

# Relationship Between Circadian Period and Size of Phase Shifts in Syrian Hamsters

STÉPHAN G. REEBBS<sup>1</sup> AND PIERRE DOUCET

*Département de biologie, Université de Moncton, Moncton, NB, E1A 3E9, Canada*

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REEBS, S.G., AND P. DOUCET. *Relationship between circadian period and size of phase shifts in Syrian hamsters*. *PHYSIOL BEHAV* **61**(5) 661–666, 1997.—In response to a light pulse, hamsters normally generate phase advances that are positively correlated with the length of their circadian period ( $\tau$ ). To determine whether this is a general property of the phase-shifting oscillator, the present study looked for a correlation between  $\tau$  and phase-advance size not only for photic but also for nonphotic shifts. Syrian hamsters, *Mesocricetus auratus*, were entrained to light–dark cycles with a periodicity of either 23.67 h (the short-T group) or 24.33 h (the long-T group); after release into constant darkness, the short-T and long-T groups exhibited short and long  $\tau$ s, respectively. These animals were then induced to run in a novel exercise wheel for 3 h, starting at circadian time (CT) 7, or were exposed to 20 min of light, starting at CT 19. The size of the ensuing phase advances did not differ between the short-T and long-T groups not only for the nonphotic stimulus but also for the photic one, an unexpected result for the photic stimulus. Within the short-T groups for photic and nonphotic stimuli, the shorter  $\tau$  was, the larger the phase advances were, another unexpected relationship. Another experiment where phase delays were induced by light pulses at CT 15 also failed to yield significant differences between the short-T and long-T groups. Independently of their after-effects on  $\tau$ , T cycles may influence the capacity of the pacemaker to phase shift in ways that are still unclear but at least similar for both photic and nonphotic shifts. © 1997 Elsevier Science Inc.

Free-running period    Phase shift    Nonphotic    Wheel running    Hamster    Circadian rhythms

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CIRCADIAN clocks have endogenous periodicities that are different from 24 h. Circadian clocks can be slow, in which case their free-running period ( $\tau$ ) is longer than 24 h, or they can be fast, in which case  $\tau$  is shorter than 24 h. During entrainment to a natural light–dark cycle with a period (T) of 24 h, animals with  $\tau > 24$  h must phase advance their activity rhythms daily, whereas animals with  $\tau < 24$  h must phase delay. Because of this observation, researchers have attempted to find a correlation between  $\tau$  and the size of phase shifts in response to a given stimulus. The expectation was that when a stimulus was given at a circadian time (CT) that caused phase advances, animals with long  $\tau$ s (who must advance daily in nature) would show larger advances than animals with short  $\tau$ s, and vice versa if the stimulus was given at a time that caused phase delays.

This expectation has been upheld in all studies to date. Syrian hamsters, *Mesocricetus auratus* (1), Djungarian hamsters, *Phodopus sungorus* (11), and mice, *Mus musculus* (1,14) have all showed larger advances in response to a light stimulus when  $\tau$  was longer or larger delays when  $\tau$  was shorter.

All of these studies have used light as the phase-shifting stimulus. Other phase-shifting stimuli have not been investigated. Consequently, we still do not know whether the relationship between  $\tau$  and the size of phase shifts is a general characteristic of the phase-shifting oscillator, or whether it is specific to the os-

illator's response to light only. For this study, we used a nonphotic stimulus, novelty-induced running, to phase-advance Syrian hamsters in an attempt to find a relation between the size of these phase advances and  $\tau$ . Nonphotic phase delays were not considered because large delays are seldom obtained with induced running (9,12).

For our experiments, we used a protocol similar to that of Daan and Pittendrigh (1). These researchers subjected mice to a long T, released them in constant darkness (DD) to obtain relatively long  $\tau$ s through after-effects, gave them a pulse of light at CT 15, measured the resulting delays, and then repeated the experiment with the same mice and a short T (and short  $\tau$ s through after-effects). Other mice received the short-T treatment first, followed by the long-T treatment. We also used T cycles to induce various  $\tau$ s in hamsters, except that we imposed different T cycles on different animals rather than subjecting the same animal to different treatments successively. Given this slight difference between the two approaches, our use of a different species, our particular attention to advances (Daan and Pittendrigh only studied delays) and the possibility to compare nonphotic and photic shifts, we did our experiment with both nonphotic and photic pulses. Moreover, in the photic experiments, we tried to obtain both advances and delays. Therefore, although it was not our original purpose, our study can be construed as an attempt to replicate the results that were previously obtained with mice

<sup>1</sup> To whom requests for reprints should be addressed. E-mail: reebss@umoncton.ca

(1), using another species and adding photic and nonphotic advances.

## MATERIALS AND METHODS

### *Animals and Housing Conditions*

Male Syrian hamsters (60 days old, Hsd:SYR from Harlan Sprague Dawley, Indianapolis, or Lak:LVG from Charles River Canada, Montreal) were housed singly in Wahman cages (25 × 15 × 13 cm) connected to running wheels (36 cm in diameter). Water and rodent lab chow were available in each cage. Room temperature was  $21 \pm 2$  °C, and lighting came from overhead incandescent bulbs. Light intensity, as measured with a Gossen Lunasix 3 lightmeter, was approximately 50 lux inside the cages during the light cycle and 0 lux during darkness. (An infrared visionscope was used to take care of the animals in the dark.) All running wheels were equipped with microswitches connected to a computer. Wheel revolutions were recorded in 6-min bins using Dataquest III hardware and software (Data Sciences Inc., obtained from Mini-Mitter Co., Sunriver, OR).

### *Prepulse Procedures*

Upon arrival in the laboratory, animals were exposed to a 14:10-h light–dark (LD) cycle synchronized with that of the supplier's breeding facilities. This exposure lasted 3–7 days and was intended to acclimate the animals to the new environment. During the following 12–16 days, the hamsters were entrained to either LD 13.84:9.83 h ( $T = 23.67$  h) or LD 14.17:10.16 h ( $T = 24.33$  h) and subsequently left to free-run in DD. The  $\tau$  tended to be short after entrainment to  $T = 23.67$  h (mean = 23.81 h; range = 23.43–24.05 h) and long after  $T = 24.33$  h (mean = 24.16 h; range = 24.01–24.38 h). Hereafter, these two groups are called “short-T” and “long-T”, respectively. In the nonphotic experiment described below, each group contained equal numbers of Hsd:SYR and Lak:LVG hamsters. In the photic experiments, only Hsd:SYR hamsters were used.

### *Pulse Procedures for Nonphotic Shifts*

After 14–23 days in DD (i.e., until  $\tau$  had been stable for at least 7 days), some animals were exposed to a single 3-h pulse of induced running starting at CT 7. Pulses of induced running took place in DD and consisted of moving a hamster from its home cage to a new running wheel (36 cm in diameter), where it remained confined without food or water. In such a situation, hamsters normally run spontaneously, and this activity induces phase advances when the pulse begins at CT 3–9 (9,12). The number of wheel revolutions during each pulse was tallied; this tally is an important step because the number of wheel revolutions influences shift size (3,5, and results below). Animals were returned to their home cages at the end of the 3 h and left to free-run for an additional 10 days.

We chose CT 7 over other times because it corresponds roughly to the middle of the negative slope on the advance portion of the phase-response curve (PRC) to 3 h of induced running (9) and also because pulses given at that time do not cause  $\tau$  changes on average (6). The  $\tau$  changes could affect the calculation of phase-shift size in confounding ways. No other CT offered such a combination of good advances and no  $\tau$  change.

### *Pulse Procedures for Photic Shifts*

After 18–22 days in DD, some animals were exposed to a 20-min pulse of light by having their cages moved to a lit room. Light came from fluorescent (cool white) bulbs, with approxi-

mately 35 lux inside the cage. Hamsters did not have access to their wheels during that time. The pulses started at either CT 19 (to induce advances) or CT 15 (to induce delays). At the end of 20 min, hamsters were moved back to their room and left in DD for another 12 days.

### *Calculation of Shift Size and Analysis*

Dataquest data were downloaded to another computer for plotting and analysis with the TAU program (J. Schull, obtained from Mini-Mitter Co., Sunriver, OR). CTs were calculated relative to activity onset, defined as CT 12. On the day of the pulse, CT 12 was obtained by extrapolating a regression line calculated for the seven activity onsets that preceded pulse time. After the pulse, a certain number of activity onsets were disregarded (the first three for the nonphotic experiment, the first five for the photic advances, and the first two for the photic delays) to eliminate the influence of transient cycles. A regression line was then calculated for the seven subsequent activity onsets. The difference between the extrapolations of the prepulse and postpulse lines on the day that followed pulse time was taken as the amount of phase shifting.

The  $\tau$  before and after the pulses was measured from the slope of the regression lines. The  $\tau$  change caused by the pulse was calculated for all animals, and it was expressed as a positive value when a lengthening had taken place. The  $\tau$  changes were small, but we nevertheless included them in the analyses.

Some animals were not included in the analyses because their rhythms showed unstable baselines or unusual behavior after the shift. This problem occurred mostly during the experiment on photic advances (Fig. 1).

Results were analyzed according to Sokal and Rohlf (16) or with statistical computer packages (Statistix 3.5 and SPSS-PC 3.0). The significance level was set at 0.05. Parametric tests were used whenever possible, but persistent heterogeneity of variance forced the use of nonparametric Kruskal–Wallis tests in a few cases. Means are reported with standard deviations.

## RESULTS

### *Nonphotic Advances*

Figure 2A shows examples of shifts obtained with the nonphotic pulses, and Fig. 3 plots shift size as a function of wheel revolutions during the pulse. For both the long-T and the short-T groups, there was a significant correlation between shift size and wheel revolutions (long-T:  $r = 0.677$ ,  $n = 25$ ,  $p < 0.001$ ; short-T:  $r = 0.643$ ,  $n = 24$ ,  $p < 0.001$ ). The two regression lines shown in Fig. 3 have the same slope, and their elevations are not significantly different from one another (analysis of covariance,  $F(1, 46) = 1.80$ ,  $p = 0.19$ ), an indication that the long-T and short-T groups did not differ in shift size (the tendency was for the short-T group to have larger advances, contrary to expectation).

For each group, the residual shifts relative to the regression line were calculated, and these “shifts corrected for wheel revolutions” were plotted as a function of prepulse  $\tau$  (Fig. 4A). For the long-T group, no significant correlation between corrected shift size and  $\tau$  was detected ( $r = 0.192$ ,  $p > 0.30$ ), but there was a significant negative correlation for the short-T group ( $r = -0.543$ ,  $p < 0.01$ ). Contrary to expectation, the shorter the prepulse  $\tau$  within this group, the larger the phase advance was.

The  $\tau$  change was close to 0 (long-T:  $0.02 \pm 0.08$  h; short-T:  $0.06 \pm 0.07$  h) and not significantly different between the two groups ( $t = 1.71$ ,  $p = 0.09$ ). In stepwise multiple regressions,  $\tau$  change never entered the equation as a determinant of shift size;

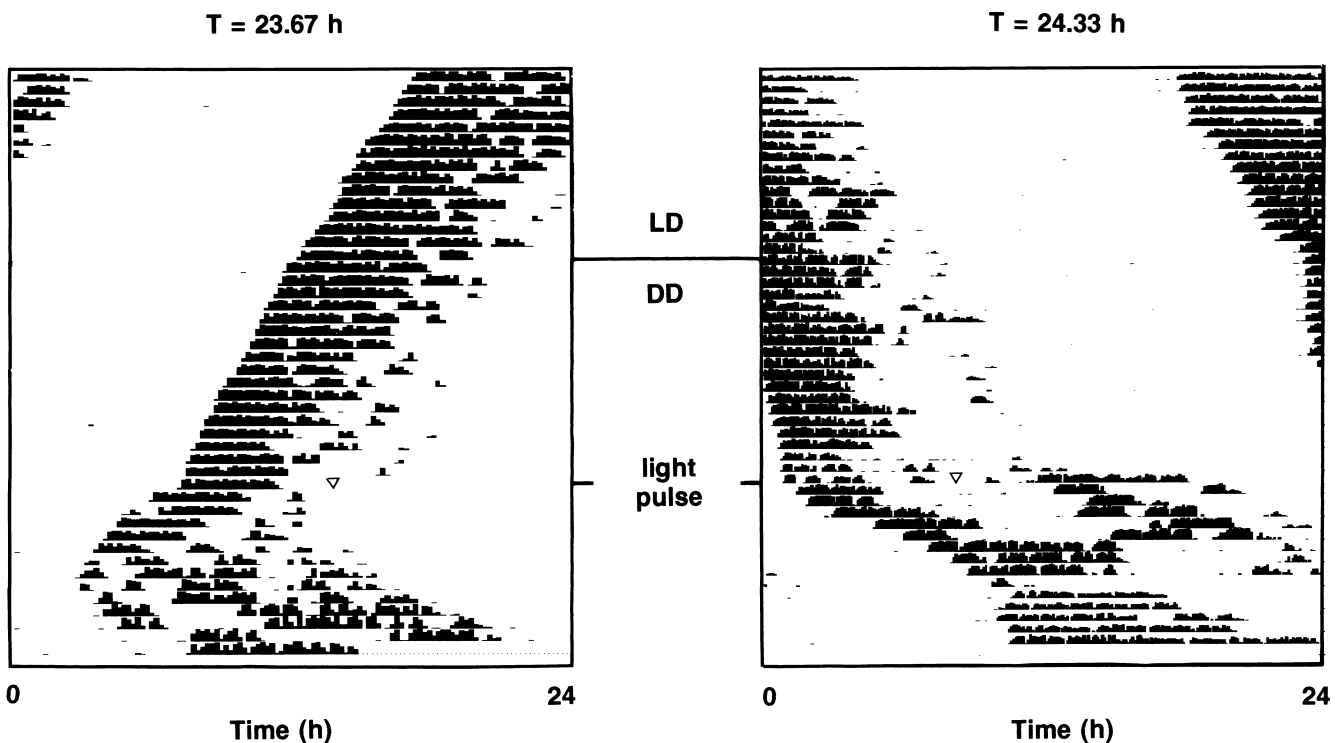


FIG. 1. Examples of actograms from hamsters that were excluded from analysis because of postpulse instability or odd behavior. In both cases here, a 20-min pulse of light was given at CT 19 (downward-pointing triangles) to induce phase advances.

only the number of revolutions and prepulse  $\tau$  did so for the short-T group, and only the number of revolutions for the long-T group.

#### Photic Advances

No significant difference was detected in photic advance size between the long-T and short-T groups (Figs. 2B, 4B). Average advance size was  $2.46 \pm 0.73$  h for the long-T animals and  $2.54 \pm 1.10$  h for the short-T animals ( $t = 0.19$ ,  $p = 0.85$ ). As in the nonphotic experiment, there was a significant negative correlation between shift size and prepulse  $\tau$  within the short-T group (Fig. 4B;  $r = -0.947$ ,  $n = 9$ ,  $p < 0.001$ ) but not within the long-T group (Fig. 4B;  $r = 0.152$ ,  $n = 10$ ,  $p > 0.50$ ). The  $\tau$  change did not correlate with shift size, even in partial correlations where the effect of prepulse  $\tau$  was taken into account (long-T:  $r = 0.344$ ,  $p > 0.30$ ; short-T:  $r = -0.050$ ,  $p > 0.50$ ). Mean  $\tau$  change was  $-0.14 \pm 0.10$  h for the long-T group and  $-0.07 \pm 0.05$  h for the short-T group; the difference was significant (Kruskall-Wallis  $t = 4.16$ ,  $p = 0.04$ ).

#### Photic Delays

No significant difference was detected in photic delay size between the long-T and short-T groups (Figs. 2C, 4C). Average shift size was  $-1.05 \pm 0.49$  h for the long-T animals and  $-0.62 \pm 0.89$  h for the short-T animals (Kruskall-Wallis  $t = 1.99$ ,  $p = 0.16$ ). Contrary to the previous two experiments, there was no significant correlation between shift size and prepulse  $\tau$  within the short-T group (Fig. 4C;  $r = 0.113$ ,  $n = 13$ ,  $p > 0.5$ ), but there was a significant positive correlation in the long-T group (Fig. 4C;  $r = 0.791$ ,  $n = 13$ ,  $p < 0.002$ ). Once again,  $\tau$  change did not correlate with shift size, even in partial correlations where

the effect of prepulse  $\tau$  was taken into account (long-T:  $r = -0.476$ ,  $p = 0.10$ ; short-T:  $r = 0.211$ ,  $p > 0.40$ ). Mean  $\tau$  change was  $-0.03 \pm 0.07$  h for the long-T group and  $-0.12 \pm 0.16$  h for the short-T group; the difference was significant (Kruskall-Wallis  $t = 6.2$ ,  $p = 0.01$ ).

#### DISCUSSION

We conducted the nonphotic experiment first. The lack of difference in shift size between the long-T and short-T groups represented a different result from that previously obtained with mice (1). This result first led us to believe that the relationship between  $\tau$  and shift size was dependent on the nature (photic vs. nonphotic) of the stimulus used. However, repeating the experiment with a photic stimulus led to results similar to those of the nonphotic experiment (compare Fig. 4A with 4B). We conclude that, in Syrian hamsters and with the procedures we used, the relationship between  $\tau$  and shift size does not depend on the nature of the phase-shifting stimulus, but it does not follow the original expectation of larger advances being associated with longer  $\tau$ s.

One possible explanation for the lack of difference between the long-T and the short-T groups is that we may have inadvertently chosen CTs where the PRCs of both groups happen to intersect. Theoretical considerations about entrainment stability dictate that, in a test of the relationship between  $\tau$  and shift size, the steepness of the negative slopes on the PRC is more important than the size of the phase shifts at a given CT (10,11). In the case of advances, for example, this slope is expected to be steeper for animals with long  $\tau$ s, but this expectation would not prevent the slope from intersecting with the same slope for short- $\tau$  animals at one CT where shift size would be indistinguishable between the two groups. To be consistent with our results, however,

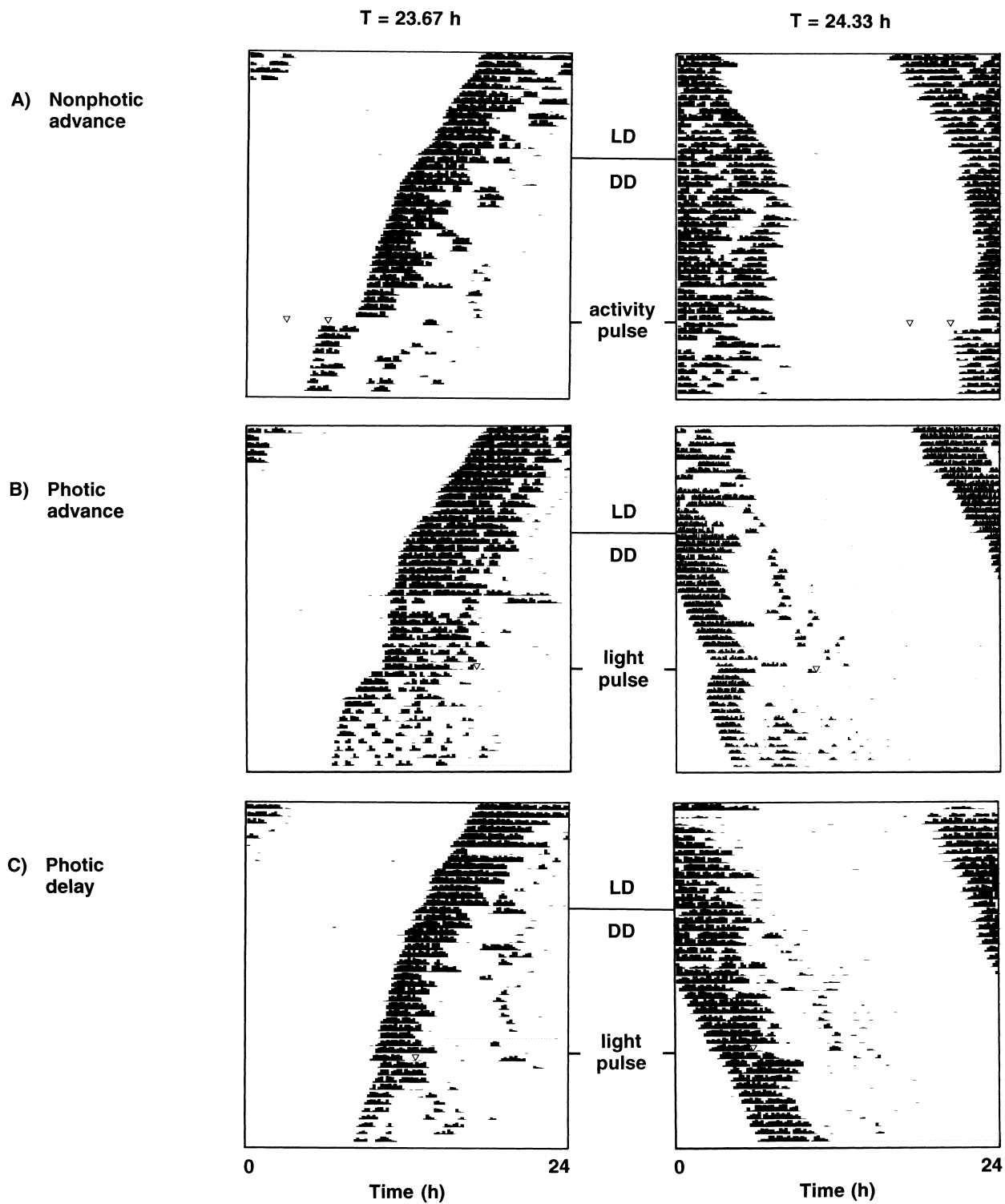


FIG. 2. Examples of actograms from hamsters subjected to (A) a 3-h pulse of novelty-induced running at CT 7, (B) a 20-min pulse of light at CT 19, or (C) a 20-min pulse of light at CT 15. Downward-pointing triangles show beginning and end of running pulses or beginning of light pulses. Animals had previously been entrained to a LD cycle with  $T = 23.67$  h or  $T = 24.33$  h.

this explanation would at once require that the intersecting point be in the middle of the negative slope of the PRC for nonphotic advances [see CT 7 in (9)], at the top of the slope for photic

advances [see CT 19 for hamsters in (1)], and in the middle of the slope for photic delays [CT 15, a place where Daan and Pittendrigh (1) obtained significantly different shifts with mice].

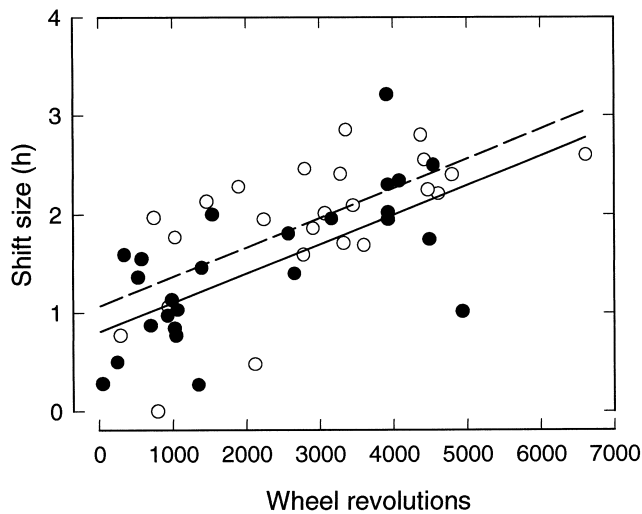


FIG. 3. Size of phase advance of hamster activity rhythms after 3 h of wheel running induced at CT 7, as a function of running level during those 3 h. Although tested in DD, hamsters had been previously entrained to  $T = 23.67$  h ( $\circ$ ) or  $24.33$  h ( $\bullet$ ). Regressions are shown for the short-T animals (dashed line) and long-T animals (solid line).

A more promising explanation is that the use of various  $T$  cycles to alter  $\tau$  may also affect, independently and in unknown ways, the capacity of the clock to phase shift. Few studies have looked at possible links between phase-shift characteristics and previous photoperiodic history. That such links may exist is exemplified by at least one previous study (18) where the shape of the (PRC) to triazolam injection [whose action is mediated by physical activity; see (7,17)] changed according to the number of days spent in DD. The influence of previous phase shifts, or  $T$  cycles (which phase shift animals daily), on subsequent shift size has not been systematically studied. The short  $T$  cycles could somehow “prime” the circadian system to better phase advance, even long after the  $T$  cycles have stopped, an idea that could explain the larger-than-expected advances that we obtained in our short- $T$  groups.

This explanation may not apply to mice (1), but so few species have been studied that general conclusions are impossible at this point. Until we know more about such phenomena in species other than mice, we can only caution against the use of animals with different photoperiodic or phase-shift histories in studies pertaining to the relationship between  $\tau$  and phase-shift size.

Another unexpected aspect of our results is the negative correlation between  $\tau$  and advance size within the short- $T$  groups (Fig. 4A,B). At first we hypothesized that the tendency to adopt a short  $\tau$  after short  $T$  cycles and the ability to phase advance were correlated consequences of clock “lability.” Labile clocks would both shorten their  $\tau$  and phase shift easily after  $T$  cycles, whereas “rigid” clocks would keep their  $\tau$  close to 24 h and would not phase shift easily. However, this hypothesis would also predict larger advances correlated with long  $\tau$ s within the long- $T$  groups, which we did not observe (Fig. 4A,B); it would also predict larger phase delays correlated with long  $\tau$ s in the long- $T$  groups, and we obtained the opposite relationship (Fig. 4C). Presently, we have no explanation for the significant correlations shown in Fig. 4.

In conclusion, we have identified a species and protocol where phase shifts may be affected by previous photoperiodic history

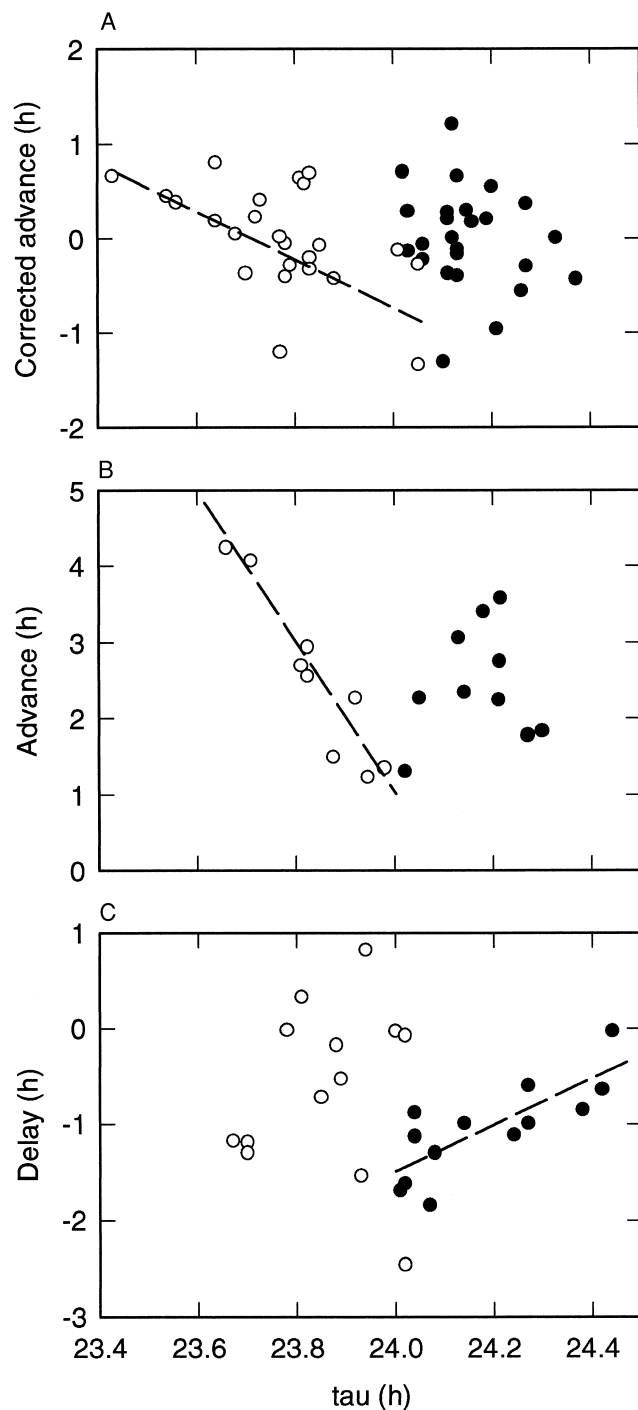


FIG. 4. (A): Size of phase advances, after correction for the effect of running level, as a function of prepulse  $\tau$  in hamsters subjected to a 3-h pulse of running at CT 7; animals had previously been entrained to  $T = 23.67$  h ( $\circ$ ) or  $T = 24.33$  h ( $\bullet$ ). The dashed line shows the regression for the only group where a significant correlation was found. (B): Size of phase advances as a function of prepulse  $\tau$  in hamsters subjected to a 20-min pulse of light at CT 19; symbols and lines as in (A). (C): Size of phase delays as a function of prepulse  $\tau$  in hamsters subjected to a 20-min pulse of light at CT 15; symbols and lines as in (A).

in ways that are inconsistent with the expected relationship between  $\tau$  and shift size, and it is a system where photic and nonphotic stimuli lead to similar results. The latter point provides a counterweight to previous experiments where many differences between photic and nonphotic shifts have been identified. Photic and nonphotic stimuli cause phase shifts that differ in size at given CTs (8), number of transients (13), the stimulus duration necessary for the shifts to take place (13), and the magnitude of  $\tau$  changes after the shift (4,6). Whereas photic input to the oscillator occurs via the eye and retinohypothalamic tract, nonphotic input caused by induced running appears to take place via

the intergeniculate leaflet of the lateral geniculate complex (2,15). But as our study has shown, photic and nonphotic effects need not always be different.

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