Range of entrainment of rat circadian rhythms to sinusoidal light-intensity cycles

SETSUO USUI, YASURO TAKAHASHI, AND TERUE OKAZAKI
Department of Psychology, Tokyo Metropolitan Institute for Neuroscience, Tokyo 183-8526, Japan

Range of entrainment of rat circadian rhythms to sinusoidal light-intensity cycles. Am J Physiol Regulatory Integrative Comp Physiol 278: R1148–R1156, 2000.—The range of entrainment of the circadian behavioral rhythm was compared between two groups of Sprague-Dawley rats (each n = 10) exposed to daily cycles of rectangular light-dark alternation (LD) and sinusoidal fluctuations of light intensity (SINE), respectively. The maximum illuminance (20 lx), the minimum illuminance (0.01 lx), and the total amount of light exposure per cycle were the same under the two lighting conditions. The periods (Ts) of both lighting cycles were lengthened stepwise from 24 through 25, 26, 26.5, 27, 27.5, and 28 h to 28.5 h in experiment 1 and were shortened stepwise from 24 through 23.5, 23, and 22.5 h to 22 h in experiment 2. Each T cycle lasted for 30 cycles. In experiment 1, 60% of rats under the LD condition entrained up to T = 28.5 h, whereas 50% of rats under the SINE condition entrained up to T = 28.5 h. In experiment 2, no animal under the LD condition entrained to T < 23.5 h, whereas 40% of rats under the SINE condition entrained down to T = 23 h and 20% of rats remained to entrain down to T = 22 h cycles. The phase angle of entrainment was systematically changed, depending on T under both conditions. These results suggest that the lower limit of entrainment is expanded under the SINE condition compared with the LD condition.

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DAILY ALTERNATION of light and darkness is the most potent time cue (zeitgeber) for circadian rhythms in many organisms. It is generally accepted that photic entrainment is achieved by daily resetting of the circadian rhythm elicited by light stimulus (14). The phase-shifting properties of brief light pulses have been extensively studied and summarized in phase response curves (PRC) in a variety of species (7). In almost all species examined, light pulses given in the early subjective night produce delay phase shifts of circadian rhythms and light pulses in late subjective night elicit phase advances, whereas daytime exposure to light produces little or no phase shift (6, 7).

In most laboratories for circadian rhythm research, light-dark (LD) alternation is achieved by abrupt lights-on and -off transitions. It is assumed that the rapid transitions between the light and dark period act as the primary phase-resetting stimuli responsible for entrainment. Under natural conditions, however, environmental light intensity varies gradually throughout the day. Most terrestrial organisms have evolved to use environmental illuminance changes to adjust their circadian rhythms to the 24-h day. Several researchers have examined the entraining properties of LD alternation with artificial twilight, and they have suggested that twilight transitions expand the entrainable range of circadian rhythm in rodents. On the other hand, Swade and Pittendrigh (18) reported that several species of rodents in the arctic region entrain their rhythms to daily illumination cycles in summer when the sun is above the horizon continuously and nautical twilight does not occur. In laboratories, circadian rhythms of rats and hamsters can be entrained by sinusoidal light intensity cycles with very small amplitudes (13, 15, 21). Ruis et al. (15) examined the entrainment of rat circadian rhythms to sinusoidal light-intensity cycles with various amplitudes as well as minimum and maximum illuminance. They found that the success of entrainment to the sinusoidal lighting cycle increases with the geometric amplitude and decreases with the geometric mean of the light-intensity cycle. Under these lighting conditions, the continuous effect of light rather than its phasic resetting effect might be responsible for the synchronization of circadian rhythms (3, 17). Unfortunately, very little is known about the role of gradual changes in environmental light intensity in the photic entrainment of the circadian rhythm. The present study was designed to compare the range of entrainment of rat circadian rhythms between rectangular LD alternation and sinusoidal light-intensity cycles (SINE).

METHOD

Animals and Housing

Male Sprague-Dawley rats were used. Each animal was housed individually in a stainless steel grid cage (40 × 23 × 20 cm) equipped with a transparent acrylic top, a tilting grid floor for detecting ambulatory activity, and a drinking spout connected with a drinkometer (O’Hara, Tokyo, Japan). The cage was placed in a sound-attenuated chamber with constant temperature (25 ± 1°C) and humidity (40 ± 10%). Food and water were given ad libitum throughout the experiment. Drinking and ambulatory activities were continuously monitored, and cumulative counts were recorded at 5-min intervals.
Light Intensity Control

Light was provided by an incandescent bulb (100V2c, Matsushita Electric Work, Osaka, Japan) fixed 36 cm above the top of each cage. Light intensity was automatically controlled in 1-min steps by a programmable illuminance-controlling system. The system consisted of a light controller (NQ26114, Matsushita Electric Work), a personal computer (PC9801VX, NEC, Tokyo, Japan), and digital illuminance meters (IM-3, Topcon, Tokyo, Japan). An illuminance sensor was attached to the top of each cage, and the measured illuminance was compared with the programmed value to correct the output of the light controller. This feedback system enabled us to avoid unexpected illuminance changes caused by occasional voltage changes of the alternating current source or by aging of the light bulb. Details regarding this system have been reported previously (20, 22).

The light intensity fluctuated as a sine function under the SINE condition and changed in a rectangular form under the LD condition. Under both conditions, the maximum and minimum illuminance were always 20 and 0.01 lx, respectively. When the period (T) of the lighting cycle was the same under both conditions, the accumulated illuminance per cycle was identical.

Procedures

Experiment 1. Twenty male rats, 9 wk old at the start of the experiment, were divided into an LD group (n = 10) and an SINE group (n = 10). At first, the rats in the LD group were put under a 12:12-h LD condition (LD 12:12), and the rats in the SINE group were exposed to a 24-h sinusoidal light-intensity cycle (SINE cycle) for 13 days, respectively. Then Ts of both lighting cycles were lengthened stepwise from 24
through 25, 26, 26.5, 27, and 28 h to 28.5 h. Each T cycle lasted for 30 cycles. After the T experiments, the rats were released into constant complete darkness (DD) for 50 days to measure the period of the free-running rhythm (tauDD). The experiment started in February and ended in November.

Experiment 2. Twenty male rats, 8 wk old at the start of the experiment, were evenly allotted into the LD and SINE groups. The rats in the LD group were exposed to LD 12:12, and the rats in the SINE group were exposed to the 24-h SINE cycle for 55 days, respectively. Then, T was shortened stepwise from 24 through 23.5, 23, and 22.5 h to 22 h. Each T cycle lasted for 30 cycles. After the T experiments, all animals were released into DD for 50 days to measure tauDD. The experiment started in February and ended in September.

Data Analysis

Although the drinking and ambulatory activity were similar throughout the experiments, we used data related to the drinking activity for the analyses, because, in general, the ambulatory activity was more diffusely distributed and less distinct in the onset and the offset of the activity period than drinking activity.

TauDD

After the drinking activity was double plotted in a standard actogram format, the onset times of the activity periods during the last 20 consecutive days in DD were determined by visual inspection. TauDD was statistically calculated from a regression line fitted to these onset times.

Entrainment to T Cycles

To determine whether a rat behavioral rhythm was entrained to each T cycle, we used the $\chi^2$ periodogram (16) for drinking activity data obtained from the last 20 cycles in each T condition. We adopted the following criteria for entrainment: 1) the most dominant and significant period of the rat rhythm is equal to the T of the zeitgeber, 2) the phase angle between the zeitgeber and the activity period of the drinking rhythm is stable during entrainment, and 3) there are no significant periodicities of behavior other than T h in the circadian range. When all of the above three criteria were filled, we regarded the rat behavioral rhythm as entrained to that T cycle.

Phase Position of the Entrained Rhythm

To determine the phase position of a circadian rhythm entrained to a T cycle, we plotted drinking activity in the manner shown in Figs. 7 and 8. As a reference zero time, we used the time of minimum illuminance for the SINE cycle and the transition time from light to dark for the LD cycle, and we plotted the drinking activity for each T cycle on this time scale. This plotting method was convenient for visualizing changes in activity period. We used data obtained from the last 10 cycles of each T condition where the rhythm phase was

Fig. 2. A double-plotted record of a rat exposed to shortening T cycles of sinusoidal light intensity (left). A $\chi^2$ periodogram of drinking activity during last 20 cycles of each T is shown at right. See Figs. 1 and 3 for further explanation.
stabilized in entrained rats, and we determined the activity period by drawing vertical regression lines best fitted to the onsets and offsets of drinking activity, respectively. The times of activity onset and offset on that time scale were then converted into zeitgeber times, where T is regarded as 24 h and ZT1800 corresponds to the time of minimum illuminance under the SINE condition and to the midpoint of the dark period under the LD condition, respectively.

RESULTS

TauDD

In experiment 1, the mean tauDD ± SE was 24.34 ± 0.12 h in the LD group and 24.37 ± 0.09 h in the SINE group. In experiment 2, it was 24.22 ± 0.11 h in the LD group and 24.21 ± 0.06 h in the SINE group. There were no significant differences in the mean tauDD between the LD and the SINE group either in experiment 1 or in experiment 2.

Entrainment to T Cycles

All the animals used in experiments 1 and 2 entrained their circadian behavioral rhythms to either the SINE cycle or the LD cycle of T = 24 h. While a rat was entrained to T cycles, the Qp value at T h in the periodogram, which reflects the strength of the periodicity of the entrained rhythm, decreased gradually as T departed further from 24 h (Figs. 1 and 2). When a rat was unable to entrain to T cycles and free ran without...
relative coordination, the periodogram revealed a single peak at a period within the circadian range but different from the given T (Fig. 3). When a rat's behavior showed clear relative coordination, its periodogram showed two peaks, one of which corresponded to a free-running period and another around the given T (Fig. 4).

The numbers of rats entrained to T cycles are summarized in Fig. 5. In experiment 1, in which T was lengthened, all the rats in the LD group could entrain up to 27.5-h cycles, whereas six rats in the SINE group entrained to T = 27.5-h cycles. The Fisher exact probability test revealed that the number of rats entrained to T = 27- or 27.5-h cycles was significantly (P < 0.04) smaller in the SINE group than in the LD group. At the longest T = 28.5 h, however, the difference in the number of entrained rats between the two groups was smaller and not significant.

In experiment 2, in which T was shortened, all rats of the LD group failed to entrain to T < 23.5-h cycles, whereas four rats in the SINE group entrained to T = 23-h cycles and two of them remained entrained to T = 22-h cycles (Fig. 5). The Fisher test revealed that the number of rats entrained to T = 23-h cycles was significantly (P < 0.04) larger in the SINE group than in the LD group.

These results suggest that the range of entrainment is wider under the SINE condition than under the LD condition when T < 24 h, whereas it tends to be narrower when T > 24 h.

Phase Position of the Entrained Rhythm

Figure 6 summarizes the phase positions of the drinking activity period in the rats entrained to T cycles in experiments 1 and 2. Because, under both the SINE and the LD conditions, the onset of the activity period was indistinct when T > 27.5 h (Fig. 7) whereas the offset of the activity period was unclear when T < 23 h (Fig. 8), we could not determine the phase position of the entrained rhythm. Within the T range from 23 to 27.5 h, a systematic change dependent on T was observed either in the phase position of the entrained rhythm or in the activity time (alpha). Under both the SINE and the LD conditions, the phase of the activity period was advanced with lengthening T and was delayed with shortening T, and the activity time was prolonged as T was lengthened. These T-dependent changes in the entrained rhythm were essentially similar between the SINE and the LD conditions.
DISCUSSION

The present study demonstrates that the range of entrainment of rat circadian rhythms to T cycles extends from 22 to 28.5 h on the SINE cycles and that the lower limit of entrainment is expanded compared with the LD cycles, i.e., rat circadian rhythms can entrain to shorter T cycles. The range of entrainment to the LD cycles extended from 23.5 to 28.5 h, which is comparable to ranges reported previously (9, 19). In the present study, 40% of rats under the SINE condition entrained to the T = 23-h cycle, whereas no rat under the LD condition did. This difference was also observed in our preliminary studies, in which 50% of rats under the sine condition entrained to a T = 23-h cycle (21), whereas almost no rats under the LD condition entrained to the T = 23-h cycle (19). On the other hand, the upper entrainable limit of T was not expanded under the SINE condition compared with the LD condition, but it tended to be longer under the LD condition as far as we examined in the present study. Because 50% or more of rats entrained up to T = 28.5 h under both lighting conditions, further studies with larger numbers of animals and with a further extension of T are necessary for determining the full range of entrainment.

The difference in the range of entrainment in the present study depends on whether environmental light intensity changes gradually or abruptly. Several researchers have examined the entraining properties of LD cycles with artificial twilight. Kavanau (8) reported that circadian wheel running rhythms in deer mice entrain to very short cycles of LD alternation with artificial twilight, and he concluded that twilight transition expands the lower limit of entrainment. However, the lower entrainable limit of a 16-h cycle, which he found, is far beyond the circadian range, and his criterion for judging entrainment was not clear. There was no such experiment with artificial twilight for more than three decades after Kavanau's study was published. Recently, Boulos et al. (1) compared the ranges of entrainment of hamster circadian rhythms to LD cycles with and without simulated dawn and dusk, finding that simulated twilight expands the upper entrainable limit of the circadian rhythm. They supposed that twilight transitions amplified the phase-shifting effects of the LD cycle and compared phase shifts elicited by rectangular light pulses with and without simulated twilight. Unexpectedly, the light pulse with twilight did not produce a larger phase shift (2). It seems necessary to reassess the effects of artificial twilight.

The SINE cycle used in the present study featured gradual transitions between light and dark similar to Boulos et al.’s (1) simulated twilight. However, the SINE cycle did not expand the upper limit of entrainment for the circadian rhythm. The differences between their results and ours suggest that the SINE cycle is different from the LD cycle with twilight in terms of its circadian rhythm-entraining properties. Alternatively, these differences may be due to the different methods for determining the upper limit of entrainment. Boulos et al. (1) lengthened T day by day, whereas we lengthened T stepwise every 30 cycles. It has been reported that such a methodological difference affects the entrainable limits of circadian rhythms in T experiments (9).

The phase relationship between the entrained circadian rhythm and the T cycle was systematically dependent on T under both the SINE and LD conditions.
During entrainment to $T = 24$-h SINE cycles, the activity period of the drinking rhythm straddled over the time of minimum illuminance. The phase of the activity period relative to the SINE cycle advanced as $T$ was lengthened and delayed as $T$ was shortened. This $T$-dependent change in the phase angle of entrainment was similar to that observed under the LD condition (9). In this phase relationship during entrainment to the SINE cycle, rats were exposed to relatively brighter light in the early subjective night of their circadian rhythms when $T > \tau$, whereas they were exposed to brighter light in the late subjective night when $T < \tau$ (Fig. 6). These findings suggest that, under the SINE condition also, light exposure in the early subjective night produces a delayed phase shift and light exposure in the late subjective night elicits an advance phase shift of the circadian rhythm, and the magnitude of the phase shift depends on the light intensity.
To explain the entrainment of circadian rhythms to sinusoidal light intensity cycles, Swade (17) proposed the theoretical construct of a velocity response curve (VRC), which describes changes in the angular velocity of the circadian oscillator affected by this continuous illumination as a function of the phase of the circadian rhythm. Daan and Pittendrigh (4) demonstrated that the VRC can be derived approximately from the PRC by its simple linear transformation. Daan (3) further developed a mathematical model that can simulate the relative coordination and entrainment to fluctuating light-intensity cycles. The use of his computer algorithm may enable us to simulate the behavior of circadian rhythms observed in our T experiment of sinusoidal light intensity. We consider that the phase relationship observed in the present study between rat circadian rhythms and the SINE cycles during entrainment can be explained by these hypotheses.

In mammals, the endogenous circadian pacemaker is located in the suprachiasmatic nucleus (SCN) of the hypothalamus. The SCN receives information about the environmental LD cycle from the retina directly via the retinohypothalamic tract and indirectly via the intergeniculate leaflet (IGL) and the geniculohypothalamic tract (see Ref. 12). Photic information conveyed to the SCN mediates photic entrainment of circadian rhythms. More than 30% of rat SCN neurons are responsive to light (10). In rats, hamsters, and ground
squirrels, light-responsive SCN neurons tonically respond to light stimulus of long duration without habituation and they alter their discharge rates monotonically in response to an increase and/or a decrease in light intensity (10, 11). Light-responsive IGL neurons in hamsters have response properties similar to those described for light-responsive SCN neurons (5). Although the functional significance of these neurons in the SCN and the IGL is still unknown, it is likely that the circadian pacemaking system continuously monitors environmental illuminance and uses this information for the entrainment of rhythms. Further studies are necessary to elucidate the response of neurons in the SCN and IGL during entrainment to fluctuating light-intensity cycles including the SINE cycle.

Perspectives

Most terrestrial organisms have evolved to adjust their circadian clocks to the 24-h day by ambient LD alternation. Under natural conditions, environmental light changes in a complex manner. However, rectangular LD cycles or light pulses with abrupt lights-on and -off transitions have been usually used in laboratory experiments. Consequently, the entraining properties of gradual changes in light intensity, in the spectral component of light, and in the position of a light source have been ignored. It has been reported that LD cycles with gradual changes in light intensity simulating natural dawn and dusk expanded the upper entrainable limit of hamster circadian rhythms. The present study revealed that the sinusoidal light-intensity cycle expands the lower entrainable limit of rat circadian rhythms. These results suggest that gradual changes in ambient light intensity act as a potent zeitgeber. In natural circumstances, however, nocturnal animals such as rats and hamsters spend most of the daytime in dark burrows, whereas diurnal animals are exposed to daylight for long periods. Probably, gradual changes in ambient illumination may be more important for entrainment in diurnal animals than in nocturnal ones. Further studies with diurnal species will be necessary to elucidate continuous effects of light on photic entrainment of the circadian rhythm.

Address for reprint requests and other correspondence: S. Usui, Dept. of Psychology, Tokyo Metropolitan Institute for Neuroscience, 2-6 Musashidai, Fuchu, Tokyo 183–8526, Japan (E-mail: usui@tmin.ac.jp).

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