently compared the upper limits of entrainment in four groups of hamsters, initially kept under conditions identical to those of the present study in all respects (Boulos et al., 1996). The period of the LD cycle was then lengthened by 5 min/day, from 24 h to 26 h. All animals in the TN and TnN groups remained entrained to the lengthening LD cycle, whereas most animals in the RN and RnN groups started to free run well before the period of the LD cycle reached 26 h. These results indicate that the inclusion of twilights raises the upper limit of entrainment and thus appears to increase the zeitgeber strength of the LD cycle, as predicted by Wever (1965). The increase in zeitgeber strength, however, was independent of nest box availability, despite the fact that, in the present study, the TN and TnN groups were the ones that showed the greatest differences in activity onset time, in the precision of activity onset, and in activity duration. This indicates that, at least under the conditions of the present study, none of these entrainment parameters accurately reflect the strength of the LD zeitgeber.

A clear relationship was found between the times of activity onset and the intraindividual variability of onset times: the earlier the onset, the greater the standard deviation of onset times. This relationship may be accounted for by the relative change in light exposure resulting from a change in onset time. In all conditions, activity onsets occurring earlier than usual would result in the animal being exposed to light in the evening for a longer duration. This would cause a larger phase delay and would tend to restore the normal phase relation between activity onset and the LD cycle. It seems reasonable to assume that the additional phase delay caused by a given increment in evening light duration would depend on the duration of evening light normally experienced by the animal (cf. Nelson and Takahashi, 1991a). A 10-min increase in light exposure duration, for example, would be expected to have less of an effect in animals normally exposed to light for 2 h in the evening than in animals normally exposed to light for only 30 min. Activity onsets would therefore be maintained within narrower limits in the latter case than in the former. In addition, if activity onset normally falls during the dusk twilight, as it did in the TN group, then an earlier onset would result not only in a longer duration of evening light exposure but also in a higher light intensity. This would tend to further limit the day-to-day variability in activity onsets in the TN group compared to the other three groups.

A relationship between the time of activity onset relative to sunset and the standard deviation of activity onset was observed in hamsters by Daan and Aschoff (1975). In that study, the day-to-day variability in onset times was lowest when onsets fell during civil twilight, the time of day when light intensity changed most rapidly. Daan and Aschoff concluded that this relationship could be entirely accounted for by a direct effect of light intensity on activity onset and, therefore, that differences in phase resetting need not be involved. However, a more rapid change in light intensity also implies that a given change in onset time will result in a greater change in the amount of light falling during the delay portion of the PRC. Such a mechanism may therefore have contributed to the observed relationship between the variability in onset times and the rate of change of light intensity.

In summary, the results suggest that the entrainment patterns observed in the different conditions are largely accounted for by the light exposure patterns of the animals—more specifically, by the balance of evening and morning light exposure. Interestingly, the major differences in entrainment pattern—those between the nest and no-nest conditions—appear to have a behavioral origin, namely the tendency of the animals to avoid light exposure in the morning when given the opportunity. The effects of twilight transitions were both more subtle and more difficult to account for in terms of light exposure, one possible exception being the greater precision of activity onsets in the TN group. The validity of these conclusions, however, can only be established by examining the relationship between entrainment and light exposure patterns under a range of lighting conditions, including different light intensity levels, photoperiods, and twilight durations. The use of shorter photoperiods would be particularly useful, as this would allow assessment of the relationship between light exposure and subsequent free-running period without the added complexity of transients. Nonetheless, long photoperiods prevail during much of the year at temperate latitudes, and results obtained under such conditions, however complex their interpretation, are necessary for a more complete understanding of circadian entrainment as it occurs in nature.

ACKNOWLEDGEMENTS

This research was supported by National Institutes of Health Grant R01 NS30528.

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