Compression and Expansion of Circadian Rhythm in Mice Under Long and Short Photoperiods

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Although the functional aspects of synchronization of the circadian pacemaker by environmental light have been extensively studied, few studies have provided systematic information about the temporal organization of behavior under light-dark cycles with varying proportions of light and darkness. In the present study, the running-wheel activity profiles of mice were investigated under short, medium, and long photoperiods. The results clearly indicated that the temporal distribution of locomotor activity in mice is modulated by photoperiod. The activity profile was compressed under long photoperiods and expanded under short photoperiods. Although negative masking by light and alteration in the state of dark adaptation may have partially accounted for the phenomenon, the major mechanism seemed to be a compression and expansion of the circadian pacemaker's cycle, as expressed in the compression and expansion of the photic phase-response curve.

Introduction

Virtually all life forms exhibit circadian rhythms—that is, endogenously-generated oscillations in biological processes with a period close to but different from 24 hours (Turek & Van Reeth, 1996). In mammals, a circadian pacemaker located in the suprachiasmatic nucleus of the hypothalamus (SCN) controls a variety of behavioral and physiological processes, including locomotor activity, body temperature, ingestive behavior, and blood hormone levels (van Esseveldt, Lehman & Boer, 2000). The cellular and molecular mechanisms responsible for the endogenous generation of circadian rhythmicity have received great attention during the last decade or so (King & Takahashi, 2000), and the synchronizing effect of the environmental light-dark cycle on the pacemaker has been consistently investigated for more than 40 years (Johnson, 1999). However, few studies have provided systematic information about the temporal organization of behavior under light-dark cycles with varying proportions of light and darkness.

Lesion and brain-transplant studies have repeatedly demonstrated that the presence of a functional SCN is essential for the occurrence of circadian rhythmicity in various behavioral and physiological variables (Earnest et al., 1999; Ralph et al., 1990; Sollars, Kimble & Pickard, 1995). In vitro recordings of neuronal activity in the SCN have established that individual neurons display circadian rhythmicity and that the period of the rhythm displayed at the tissue level corresponds to the mean of the periods of oscillation of the individual cells (Herzog, Takahashi & Block, 1998; Shirakawa et al., 2000; Welsh et al.,

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1995). Studies at the molecular level have identified several genes and associated proteins that seem to establish a multi-gene feedback loop responsible for the rhythmicity observed at the cellular level (Gekakis et al., 1998; Shearman et al., 2000).

An endogenous clock can be beneficial to the organism's adaptation to the temporal structure of the environment only if the clock can be synchronized (entrained) by environmental time cues (Daan, 1981). The daily light-dark cycle is one of the most conspicuous time cues in the natural environment. Photic stimulation of the eyes has been shown to directly stimulate the SCN via a specialized monosynaptic pathway—the retinohypothalamic tract (Aggelopoulos & Meissl, 2000; Moore, 1973; Senseman & Rea, 1994). According to the widely-accepted non-parametric theory of entrainment, synchronization is attained not by an acceleration or deceleration of the pacemaker but by discrete daily phase shifts that equal the difference between the period of the light-dark cycle and the period of the pacemaker (Pittendrigh, 1981). The theory is called non-parametric because light is believed to affect the pacemaker by inducing discrete phase shifts rather than by parametrically slowing or accelerating the pacemaker.

Although much has been learned about how photic stimulation modulates the phase of the circadian pacemaker and the period of the expressed rhythms, relatively little attention has been given to the effects of illumination on the wave form of circadian rhythms. The lack of attention given to wave form is understandable because of the traditional practice of plotting rhythm data in the actogram format, a format in which detailed wave-form information is sacrificed in favor of greater temporal resolution over consecutive days (Reffinetti, 1992). Nevertheless, some researchers have analyzed the wave form of activity rhythms. In one study on rats, the daily duration of drinking and feeding activity was found to be progressively compressed as the duration of the dark phase of the light-dark cycle was shortened from 15 h to 12 h, and then to 9 h per day (Rosenwasser, Boulos & Terman, 1983). In diurnal animals, gradual shortening of the light phase of the light-dark cycle caused a gradual compression of the rhythm of locomotor activity in chipmunks (DeCoursey, 1972), of the rhythm of drinking activity in squirrel monkeys (Sulzman, Fuller & Moore-Ede, 1982), and of the rhythm of feeding activity in pigeons (Basco, Rashotte & Stephan, 1996).

The goals of the present study were to evaluate the compression and expansion of the circadian rhythm of locomotor activity in mice maintained under long and short photoperiods and to investigate the mechanism by which the compression and expansion are achieved. Among several potential mechanisms responsible for compression and expansion, the simplest one is perhaps negative masking (i.e., a parametric effect consisting of the inhibition of locomotor activity by light in nocturnal animals). To assess the role of negative masking in the compression of the active phase ($\alpha$) of the activity rhythm, the pattern of running-wheel activity of animals maintained under various light-dark cycles was compared with that of the same animals on the first day after transfer to constant darkness. Other potential mechanisms responsible for rhythm compression and expansion include an alteration in rhythm expression due to a non-parametric effect of photic stimulation on the pacemaker (Pittendrigh, 1981), a parametric compression or decomposition of the circadian pacemaker's cycle (Sumová, Trávníčková & Illnerová, 1995), and a change in photic sensitivity of the pacemaker caused by the alteration in the duration of daily photic exposure (Daymude & Reffinetti, 1999). These possibilities were assessed by examination of the locomotor activity rhythm under a skeleton photoperiod and by evaluation of phase-response curves depicting phase shifts in response to discrete light pulses after exposure to light-dark cycles with different photoperiods.
Method

Subjects

Male, 3-month-old mice (CD-1 strain) were purchased from Charles River Laboratories (Wilmington, MA) and housed individually in polypropylene cages (24 x 36 x 19 cm) lined with wood shavings under a 24-h light-dark cycle (LD 12:12). Food pellets (Purina lab diet) and water were provided ad libitum.

Apparatus

The animal cages were maintained inside individual light-tight, ventilated chambers at 24°C under varying lighting regimes controlled by programmable timers. In all cases, illumination was provided by individual white fluorescent bulbs located on the ceiling of the chambers. Illuminance 10 cm above the floor of the animal cages was 300 lux. Changes of water bottles and refilling of food hoppers were conducted during the light phase of the light-dark cycle whenever possible. For animals maintained in constant darkness, animal care procedures were conducted under dim red light (< 1 lux).

For the measurement of locomotor activity, a metallic running wheel (12 cm diameter) was attached to each animal cage. Magnetic switches attached to the running wheels were connected to a data acquisition system (A-BUS, Alpha Products, Darien, CT) that recorded the number of wheel revolutions in 6-min bins (i.e., 0.1 h). Data were transferred later to a different computer for analysis.

Procedure

Animals were kept under a 24-h light-dark cycle with 12 hours of light per day (LD 12:12) prior to the beginning of the experiments. To evaluate the compression and expansion of the circadian rhythm of locomotor activity under different photoperiods, groups of 30 mice were kept for 28 days under LD 16:8 (long photoperiod), LD 12:12 (medium photoperiod), or LD 8:16 (short photoperiod). An additional group of 30 mice was kept under a skeleton photoperiod with only 15 min of light per day (LD 0.25:23.75), which is sufficient to maintain entrainment but exposes the animals to much less light than full light-dark cycles do. To facilitate the transition from the preceding full light-dark cycle to the skeleton photoperiod, the 15 min of light stimulation were set to start 30 min before the light-to-dark transition of the preceding LD 12:12.

Data from the last 6 of the 28 days were used for analysis. Running-wheel revolutions in each of the 240 daily 6-min bins were averaged over the six days for each animal. To prevent highly active animals from skewing the group means, the absolute activity scores were first converted into percentages of the number of revolutions in the highest bin (i.e., the bin with highest number of revolutions) for each day for each animal. The daily averages for the individual animals were then averaged over all 30 animals in each group. These grand-mean activity profiles were plotted in Cartesian coordinates for visual inspection and were compared by the Kolmogorov-Smirnov test (Siegel, 1956) to determine statistical significance of the differences in temporal profile. The duration of the active phase of the activity rhythm (α) for each group profile was computed as the interval between the time at which the profile ascended past the level of 10 percent of maximal activity and the time at which the profile descended past the 10 percent level. If the 10
percent level was crossed more than twice, the first ascension and last descent were used for the computation of $\alpha$.

To evaluate the effect of photic masking on the activity rhythm, all four groups of mice were transferred to constant darkness (DD) at the end of the 28th day. Because the effects of the preceding light-dark cycles were expected to vanish over time, only data from the first day in DD were used. Group profiles and $\alpha$ were computed in the same manner as described earlier. To allow comparison between the profiles for light-dark cycle and constant darkness, the profiles for DD were expressed in geophysical time without correction for variations in free-running period. To evaluate whether this could have caused a distortion in the DD profiles, a fifth group of 30 mice was maintained in DD throughout the experiment, and its activity profile was computed for the 29th day. The profile was computed twice, once with geophysical time and once with circadian time. Circadian time ($t'$) was calculated as $t' = t \cdot 24/\tau$, where $t$ is geophysical time and $\tau$ is the free-running period as determined by the chi-square periodogram procedure (Sokolove & Bushell, 1978) using the activity data for Days 19 through 28.

Presentation of discrete light pulses for the construction of phase-response curves would ideally take place on the first day after release from the light-dark cycle in order to capture the full effect of the preceding treatment. However, determination of the exact pre-pulse phase of the activity rhythm requires several days of data. As a compromise between the need to present the light pulses as soon as possible and the need to have several daily onsets for computation of circadian phase, light pulses were presented on the 6th day after release into DD. Activity records for each animal were plotted as actograms, and circadian times were determined from the actograms using the daily onset of activity as circadian time 12 (CT 12). Light pulses of 60-min duration were administered in each animal's own chamber without physical disturbance of the animals. Each animal received one pulse. As there were 30 animals per group, one animal was pulsed at each circadian hour (from CT 0 to CT 23), and the 6 extra animals were pulsed one per circadian hour between CT 13 and CT 18. Phase shifts were determined by drawing separate eye-fit lines through the onsets for 5 days before and 7 days after the pulse and calculating the time between the two lines on the first cycle following the pulse (Repinetti, 1992). The shifts were plotted as a function of circadian time in 3-h bins for each group (those previously kept under LD 16:8, LD 12:12, LD 8:16, and LD 0.25:23.75). Group means were compared with a factorial ANOVA (with animal group and time bin as factors).

**Results**

A representative sample of the locomotor activity records of mice maintained under a long photoperiod (LD 16:8) and a short photoperiod (LD 8:16) is shown in Figure 1. To allow greater temporal resolution for visual inspection, only three days are shown for each animal. Under the long photoperiod (short night), activity initiates abruptly at the time of lights off and terminates abruptly a little after the time of lights on. Activity also initiates abruptly at the time of lights off under the short photoperiod (long night) but ascends to lower levels and is interrupted several times until termination at the time of lights on. As a consequence, the total amount of activity per day is conserved despite the expansion of the active phase.

The mean total activity of all animals in the four groups (LD 16:8, LD 12:12, LD 8:16, and LD 0.25:23.75) was 17,245 revolutions per animal per day. This corresponds to a running distance of 6 km per day. Although some mice ran more than others (best runner:
Fig. 1. Three-day segments of the records of running-wheel activity of two representative mice, one kept under a long photoperiod (LD 16:8) and one kept under a short photoperiod (LD 8:16). The data are plotted with 6-min (0.1 h) resolution. The white and black bars above the activity records indicate the duration of the light and dark phases of the light-dark cycle, respectively.

28,197 revolutions per day; worst runner: 3,546 revolutions per day), there were no significant differences among the means for the four groups, $F(3, 116) = 2.21, p > .05$.

To prevent highly active animals from skewing the group means, the absolute activity scores were converted into percentages of the number of revolutions in the highest bin for each day for each animal prior to group comparisons. Mean activity profiles computed for the 30 animals in each group (based on 6 days for each animal) are shown in Figure 2. To avoid cluttering of the figure, standard errors of the means are plotted only in 2-h intervals, even though the means are plotted in 6-min intervals. Under all four LD cycles, locomotor activity increased sharply at the time of lights off and decreased gradually over the course of many hours. As indicated in each panel, $\alpha$ ranged from 9.5 h under LD 16:8 to 15.9 h under LD 0.25:23.75. Both under LD 16:8 and LD 0.25:23.75, a small increase in activity preceded the sharp rise at lights off. Clusters of activity as those seen in individual records under LD 8:16 (cf. Figure 1) were not present in the averaged profiles because the period of these ultradian oscillations was not consistent between animals, which resulted in the averaging out of the oscillations. Kolmogorov-Smirnov tests were used to compare the four profiles. The Kolmogorov-Smirnov test is a test of similarity of distributions and, consequently, it evaluates whether the differences between two wave forms are greater than that expected by random fluctuations. Thus, it constitutes a holistic test of similarity, distinct from specific comparisons of $\alpha$, peak activity time, or magnitude of peak. With the level of significance adjusted for multiple comparisons, each activity profile was found to be significantly different from all others (familywise $p < .05$) except that LD 12:12 did not differ significantly from LD 0.25:23.75.
Mean activity profiles computed for the 30 animals in each group during the first day in DD immediately after the 28 days under LD cycles are shown in Figure 3. A sharp increase in activity at the time when the lights were previously turned off was seen, even though in an attenuated form, in all groups except the one previously exposed to LD 16:8. In this group, activity increased gradually, starting several hours prior to the previous time of lights off. In all groups, $\alpha$ was expanded by a few hours (cf. Figure 2). As determined by Kolmogorov-Smirnov tests, all profiles were significantly different from each other and from the profiles obtained under the LD cycles (familywise $p < .05$).

In the additional group of mice that was maintained in DD throughout the experiment, free-running periods ranged from 23.1 to 24.0 h, with a mean of 23.6 h. The activity profile of this group, expressed both in geophysical time and in circadian time, is shown in Figure 4. Because the geophysical time of activity onsets varies greatly in free-running animals, the records from the individual animals were aligned in reference to onset time before averaging. Except for the beginning of a new circadian cycle of activity at the end of the day in the top panel, the two profiles were quite similar. Activity increased sharply at first, decreased slightly 3 h later, rebounded for a few hours, and then gradually decreased over about 8 h. The calculated $\alpha$ was 12.5 h. As determined by the Kolmogorov-Smirnov test, the activity profile was significantly different from all other profiles determined for the LD and DD conditions (familywise $p < .05$).

Figure 5 shows representative records of activity (in actogram format) depicting phase shifts evoked by single 60-min light pulses. A pulse presented at CT 17 evoked a phase delay of 2.8 h (A); a pulse presented at CT 22 evoked a phase advance of 1.2 h (B); and a pulse presented at CT 18 evoked no phase shift despite a change in free-running period (C).

Figure 6 shows the phase-response curves (PRCs) depicting the phase shifts associated with light pulses presented at different circadian times (in 3-h bins) to animals previously held under the four different LD cycles. Although the graphs are not technically curves but bar graphs, I refer to them as PRCs because they describe the relationship between circadian phase and magnitude of phase shift in the same manner as continuous PRCs do. For instance, the top panel shows that animals maintained under LD 16:8 responded to light pulses presented at CT 13 (i.e., from CT 12 to CT 14) with a mean phase delay of 0.9 h (and standard error of 0.45 h). Likewise, animals maintained under LD 16:8 responded to light pulses presented at CT 19 (i.e., from CT 18 to CT 20) with a mean phase advance of 1.4 h (and standard error of 0.29 h).

Inspection of Figure 6 reveals that, in all four groups, phase delays were generally evoked by pulses presented during early subjective night and phase advances were evoked by pulses presented during late subjective night. Small phase advances were evoked during subjective day. Factorial ANOVA indicated a significant effect of light regimen, $F(3, 88) = 4.23, p < .01$, and of time bin, $F(7, 88) = 18.06, p < .01$, but no interaction between the two factors, $F(21, 88) = 1.51, p > .05$. The most evident inter-group difference was a widening of the phase-delay region as the photoperiod became shorter. Thus, the mean phase delay at the CT 16 bin was significantly smaller in the LD 16:8 group than in the other groups, and the mean phase delay at the CT 19 bin was significantly greater in the LD 0.25:23.75 group than in the other groups. A less remarkable inter-group difference was the presence of a dead zone of the PRC during subjective day in the LD 12:12 group but not in the other groups—although, due to relatively large variances, only the mean at the CT 4 bin differed significantly between the LD 12:12 group and the LD 16:8 group.
Fig. 2. Locomotor activity profiles of groups of 30 mice maintained under four different light-dark cycles. Group means are plotted with 6-min (0.1 h) resolution. To improve readability, standard errors of the means are plotted only in 2-h intervals. The white and black bars above the activity profiles indicate the duration of the light and dark phases of the light-dark cycle, respectively. The duration of the active phase (activity at or above 10%) is denoted by $\alpha$. 
Fig. 3. Locomotor activity profiles of groups of 30 mice as recorded in constant darkness on the first day after release from four different light-dark cycles. Group means are plotted with 6-min (0.1 h) resolution. To improve readability, standard errors of the means are plotted only in 2-h intervals. The white and striped bars above the activity profiles indicate the duration of the light and dark phases of the preceding light-dark cycle, respectively. The duration of the active phase (activity at or above 10%) is denoted by $\alpha$. 
Discussion

Mice in this study free-ran in constant darkness with a mean period of 23.6 h, which is consistent with values obtained in previous studies (Daan & Pittendrigh, 1976; Edgar & Dement, 1991; Mistlberger et al., 1998) and responded to photic stimulation by phase-shifting after brief light pulses and by entraining to light-dark cycles in a manner similar to that described in previous studies (Benloucif et al., 1999; Khammanivong & Nelson, 2000; Mistlberger & Holmes, 2000).

The daily profile of locomotor activity of mice maintained under light-dark cycles was clearly affected by the duration of the light phase of the cycle (Figure 2). If one assumes
that the activity profile of mice maintained in constant darkness for several weeks is the natural profile of these animals (Figure 4), then it can be said that LD 16:8 and LD 12:12 caused a compression of the activity profile, whereas LD 8:16 and LD 0.25:23.75 caused an expansion of the profile. A similar modulation of $\alpha$ by photoperiod has been observed in other species (Basco, Rashotte & Stephan, 1996; DeCoursey, 1972; Rosenwasser, Boulos & Terman, 1983; Sulzman, Fuller & Moore-Ede, 1982). Noteworthy is the fact that the total amount of daily activity, as reflected in the number of wheel revolutions, did not depend on the photoperiod. Thus, the amount of activity was conserved as the duration of the photoperiod varied. Similarly, conservation of the amount of activity after shortening of the circadian cycle has been described in golden hamsters (Refinetti & Menaker, 1997).

That negative masking by light plays a role in the compression of the activity profile was demonstrated by the expansion of the profiles on the first day after transfer to constant darkness (Figure 3). However, not only was this expansion observed in all four groups of animals (including those that were already expanded) but it also exceeded in all four cases the $\alpha$ of animals maintained in DD. This indicates that negative masking is only one of multiple factors affecting the activity profile under LD. Other potential mechanisms responsible for rhythm compression and expansion include an alteration in rhythm expression due to a non-parametric effect of photic stimulation on the pacemaker (Pittendrigh, 1981), a parametric compression or decompression of the circadian pacemaker’s cycle (Sumová, Trávnícková & Illnerová, 1995), and a change in photic sensitivity of the pacemaker caused by the alteration in the duration of daily photic exposure (Daymude & Refinetti, 1999).

The potential role of non-parametric effects of photic stimulation can be partially evaluated by a comparison of activity profiles observed under DD and under a skeleton photoperiod (here, LD 0.25:23.75). Because the free-running period of the mice (in DD) was shorter than 24.0 h, and because the animals were entrained by the skeleton photoperiod, it can be inferred that the 15 min of light per day were sufficient to phase delay the circadian pacemaker by 24 min (i.e., 0.4 h) each day. Therefore, an entraining non-parametric effect of photic stimulation on the pacemaker was present. Because a single 15-min pulse per day provides only a brief interval of photic stimulation, a parametric effect of light can be excluded. Thus, comparison of the activity profiles under DD (Figure 4) and under LD 0.25:23.75 (Figure 2, bottom) provides a test for the presence of a non-parametric effect of
Fig. 6. Phase-response curves depicting the phase shifts of the activity rhythm elicited by light pulses presented at various circadian times for groups of 30 mice previously maintained under four different light-dark cycles. Each bar indicates the mean shift (± SE) of animals that received light pulses during the designated 3-h bin. Letters: a = significantly different from LD 16:8, b = significantly different from LD 0.25:23.75 (p < .01, protected t test).
light on the activity profile. If one discards the “anticipatory” activity observed between 0400 and 0600 under the LD 0.25:23.75, the remainder of the activity profile is very similar to that under DD, which suggests the absence of a substantive non-parametric effect of light on the activity profile (despite the presence of a phase-setting non-parametric effect, as previously mentioned).

Because prolonged exposure to constant darkness significantly increases the pacemaker’s phase-shifting response to light pulses (Daymude & Refinetti, 1999; Shimomura & Menaker, 1994), it is possible that exposure to short photoperiods produces a greater degree of “dark adaptation” than exposure to long photoperiods does. Greater dark adaptation would result in greater light-induced phase shifts, possibly affecting the phase angle of entrainment and the profile of locomotor activity under a light-dark cycle. Should this be true, the amplitude of the phase-response curve would be greater in animals previously maintained under short photoperiods than in animals maintained under long photoperiods. The experimental results (Figure 6) are consistent with this hypothesis. Particularly the magnitude of phase delays in the region of CT 16 was greater in animals maintained under LD 8:16 than in animals maintained under LD 12:12, and greater in animals maintained under LD 12:12 than under LD 16:8. On the other hand, the increased amplitude of the PRC was accompanied by a widening of the sensitive zone. For instance, under LD 16:8 the phase-delay region encompassed 7 circadian hours (CT 9 to CT 16), whereas under LD 8:16 it encompassed 10 circadian hours (CT 10 to CT 20). This widening of the sensitive zone could be a simple consequence of the increased amplitude of the PRC resulting from the long exposure to darkness (e.g., Shimomura & Menaker, 1994). However, the results in Figure 6 suggest that the primary effect of changes in photoperiod is the widening of the phase-delay region. Furthermore, the fact that the duration of the active phase of the activity profiles (\( \alpha \)) on the first day in DD was proportional to the duration of the dark phase of the preceding light-dark cycles (Figure 3) strongly suggests that the light-dark cycles had a chronic parametric effect on the pacemaker’s cycle. Previous studies in rats and hamsters have found both a widening of the sensitive zone of the PRC in animals maintained under short photoperiods (Sumová, Trávníčková & Illnerová, 1995; Vuillez et al., 1996) and a concomitant expansion of the profile of neuronal activity in the suprachiasmatic nucleus (Mrugala et al., 2000; Sumová, Trávníčková & Illnerová, 2000). Thus, the widening of the sensitive zone of the PRC in shorter photoperiods (or, in the present case, the widening of the phase-delay region and consequent compression of the phase-advance region) is most likely the consequence of a specific widening of the sensitive zone of the pacemaker’s cycle. This widening of the sensitive zone of the pacemaker’s cycle may be due to alterations in the coupling of its two putative oscillators (de la Iglesia et al., 2000).

In conclusion, the temporal distribution of locomotor activity in mice is modulated by photoperiod. The activity profile is compressed under long photoperiods and expanded under short photoperiods. Although negative masking by light and alterations in the state of dark adaptation may partially account for the phenomenon, the major mechanism seems to be a compression and expansion of the circadian pacemaker’s cycle. It should be noticed that, whereas responsiveness to variations in photoperiod is of utmost importance for seasonally-reproductive animals (Underwood, Wassmer & Page, 1997), mice are not seasonal breeders and are unlikely to benefit from this responsiveness. On the other hand, rodents traditionally considered not to be seasonally reproductive, such as the laboratory rat and house mouse, have been shown to be responsive to variations in photoperiod (Heideman & Sylvester, 1997; Nelson, 1990). Thus, the basic mechanisms of responsive-
ness to photoperiod are likely present in both seasonally- and nonseasonally-reproductive animals.

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