Activity rhythm of golden hamster (Mesocricetus auratus) can be entrained to a 19-h light-dark cycle

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Chiesa, Juan J., Montserrat Anglès-Pujolràs, Antoni Díez-Noguera, and Trinitat Cambras. Activity rhythm of golden hamster (Mesocricetus auratus) can be entrained to a 19-h light-dark cycle. Am J Physiol Regul Integr Comp Physiol 289: R998–R1005, 2005; doi:10.1152/ajpregu.00139.2005.—Both temporary access to a running wheel and temporary exposure to light systematically influence the phase producing entrainment of the circadian activity rhythm in the golden hamster (Mesocricetus auratus). However, precise determination of entrainment limits remains methodologically difficult, because such calculations may be influenced by varying experimental paradigms. In this study, effects on the entrainment of the activity pattern during successive light-dark (LD) cycles of stepwise decreasing periods, as well as wheel running activity, were investigated. In particular, the hamster activity rhythm under LD cycles with a period (T) shorter than 22 h was studied, i.e., when the LD cycle itself had been shown to be an insufficiently strong zeitgeber to synchronize activity rhythms. Indeed, it was confirmed that animals without a wheel do not entrain under 11:11-h LD cycles (T = 22 h). Subsequently providing hamsters continuous access to a running wheel established entrainment to T = 22 h. Moreover, this paradigm underwent further reductions of the T period to T = 19.6 h without loss of entrainment. Furthermore, restricting access to the wheel did not result in loss of entrainment, while even entrainment to T = 19 h was observed. To explain this observed shift in the lower entrainment limit, our speculation centers on changes in pacemaker response facilitated by stepwise changes of T spaced very far apart, thus allowing time for adaptation.

entrainment limits; wheel running; circadian rhythms

ENTRAINMENT OF CIRCADIAN OSCILLATORS produces synchronization between inner and outer temporal programs and involves cyclic environmental cues (zeitgeber) within the species’ ecologic niche. Self-sustained endogenous oscillators with a free-running period (τ) can be entrained to zeitgeber cycles whose period lengths (T) differ from τ. Entrainment is achieved when 1) τ progressively changes until it equals T, 2) a stable phase relationship is reached between the oscillator and the zeitgeber, and 3) the rhythm oscillates once in constant darkness from the initial phase attained when the zeitgeber was present. However, entrainment can only be produced within a limited range of periods T that encompass the range of entrainment (2). This range varies depending on the animal species (3), age (21), and the strength of the zeitgeber (2). Outside the range of entrainment, the oscillator free-runs with a period close to that measured in constant conditions (1) or with an intermediate one, when its frequency is periodically modulated by the zeitgeber, the so-called relative coordination phenomenon (2).

The study of entrainment range, as well as the precise determination of its limits, presents some difficulties, such as observation of unstable or “relative entrainment” toward the limits of the range, resulting in periodic fluctuations of τ (65). Moreover, exposure of organisms to a given zeitgeber at the limits of entrainment might lead to a situation in which various rhythms have different ranges of entrainment (1), as has been observed in humans as fractional desynchronization (23, 64) or in rats as dissociation patterns (17). Indeed, some studies have suggested that a circadian system submitted to extreme short or long T periods might become arrhythmic and would then entrain to an unlimited range of T cycles (2).

Consequently, the different methodological approaches might produce different results. To date, the limits of photic entrainment of the hamster activity rhythm to a given zeitgeber have been studied using one of the following procedures: 1) exposure of animals to distinct T cycle periods across the studied range (3); 2) exposure during progressively increasing (8), or both increasing and decreasing (9, 10), T cycle periods starting from T = 24 h until loss of entrainment; and 3) by prediction, using the known τ and the respective phase-response curve for the hamster in such a manner that both the lower and upper limits are defined by τ minus the maximum phase advance or τ plus the maximum phase delay, respectively (44). In each of these scenarios, there remains a short time of “adaptation” before the exposure to the newly introduced condition: in 1, the animals are transferred to the new T cycle directly from T = 24 h, so adaptation time is zero; in 2, the new condition is changed gradually (daily), and adaptation time depends on the derivative of the change in T per day. However, it has not been established whether stepwise changes, with a longer time for adaptation, facilitate entrainment to a new T cycle.

Although daily light-dark transitions are the dominant zeitgeber for epigeous organisms, a wide array of nonphotic stimuli may prove effective entraining agents in rodents. Therefore, cycles of food availability (7, 37), ambient temperature (24), social interactions (27), and the induction of wheel running activity (20, 26, 30, 51, 61) may entrain or modify the phase of free-running rhythm when experimentally manipulated. In addition, activity induced by behavioral manipulations has been shown to accelerate the rate of reentrainment in hamsters (41, 52). It is known that nonphotic events induce the behavioral activation (arousal) in animals with concomitantly increased activity levels. Janik and Mrosovsky (29) showed that larger phase advances occurred accompanied by higher wheel running activity.
In addition, the periodicity of free-running rhythm appears to be inversely related to the amount of wheel running activity in some rodent species, such as in hamsters (40, 62) and rats (68, 69). However, several studies in mice suggest that the strength of this correlation is species and strain dependent (6, 20). Behavioral activity or vigilance state has been shown to modify the spontaneous firing activity rhythm of the hypothalamic suprachiasmatic nucleus of freely moving animals (15, 55), thereby suggesting a direct feedback of the behavioral state on the circadian clock. However, the function of activity feedback on the circadian clock as found in nature, when such arousing events most likely occur at night, remains unclear. Because of the powerful resetting effects of light, the role of nonphotic stimuli such as wheel running during light-dark (LD) cycles remains difficult to assess in the laboratory. In addition, the interaction between wheel running and light is not well understood, and activity feedback on the circadian clock may become more apparent when studied near the limits of photic entrainment.

The aims of this study were twofold: 1) to investigate the influence of wheel running activity on entrainment, and 2) to study entrainment of the activity rhythm at various LD cycles, stepwise decreasing period T further below the known lower limit of entrainment.

MATERIALS AND METHODS

Animals and experimental paradigm. Eight 2-mo-old male golden hamsters (Mesocricetus auratus) arrived at the laboratory directly from the provider (Harlan France, Gannat, France). The animals were housed isolated in individual transparent metacrylate cages measuring 22 × 22 × 15 cm (Panlab, Barcelona, Spain) and covered with a stainless steel grid, with wood shaving bedding (Souralit, La Rioja, Spain). Cages were maintained in sound-proof humidity- and temperature-controlled chambers (room temperature and humidity were between 18 and 22°C and 50 and 80%, respectively) with time-adjustable illumination cycles. Illuminance during the light phase was supplied by two 36-W Mazdafluor fluorescent tubes, producing ~300 lux of reflected cool white light at cage level and a dim red light of ~0.1 lux during the dark phase (Mavalux 5032B digital luxmeter; Gossen-Metrawatt-Camille Bauer). After 30 cycles, the animals were transferred to transparent metacrylate cages measuring 48 × 22 × 15 cm, equipped with running wheels (diameter = 30 cm), where they remained until the end of the experiment. During the entire experiment, the animals received food (Harlan Teklad 2040 Global Diets) and tap water ad libitum. Cage cleaning, as well as food and water supply, was conducted once a week at irregular daylight hours.

The animals were subjected to symmetrical LD cycles whose period (T) was changed sequentially from T = 22 h (T22) to T = 19 h (T19). At the end of the experiment, the animals were transferred to constant darkness (DD), with lights off maintained from the last scotophase in LD. The sequence of LD cycles with their respective extensions and wheel availability was as shown in Table 1. As at LD T20, the lower limit of entrainment was greatly exceeded; stage 4 lasted twice the duration, attaining a stable rhythmic pattern before a further shortening of LD.

Motor activity and wheel running measurement. In stage 0, spontaneous motor activity (MA) of the animals was measured by means of an activity meter with two perpendicular infrared light beams crossing the cage at a height of 7 cm. From stage 1 until the end of the experiment, MA was measured with an activity meter consisting of one infrared light beam crossing the cage at a height of 7 cm and placed in a manner to avoid wheel-related movement. Each beam interruption represented an activity count that was measured and compiled in bins of 15 min, obtaining regularly sampled time series.

When the running wheel was available for animals, wheel running activity (WR) was acquired by means of magnetic switches activated by a magnet placed on the wheel axis. Wheel revolutions were compiled in bins of 15 min, and two independent types of animal activity, MA and WR, were recorded simultaneously. All data samples were acquired through parallel channels and stored in a computer for further analysis. LD cycles were recorded simultaneously with activity in a separate channel connected to a photocell pulse generator.

Time-series and statistical analysis. Time-series analysis and statistical comparisons were carried out separately for each data set of MA and WR signals. Detection of MA and WR rhythms at each LD stage was conducted with a χ² periodogram (58), obtaining both the percentage of variance (RV) explained by the rhythm (11) and its period estimation. The period range examined was from 16 to 26 h. P level for significant periodicities was set at 0.05.

Daily phases of WR rhythm during each LD stage were calculated using least-squares data fitting to a cosine function with a period equal to T. Each set of daily phases was studied with circular statistics to assess the directedness of phase distribution along respective T cycles, using a Rayleigh z-test (4). This test obtains an r vector with its origin at the center of a circumference of radius one, where the daily estimated phases of the WR rhythm are distributed. The direction and length of the r vector was calculated as the vectorial mean of the unitary vectors associated with daily phases. Thus the length of r (between 0 and 1) is proportional to the degree of phase homogeneity during the respective T cycles and may be considered a measure of the rhythm’s phase stability during successive days. Therefore, higher r values indicate a greater constancy of rhythm phase as a consequence of the pacemaker’s stable response to the LD cycles (because the rhythm starts at similar phases during successive zeitgeber cycles), and lower r values correlate to unstable entrainment, when most spontaneous oscillation prevails during LD cycles.

The daily mean waveform was calculated at each stage for WR activity. The phase angle was calculated on waveforms, the time span for changes occurring in the waveform, the time span for total activity at tmax, and r9251. To better understand the changes occurring in the waveform, the time span for α, αs, and αf were calculated at the different stages.

Double-plot graphic analysis was used to observe changes in the rhythm during the different stages and to evaluate the temporal

Table 1. Sequence of LD cycles

<table>
<thead>
<tr>
<th>Stage</th>
<th>Illuminance Conditions</th>
<th>No. of Cycles</th>
<th>Wheel Availability</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>T22 (11:11 h)</td>
<td>30</td>
<td>–</td>
</tr>
<tr>
<td>1</td>
<td>T22 (11:11 h)</td>
<td>28</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>T21 (10.5:10.5 h)</td>
<td>24</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>T20.4 (10.2:10.2 h)</td>
<td>30</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>T20 (10.10 h)</td>
<td>64</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>T19.6 (9.8:9.8 h)</td>
<td>26</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>T19.6 (9.8:9.8 h)</td>
<td>32</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>T19 (9.5:9.5 h)</td>
<td>12</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>DD</td>
<td>16</td>
<td>–</td>
</tr>
</tbody>
</table>
relationship between the rhythm under T19 and under DD. In addition, the onset of the MA rhythm during the first five cycles in DD was calculated by projecting an eye-fitted vertical line in actograms plotted at modulo $\tau$. This line was projected through the last cycle in T19 to estimate the phase of the rhythm in LD. Individually obtained phases were evaluated to discriminate between randomness or one-sided distribution along the T19 cycle by means of a Rayleigh $z$-test.

MA rhythm in DD was analyzed with a regressive periodogram by using four harmonics to avoid waveform interference (32). The period range examined was from 20 to 26 h, and $P$ level for significant periodicities was set at 0.05. Analysis was done by dividing the entire DD stage into two consecutive parts of 8 days ($S_1$ and $S_2$). Aftereffects of previous entrainment were studied by comparing the $\tau$ values obtained between $S_1$ and $S_2$. Only significant periodicities were included for this comparison.

Statistical analysis of variance (ANOVA) was carried out to perform comparisons. Normal distributions and homogeneity of variances for categories (the stages) of the previously defined variables were found, except for the phase angle, which was log-transformed to achieve homogeneity (Kolmogorov-Smirnoff test, $P > 0.1$, and Levene test, $P > 0.05$) (57). Regression analysis was performed to assess stage differences for the phase angle, mean WR activity per cycle, $\%WR \alpha$, and the time span of $\alpha$, $\alpha_0$, and $\alpha_0$. Correlation analysis was done to study the relationship between the PV of the rhythm and phase stability, and a $t$-test for dependent samples to compare the $\tau$ between $S_1$ and $S_2$. Heterogeneity of variances was found for phase stability and, because mathematical transformation did not attain homogeneity, nonparametric Kruskal-Wallis ANOVA and Mann-Whitney $U$-test were utilized for this variable. The level of statistical significance for all tests was defined at $P = 0.05$. Obtained $P$ values lower than 0.01 are reported as 0.01.

Graphs and calculations were made using the integrated package for analysis in chronobiology “El Temps” (http://www.ub.es/dptisiv/soft/ElTemps/). For statistical analysis and comparisons, STATISTICA software was utilized (StatSoft).

RESULTS

Running wheel transference. The entire MA record of a representative animal is displayed in Fig. 1A on a double-plotted actogram at modulo T20. During the first 30 cycles at T22, all animals exhibited nonentrained MA rhythms with relative coordination patterns (mean $\pm$ SE of rhythm period: 22.5 ± 0.24). On the 30th cycle, hamsters were transferred to cages with running wheels. Providing a running wheel led to entrainment for both MA and WR rhythms, with the activity phase confined to the scotophase. Further reductions of the LD cycle period to T19.6 were conducted without loss of entrainment. However, subsequent blocking of the wheel did not result in loss of entrainment during the study period of 32 cycles, nor did it result in loss of entrainment during a further 12 cycles at T19. Only periods equal to $T$ were detected significantly in periodograms calculated for each LD part, for both MA and WR rhythms. An actogram for one representative animal at modulo T22 displays the MA rhythm during the first 52 cycles of the study both at stage 0 and stage 1 (Fig. 1B). Similar patterns of MA were observed on actograms for all the animals in the experiment.

Phase stability. The phase changes of WR rhythms are clearly visible in single-plotted actograms at modulo $T$ (Fig. 2A). Daily phase of the overt WR rhythms shows day-to-day instabilities that increased with the shortening of the $T$ cycle. However, the rhythm generally remains confined to the scoto-
In addition, variations of the compression. Comparable patterns were observed for all animals. The phase when considered as a whole. Also, this can be observed in the general MA rhythm (Fig. 2B). Note that these patterns are different from relative coordination, where angular frequency decelerates and accelerates together with α decompression and compression. Comparable patterns were observed for all animals. In addition, variations of the τ vector (Fig. 3A) manifested a higher phase stability at T22 and T21, whereas they diminished from T20.4 to T19.6 (Kruskal-Wallis ANOVA, P < 0.01; Mann-Whitney U-test, T22 and T21 higher than T20.4, T20, and T19.6, P < 0.01). This relationship could be interpreted as an objective measure of that described in the actograms of Fig. 2. Furthermore, the percentage of variance explained by the rhythm (Fig. 3B) exhibits this same tendency (correlation: r = 0.85, P < 0.01), suggesting that the robustness of rhythm is related to phase stability as a feature of entrainment.

Phase angle. Figure 4 exhibits the phase angle variation as a response to T cycle changes calculated for the WR rhythm. The phase angle value was positive, increasing from ~1.5 h ZT at T22 to 3 h ZT at T19.6, when the WR onset progressively phase-lagged light offset as the period of the T cycle decreased. A significant linear regression was found for the phase angle vs. T cycle relationship (regression: r = 0.35, P < 0.05). Therefore, these modifications of the phase relationship between T cycle and the rhythm might indicate active changes in the pacemaker activity.

Changes in WR activity. Calculations on WR activity derived from waveforms were performed to study the amount and distribution of activity at α phase. Figure 3C shows mean wheel revolutions related to T cycle. Lowering the T cycle caused a decrease in mean WR activity (regression: r = 0.63, P < 0.01). However, the distribution of WR activity at α phase also changed in relation to T cycle. Figure 3D shows variations in the percentage of running activity at the αi interval. Percentage of WR at αi increased significantly as the T cycle shortened (regression: r = 0.68, P < 0.01). Moreover, duration for αi decreased significantly in relation to T cycle shortening (regression: r = 0.7, P < 0.01), but no changes on αi were detected (regression: P > 0.1) (Fig. 3E). Changes in duration of α were found to be proportional to changes on T (data not shown). These results indicate that the incremented percentages of WR at αi, found at the beginning of α in relation to T cycle shortening, may be associated with changes in activity distribution during the α phase.

Phase control of rhythm. Temporal coincidence between the rhythm under LD and that under DD on the LD-DD transition suggests that the internal phase of the circadian clock was controlled by the LD zeitgeber. After the animals were transferred from LD T19 to DD, a significant phase grouping was found (Rayleigh z-test: r = 0.94, P < 0.01). Because constant darkness was initiated from the last scotophase in LD, no effects on the rhythm phase in constant darkness can be attributed to lights off. The phase control of rhythm also was evident in the actograms. Figure 1C displays a double-plotted actogram at modulo T19 for one representative animal during the transition from LD T19 to DD. MA rhythms began to exhibit free-run from the phase held during the preceding LD conditions.

Aftereffects. Free-running period changes in constant darkness were assessed to verify the presence of aftereffects of previous entrainment. A significant increment of τ was observed during the 16 days in DD, closely approximating the spontaneous value of 24 h (t-test for dependent samples: P < 0.05, S1: τ = 23.1 ± 0.2 vs. S2: τ = 23.8 ± 0.1). Two nonsignificant periods were detected at periodograms at both S1 and S2 and were excluded from this comparison.

DISCUSSION

In this study, entrainment of the circadian activity pattern in hamsters, subject to various stepwise decreasing T cycles, was assessed. Starting at T22, data confirmed that animals without...
a wheel exhibited nonentrained activity rhythms with relative coordination patterns. Continuous access to a running wheel rapidly accelerated entrainment to T22 and may well have contributed to the maintenance of entrainment during further T cycle reductions to T19.6. However, when the access to the wheel was removed at T19.6, there was no loss of entrainment,

nor when T was reduced to T19. Therefore, these data suggest that during T22 the spontaneous WR activity contributed to drive the phase of the pacemaker via feedback mechanisms and that once entrainment was acquired (during the following shortened T cycles), it became partially independent of WR.

Entrainment to short T cycles has already been described, but not entrainment to a T as short as T19. A study by Carmichael et al. (12) reported entrainment in hamster to T cycles close to T21.5. This paradigm was based on a stepwise decreases in T cycle of 15 min/day or less from 14:10-h LD, maintaining a 14-h fixed photophase and shortening the scotophase. Also, Boulos et al. (9) reported the lower entrainment limit for hamster activity rhythms in LD square cycles as being close to T22. In that study, the animals were exposed to LD cycles with a light intensity of 10 lux, with changes in T cycle of 5 min/day from T24 to T21.5, whereas the present data were obtained using a stepwise decreasing protocol, which could have allowed sufficient time for adaptation. This suggests that entrainment limits are not fixed but depend largely on the methods used to evaluate them. Furthermore, Boulos et al. (9) indicated that a precise estimation of the entrainment limits is possible “in a relatively short period of time” with a 5 min/day rate of change of T. However, this takes the implicit assumption that adaptation time determines the limits of entrainment.

Usui et al. (60) studied the entrainment range of activity rhythms in rats, assessing stepwise changes in T every 30 cycles, and reported a value near 28.5 h for the upper limit and 23.5 h for the lower one, whereas Boulos et al. (9) reported
values that were close to 25.5 and 22 h, respectively. Moreover, it has been reported in rats that the upper entrainment limit of feeding activity rhythm occurs at 28 h if the animals are exposed directly to T cycles from T24, and only at 26 h 44 min with a 10 min/day increase in T cycle from T24 to T30 (36). These studies suggest that pacemaker function is differentially affected depending on previous environmental history or on the time required to adapt to changing conditions.

According to the present results, adaptation time for a T cycle could be considered an important aspect of the experimental paradigm in assessing the entrainment limits of hamster activity rhythm to LD square cycles, at least for the lower one. This suggests that the range of entrainment is more a dynamic than a static property of the system. If the period of circadian rhythm is inversely related to the intrinsic velocity of the endogenous oscillator, ωi, the upper and lower limits of entrainment can be associated with velocities ωU and ωL, respectively. In Fig. 5, we have represented the expected changes in these values from the theoretical point of view. According to the graph in Fig. 5, both a stable free-running and an entrained rhythm will be represented as horizontal lines, because the angular velocity remains constant over time. Change in velocity, when T cycle is imposed, is represented as a vertical displacement on the graph. Under these conditions, the system exhibits a sort of inertia in modifying its internal velocity. Entrainment beyond a certain limit is not possible because the system is unable to maintain the acceleration (change of velocity over time) imposed by the zeitgeber. In light of this experimental evidence, a border separating the regions where entrainment is and is not possible can be drawn. Furthermore, it can be assumed that this border must have a horizontal asymptote, representing the real limits for entrainment, adopting the concept of “dynamic range of entrainment” instead of that currently used (static).

The mechanism by which these differences are produced cannot be determined with the present data. However, it is known that in most physiological processes, sufficient adaptation time is necessary for setting compensation mechanisms, particularly when response to a new environmental condition is nearing the limits of the system adaptability.

Although the adaptation time to a T cycle seems to be an important factor to improve entrainment, the systematic changes found in the amount of WR activity also suggest the role of a possible activity feedback. Mean WR activity diminished when the T cycle was shortened, when shorter τ or larger phase advances are typically required to entrain. However, distribution of WR activity, as a percentage of running during the initial part of the α phase, increased significantly from T22 to T19.6. Thus it is possible that not only the amount of volitional WR activity but also its distribution in the α phase should be considered as a feedback-related variable. Edgar et al. (20) reported that a shorter τ was observed in mice when intense activity was performed during early subjective night, whereas a longer τ occurred when activity was concentrated at its end. In addition, Mistlberger and Holmes (38) reported that the activity feedback over the phase angle of entrainment in mice that had been exposed to different LD cycles less than T24 occurred mainly during the first 2 h at α phase. These findings suggest that the feedback contribution of WR activity on entrainment could depend on the activity levels that occur at certain circadian phases. The ecologic function of this mechanism may be related to instances of entrainment reinforcement, whereby occurrences of behavioral significance may concentrate the animal’s temporal perception of its environment (e.g., encounters with mating opportunities, food sources, or a predator’s presence) (27). However, because entrainment was achieved at T19.6 and T19 without running wheel access, the contribution to entrainment of continuous wheel running, once the circadian system has been already entrained, is not so important as expected. Gorman et al. (25) reported that the presence or absence of a running wheel had only minor effects on entrainment to lengthening T cycles. Although the shortening of τ by activity feedback could be accounted to explain entrainment, at least to T22, we observed in the laboratory (unpublished observations) that hamsters did not entrain to T27, but they did when the wheel was available. Therefore, the precise role of activity feedback on entrainment remains to be established.

Control of the overt rhythm’s period was observed during the entire experiment, because the same period as the external LD was acquired by both WR and MA rhythms. In addition, phase angle was positive, increasing systematically from ~1.5 h ZT at T22 to 3 h ZT at T19.6. With the use of a single discrete phase reference, these results are in agreement with the expected phase relationship on steady-state entrainment of a rhythm by a high-frequency zeitgeber (2). However, the temporal structure of hamster WR activity throughout the day seems to be more complex than a single and continuous α phase and would be regulated by a coupled multisaccillatory system. Pittendrigh and Daan (46) suggested that changes in activity peaks within α reflect the behavior of two coupled oscillators and the phase relationship between them. Moreover, the possibility that each component of a complex but regular activity pattern may be under the control of a separate circadian

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**Fig. 5.** Graphic representation of the concept of dynamic limits of entrainment. On the ordinates, the intrinsic angular velocity of the oscillator (ωi) is represented, and on the abscissas, time (t), to show how ω evolves through time as a consequence of changes in the period (T) of zeitgeber. The horizontal arrow starting at a represents a system running at constant velocity (entrained or free running). The vertical arrow represents a theoretically instantaneous increase in ω as a consequence of a direct transition to a new T. b is the maximum velocity that the system can assume after such a transition (conventional limit of entrainment). Gradual modifications of T constitute an intermediate situation that can be graphically represented as inclined lines, with slopes proportional to the rates of change of T. The thick curve indicates the maximum velocity that the system can assume under these different conditions (c), defining the “dynamic limit of entrainment.” Considering all the possible rates of change, the dotted line represents the asymptote at which tends the real absolute limit of entrainment.
oscillator was suggested (22). Furthermore, it has been proposed that activity time appears as a “window” controlled by the pacemaker during which expression of different WR activity components, controlled by additional circadian oscillators, is permitted (14). Within this framework, daily WR activity patterns can be considered a function of the phase relationship between the window pacemaker and the activity components. A comparable scenario is illustrated in Fig. 2A at T20 and T19.6 stages, where the overt WR rhythm remains entrained but also seems to present rhythmic components that tend to free-run. Although the overt rhythm observed at T20 and T19.6 appeared to be partially driven by light, this influence was sufficient to drive the pacemaker’s phase, as can be shown when the phase of free-running rhythm is observed after being released in constant darkness.

Despite entrainment, it is clear that hamsters do not exhibit the same phase stability under the different T cycle. This suggests that the temporal pattern of WR activity is regulated by a coupled multisessory circadian system, a hypothesis that has been studied using different theoretical (18, 45, 54, 59, 66) and empirical approaches (28, 34, 35, 43, 63, 70). Moreover, this coupling strength seems to be modulated mainly by light intensity, which under specific conditions can prompt a functional reorganization of the oscillatory components of circadian system (17, 33, 42, 49). Therefore, cycle-to-cycle phase stability of the overt rhythm with respect to the zeitgeber phase could be interpreted as the “degree of entrainment” of the system, allowing one to imagine the entrainment process as a continuous or gradual one. Indeed, the phase stability of the overt rhythm could indicate the entrainment degree of the system as a measure of the temporal organization of the phases of the different oscillators. T cycles shorter than that of the spontaneous frequency of the system produce phase instability and a “low entrained” system with more free-running components. However, because the amount of light per cycle remains the same at different T cycles, the incremental phase instability that occurs at lower T cycles must depend on the τ/T relationship. In addition, the present results suggest a relationship between entrainment degree and the robustness of the output signal. This suggests an interesting hypothesis, namely, that rhythm variability may be a quantitative function of the coupling level, as well as a measure of the system’s functional coherence (67).

At the end of the experiment, changes in the free-running period from 23.1 to 23.8 h were observed under DD. It has been shown that the continuous effects of the LD cycle can modify the pacemaker period to compensate for differences with the T period (5, 16, 47). Remarkably, detecting values below 24 h, specifically 23.1 h over eight cycles at DD after release from LD, proved strong evidence in demonstrating how pacemaker period is affected by the LD cycle.

Although the present study cannot demonstrate the existence of discrete entrainment, it does suggest that the history of entrainment may affect the phase-resetting mechanism of the pacemaker. Rees and Doucet (50) reported that T cycle frequency may influence the responsiveness of the pacemaker to both light and induced wheel running; whereas shorter T cycles produced shorter τ when free-run under DD, large phase advances were induced after light and activity pulses, contrary to the expected relationship between τ and phase shifts (13, 48, 56). This evidence makes it clear that the sensitivity and/or responsiveness of the pacemaker to resetting stimuli (the resetting contour in a state plane; see Ref. 31 for revision) are not solely characteristics of a “phase only system,” which always responds with the same phase-response curve (53), but they may be affected by previous illumination and entrainment history.

In conclusion, as far as we know, this is the first time that the activity rhythm of the hamster has been entrained to a T19 cycle. We believe that once entrainment has been established (a process in which access to the running wheel may have an important role), the time of adaptation before a new change of the T cycle may be considered an important factor to evaluate the “real” dynamic entrainment limits, whose values depend not only on the sum of τ ± the maximum phase shift observed at the phase-response curve but also on the rate of change of the imposed T cycle.

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REFERENCES


