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Photoperiod differentially modulates photic and nonphotic phase response curves of hamsters

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Evans, J. A., J. A. Elliott, and M. R. Gorman. Photoperiod differentially modulates photic and nonphotic phase response curves of hamsters. *Am J Physiol Regul Integr Comp Physiol* 286: R539–R546, 2004. First published November 26, 2003; 10.1152/ajpregu.00456.2003.—Circadian pacemakers respond to light pulses with phase adjustments that allow for daily synchronization to 24-h light-dark cycles. In Syrian hamsters, *Mesocricetus auratus*, light-induced phase shifts are larger after entrainment to short daylengths (e.g., 10 h light:14 h dark) vs. long daylengths (e.g., 14 h light:10 h dark). The present study assessed whether photoperiodic modulation of phase resetting magnitude extends to nonphotic perturbations of the circadian rhythm and, if so, whether the relationship parallels that of photic responses. Male Syrian hamsters, entrained for 31 days to either short or long daylengths, were transferred to novel wheel running cages for 2 h at times spanning the entire circadian cycle. Phase shifts induced by this stimulus varied with the circadian time of exposure, but the amplitude of the resulting phase response curve was not markedly influenced by photoperiod. Previously reported photoperiodic effects on photic phase resetting were verified under the current paradigm using 15-min light pulses. Photoperiodic modulation of phase resetting magnitude is input specific and may reflect alterations in the transmission of photic stimuli.

circadian rhythm; novel wheel running; free running period; tau response curve; pacemaker amplitude

FOR HEURISTIC PURPOSES, the circadian system can be conceptualized as three distinct components: a central pacemaker oscillating with a period near 24 h, input pathways that relay temporal cues to the pacemaker, and output pathways that couple the pacemaker to effector systems (22). The suprachiasmatic nucleus (SCN) of the hypothalamus is the principal circadian pacemaker for those mammalian species studied to date (42). Retinal and thalamic afferents to the SCN allow entrainment to the 24-h day via induced changes in the phase and period of the central pacemaker. Finally, overt physiological and behavioral rhythms (e.g., hormonal fluctuations and the sleep:wake cycle) are driven by the SCN through diverse neural and humoral output pathways.

Nearly all organisms rely on photic input for circadian entrainment. Discrete light pulses shift pacemaker phase, and phase shifts can be plotted as a function of the endogenous time of light pulse application to yield a phase response curve (PRC). In most mammals, light pulses induce phase delays and advances during early and late subjective night, respectively, but produce negligible phase shifts during subjective day (12, 32). Within the SCN, light pulses that generate phase shifts induce increased expression of the protooncogene c-Fos and an upregulation of the central clock genes, *Per1* or *Per2* (14, 45).

In addition to altering pacemaker period and phase, entraining light regimens can alter the waveform of pacemaker-driven functions. Several nocturnally expressed markers of circadian phase (e.g., elevated melatonin secretion and locomotor activity in nocturnal rodents) are expressed for a longer duration during short day (SD, e.g., 10 h light:14 h dark) than during long day entrainment (LD, e.g., 14 h light:10 h dark) (6, 10). The converse is also true of the subjective day markers, SCN electrical activity and endogenous c-Fos and *mper* expression (19, 20, 29, 38). Moreover, photoperiod alters the fraction of the circadian cycle during which c-Fos can be elicited by light pulses (41). Thus entraining light:dark regimens cause a suite of diurnal and nocturnal events to reflect the duration of daily light and dark phases.

Parallel to its effects on molecular markers of light responsiveness, photoperiod modulates the waveform of the photic PRC. After SD entrainment, Syrian hamsters exhibit light-induced phase shifts over a wider range of times, with peak shifts markedly greater than those exhibited after LD entrainment (31). This photoperiodic difference in phase shift magnitude may reflect a switch from weak (type 1) to strong (type 0) resetting, which would imply that the circadian pacemaker has a variable amplitude (16, 44). Accordingly, it has been proposed that enhanced SD photic responsiveness reflects a dampened pacemaker amplitude (31). While a reduction in pacemaker amplitude may account for potentiated phase shifting, strong resetting may also be elicited by increases in stimulus strength (5, 13, 34, 44). SD entrainment could alter the photic input impinging on the circadian pacemaker and thereby increase responsiveness to this particular input. To our knowledge, it remains to be determined whether photoperiod alters photic resetting through a change in pacemaker amplitude or in the circadian sensitivity to discrete light pulses.

Although light is the most studied zeitgeber, nonphotic cues can also entrain the circadian clock. In contrast to light, various nonphotic stimuli (e.g., social interactions, triazolam injections, and physical activity) induce phase advances in subjective day and smaller phase delays throughout subjective night (for review, see Ref. 24). Physiologically, exposure to nonphotic stimuli produces an acute downregulation of clock genes cycling within the SCN and high c-Fos expression within the intergeniculate leaflet (IGL) of the thalamus, but not in the SCN (18, 21). The distinct mechanisms of photic and nonphotic resetting suggest means of investigating how photoperiod alters photic phase resetting. If pacemaker amplitude is altered by photoperiod, then photic and nonphotic PRCs should have a parallel dependence on photoperiod.

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The present study assesses whether photoperiod affects the magnitude of phase shifts after exposure to a nonphotic stimulus, novel wheel running (NWR). If SD animals do not display larger phase shift responses than LD animals to this nonphotic stimulus, then SD-induced augmentation of photic resetting may be due to changes in the light input pathway. Alternatively, joint enhancement of photic and nonphotic phase resetting after SD entrainment would support the hypothesis that pacemaker amplitude is modulated by photoperiod. Our results suggest that the amplitude of the nonphotic PRC is unaffected by photoperiod and thus do not support the hypothesis that pacemaker amplitude is decreased by SD entrainment.

METHODS

Animals and Husbandry

Male Syrian hamsters (*Mesocricetus auratus*, $n = 96$), 3–4 wk of age (HsdHan:AURA, Harlan, Indianapolis, IN), were assigned for the duration of the study to either a LD (14 h light: 10 h dark, 14L:10D, lights on: 0300 PST) or a SD condition (10L:14D, lights on: 0700 PST). Fluorescent bulbs provided photophase illumination of 50–75 lx at the cage floor with complete darkness during the scotophase. Ambient temperature was maintained at 22 ± 2°C. Food (Purina Rodent Chow no. 5001, St. Louis, MO) and water were available ad libitum. This study was conducted in compliance with all rules and regulations of the Institutional Animal Care and Use Committee, University of California, San Diego.

Animals were housed in cylindrical polyethylene cages (35 cm height × 26 cm diameter) without a running wheel (*experiment 1*) or in polypropylene cages (48 × 27 × 20 cm) equipped with a wheel (17 cm diameter, *experiment 2*). In both experiments, cages were located in light-tight, ventilated chambers (12 cages/chamber), except during 3-wk intervals between successive pulses (see below) when LD and SD animals were reentrained to their respective photoperiods. At this time, cages were placed on open racks in separate rooms.

Since photoperiod alters gonadal hormone concentrations, which in turn influence wheel running behavior in this species (23), a between-photoperiod comparison in intact animals might be confounded by group differences in NWR behavior. Accordingly, we equalized testosterone levels between photoperiodic groups through bilateral orchidectomy. We opted not to control for group differences in other photoperiod-driven variables, such as the daily pattern of melatonin secretion or gonadotropin concentrations, and similarly, no effort was made to retain SD animals in a photoperiodic state. The above factors, however, are not known to markedly alter NWR- or light-induced phase resetting. Before all manipulations described below, animals were castrated under pentobarbital sodium anesthesia (65 mg/kg) at 4–5 wk of age. Two weeks after castration, hamsters received a 2-h introductory NWR bout during late-subjective day to provide animals with wheel-running experience before data collection.

Experiment 1

Phase shifts were studied under a repeated Aschoff type II design (1). Each round of the experiment commenced with 31 days of entrainment to LD or SD. Animals were then exposed to constant darkness (DD) beginning at the light-to-dark transition (designated zeitgeber time, ZT 12). NWR pulses were delivered after 25–49 h in DD at every odd ZT. Transferred under dim red illumination, animals were locked into dark Wahmann wheels (34-cm diameter) for 2 h, where they were left to run of their own volition. Randomly selected control animals from each photoperiodic group remained in their home cages. On return, all hamsters were monitored for an additional 9–10 days and then reentrained to their previously experienced photocycle. At no time during the experiment was the time of NWR stimulation repeated for an individual animal. To align phase-resetting responses, the difference between activity onset on the first day in DD (CT 12) and ZT 12 was used to arithmetically transform NWR pulse time into circadian time (CT). Table 1 summarizes this protocol and indicates sample size for each round.

To replicate previously reported photoperiodic differences in photic phase shifting, a subset of animals ($n = 45$, see Table 1) was exposed to a 15-min light pulse (light intensity 100–460 lx) at approximately CT 14 or CT 20. Projected CT for light pulses was determined by each chamber's median time of activity onset on the first day in DD. To minimize inadvertent nonphotic stimulation associated with removing cages from the housing chambers, all animals within a chamber were pulsed simultaneously.

Experiment 2

The low between-subject variability in NWR exhibited by LD and SD animals in *experiment 1* contrasts with that commonly reported in studies of animals with home cage wheels; consequently, *experiment 2* was conducted to assess the impact of home cage wheel provision on group NWR activity levels. After the conclusion of *experiment 1*, LD and SD animals were reentrained for 4 wk and then given home cage wheels 1 h past the expected time of activity onset. After 3 additional weeks, NWR pulses were conducted, in a manner identical to that described above, at CT 01, CT 07, and CT 21. Projected CT of NWR pulses was determined by each animal's time of activity onset on the first day in DD. The experiment was terminated after the NWR stimulus.

Data Collection

For both experiments, the intensity of NWR was quantified by summing the number of wheel revolutions recorded by magnetic contacts. Home cage activity was recorded via passive infrared motion detectors (Coral Plus by Visonic, Bloomfield, CT) mounted ~32 cm above the cage floor (*experiment 1*) or through home cage wheels equipped with magnetic contacts (*experiment 2*). NWR and home cage activity were recorded and compiled into 6-min bins by DataQuest III or VitalView software, respectively (Mini-Mitter, Sun River, OR). Actograms were analyzed using ClockLab software (Actimetrics, Evanston, IL).

To confirm the presence of distinct LD and SD activity profiles, a 24-h histogram was produced for each hamster by averaging counts in each 6-min bin over the last 8–9 days under entrained conditions. For each histogram, activity onset was defined as the first 6-min time bin after noon with average counts above overall daily mean levels, and activity offset was the last time point preceded by a bin exceeding this

Table 1. *Summary of experimental protocol and sample size for each round*

Day	Manipulation
14	Cage change
21	Activity recording begins
28	Cage change
31	Release into constant darkness
32	NWR pulses
42/1	Transfer to open racks for reentrainment

	NWR *n*		NWR Control *n*		Light *n*	
Age (±2 wk)	LD	SD	LD	SD	LD	SD
11	33	27	6	5		
18	42	38	6	6		
23	19	15	2	1	24	21
29	16	16	3	3		

LD, long day; SD, short day; NWR, novel wheel running; *n*, sample size.

threshold. The duration of activity, α, was calculated as the time difference between activity onset and offset. Activity onset was later transformed into the phase angle of entrainment to the light/dark transition (ψ_{onset}).

In *experiment 1*, phase shifts were measured by determining the displacement between activity onset on the first day in DD and activity onset predicted by a regression line fit to postpulse activity onsets (6 days were used excluding the first 3 postpulse days to allow for transients; on 1 occasion, fewer days were used for 6 animals). Pre- and postpulse activity onsets were identified visually by the abrupt transition from a state of low to high activity. The slope of the postpulse regression line was used to calculate each animal's free-running period (τ). Phase shift and τ values for nonpulsed controls were calculated in the same fashion and used to adjust for the effects of release into DD.

Statistical Analyses

Animals were used repeatedly in successive rounds, yet the round of the pulse was not a statistically significant factor in any of the final analyses, indicating that entrainment parameters, phase shift, and tau responses were not systematically affected by age or prior testing. Consequently, this factor was removed from all analyses and is not reported here. Unless otherwise stated, statistical tests were conducted with JMPIN software (SAS Institute, Cary, NC) and considered significant if $P < 0.05$.

In *experiments 1* and *2*, LD and SD differences in entrainment parameters (α and ψ_{onset}) were assessed with Student's *t*-tests. Photoperiodic comparisons of light-induced phase shifts were also conducted with *t*-tests at each pulse time.

For *experiment 1*, CT of NWR exposure was rounded into 2-h time bins. NWR revolutions, phase shifts, and τ responses were initially submitted to factorial ANOVA with CT, photoperiod, and the photoperiod × CT interaction as factors. LD and SD data were separated for further analysis with one-way ANOVA. Controls were included in each one-way ANOVA model, and least-squares contrasts were used to compare pulsed and nonpulsed phase shift and period responses at each CT bin. At each CT bin with a significant effect of NWR, we conducted post hoc comparisons of LD and SD phase shift responses.

Although ANOVA is commonly used, its utility in the analysis of PRC data is limited in several respects (15). For example, ANOVA encodes the time of stimulus application as a noncontiguous independent variable that obscures both the linear and cyclical nature of time (e.g., CT 23 is midway between CT 21 and CT 1). Representation of the dependent variable (i.e., the phase shift) is also problematic when large phase shifts must be labeled as either advances or delays since the continuity between the two responses is obscured. Finally, ANOVA does not permit further analysis of PRC properties important for the assessment of phase-resetting rhythms (e.g., amplitude, inflection point). To address these shortcomings, we supplemented our analysis of each PRC with a recently developed PRC bisection test, which is designed to permit tests of PRC robustness and between-group comparisons of PRC amplitude (15).

The PRC bisection test represents independent and dependent variables in circular coordinates. The precise CT of the NWR pulse and the magnitude of each phase shift are transformed into angular degrees (0 to 360, where 180 = CT 12). The circular distribution of the independent variable is bisected into every possible set of two hemicircles. For each bisection, PRC amplitude is calculated from the difference between the average phase shift in the two hemicircles, and the bisection that yields the greatest contrast is taken as the optimal bisection. To determine if the PRC is significantly robust, the bisection point with the largest amplitude score is then compared with a distribution of random divisions generated through a Monte Carlo procedure. Photoperiodic differences in PRC amplitude score were assessed with a Wilcoxon rank sum test. Last, the area under the curve (AUC) for each NWR-induced PRC was calculated and is reported here to supplement the PRC bisection test.

For *experiment 2*, NWR behavior was initially submitted to factorial ANOVA with CT, photoperiod, and the photoperiod × CT interaction as factors. Post hoc comparisons were conducted with Tukey's honestly significant difference (HSD) test. To assess differences in NWR behavior across *experiments 1* and *2*, each photoperiodic group's data from NWR pulses performed at the same subjective time bin (CT 01, CT 07, and CT 21) were compared with the use of a one-way ANOVA, Tukey's HSD, and Levene's test for heterogeneity of variance.

RESULTS

Experiment 1

Entrainment parameters. As expected, entrainment to SD and LD photoperiods produced robust differences in the phasing of home cage activity rhythms (Fig. 1). During the entrainment portion of each round, SD animals expressed longer active phases than LD animals [e.g., round 1 α: SD = 11.71 ± 0.15 h, LD = 9.68 ± 0.15 h; $t(89) = -9.65$, $P < 0.001$] and initiated activity later in the scotophase [e.g., round 1 ψ_{onset}: SD = −1.62 ± 0.1 h, LD = 0.11 ± 0.1 h; $t(89) = 12.0$, $P < 0.001$]. Photoperiodic differences in α and ψ_{onset} were consistently generated before each subsequent round ($P < 0.05$ in all cases).

Activity levels within novel wheels. Nearly all animals engaged in robust wheel running throughout the 2 h exposure to novel wheels, with no significant difference between LD and SD running levels [$F(1,182) = 0.001$, $P > 0.95$]. NWR levels varied with time of day [$F(11,182) = 4.31$, $P < 0.001$], but the daily rhythm in NWR activity did not differ between photoperiodic conditions [photoperiod × CT: $F(11,182) = 0.74$, $P > 0.65$]. In both photoperiods, NWR levels increased as subjective night approached, reached a maximum during mid to late subjective night, and then fell to a minimum early in subjective day (Fig. 2).

Phase and τ response curves. Phase-shifting responses fluctuated with time of day [Fig. 3, $F(11,182) = 2.22$, $P < 0.05$]. There was no main effect of photoperiodic condition [$F(1,182) = 0.01$, $P > 0.9$], nor did the LD and SD PRCs differ from one another [photoperiod × CT: $F(11,182) = 1.4$, $P > 0.1$]. Relative to control values (SD = 0.06 ± 0.23 h, LD = 0.45 ± 0.21 h), significant phase advances and delays were reserved for SD (CT 09, $P < 0.05$) and LD animals (CT 21, $P < 0.05$), respectively. However, at neither time point did SD and LD phase shifts differ significantly from one another (CT 09: SD = 0.88 ± 0.32; LD = 0.06 ± 0.34; $P = 0.08$; CT 21: SD = −0.81 ± 0.44; LD = −0.76 ± 0.32; $P > 0.9$).

The PRC bisection test confirmed that NWR-induced PRCs for LD and SD were each significantly robust ($P < 0.05$ in both cases). For the SD and LD PRCs, the switch from phase advances to phase delays was CT 10.63 and 13.18, respectively, which are both consistent with previous reports. PRC amplitude scores for SD and LD NWR-induced resetting curves did not differ significantly (10.61 ± 0.39 and 11.19 ± 0.39, respectively; $Z = 0.39$, $P > 0.5$). The AUC for the SD NWR-induced PRC was reduced by 33% relative to the LD AUC (5.69 and 8.48 h^2, respectively).

Post-NWR τ differed between photoperiods (Fig. 4, Table 2). SD animals expressed significantly longer τs than LD animals after NWR pulses [$F(1,182) = 34.5$, $P < 0.001$].

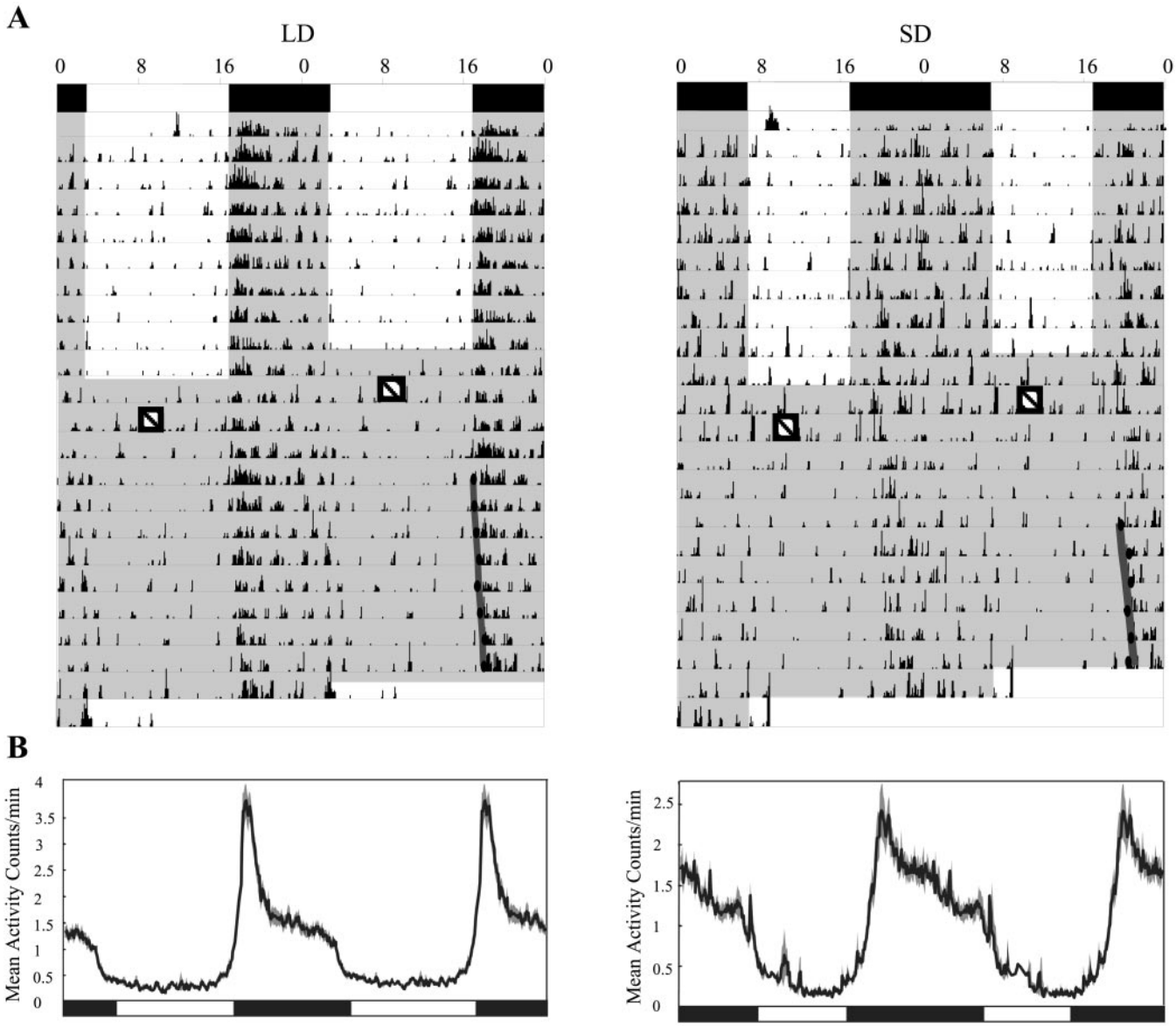

Fig. 1. Long day (LD) and short day (SD) entrainment patterns, with illustration of the experimental paradigm used to study phase shifts induced by novel wheel running (NWR). *A*: representative double-plotted actograms depicting both entrained and free-running activity. White and black bars above actograms represent the light and dark phases, respectively, as do related shading within actograms. The change in the pattern of the internal shading indicates the switch to constant darkness. Hatched boxes represent times hamsters were removed from their home cages for NWR pulses. *B*: 24-h histograms averaging activity exhibited by all animals within each photoperiod during the monitored entrainment interval before the first NWR pulse. LD sample size = 39; SD sample size = 32. SE is indicated by gray shading.

There was no main effect of the phase of NWR [$F(11,182) = 1.5$, $P > 0.1$], but the phase of NWR differentially affected the τ of LD and SD animals [photoperiod × CT: $F(11,182) = 1.8$, $P = 0.05$]. When analyzed separately, SD animals displayed a robust fluctuation of post-NWR τs [$F(11,84) = 2.15$, $P < 0.05$], while LD animals did not [$F(11,98) = 0.79$, $P > 0.6$]. Likewise, SD animals expressed longer τs after NWR pulses at CT 7 and CT 9 ($P < 0.05$) while LD animals did not differ from controls at any pulse time ($P > 0.05$).

Light-induced phase shifts. Consistent with previous reports, light pulses induced larger phase delays [CT 14, Fig. 5, $t(20) = 4.5$, $P < 0.001$] and phase advances [CT 20, Fig. 5, $t(21) = -2.56$, $P < 0.05$] in SD vs. LD animals. Postpulse τ did not differ between photoperiods at either CT 14 [$t(20) = -1.77$, $P = 0.09$] or CT 20 [$t(21) = -0.79$, $P = 0.4$].

Experiment 2

As in *experiment 1*, SD animals expressed longer active phases than LD animals [α: SD = 9.66 ± 0.24 h, LD = 8.22 ± 0.12 h; $t(91) = -5.44$, $P < 0.001$] and initiated activity later in the scotophase [ψ_{onset}: SD = −2.35 ± 0.14 h, LD = −0.68 ± 0.03 h; $t(91) = -11.82$, $P < 0.001$].

NWR activity was similar between photoperiods [$F(1,65) = 0.19$, $P > 0.6$] and varied by time of day [$F(2,65) = 13.37$, $P < 0.001$] in a manner similar for LD and SD animals

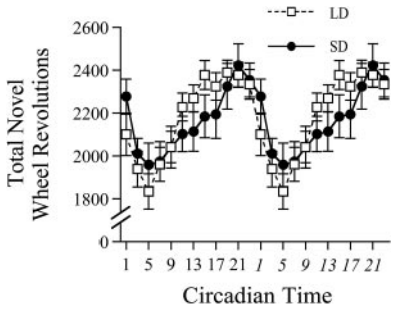

Fig. 2. Mean novel wheel running levels (±SE) for LD and SD animals across the circadian cycle. Dark periods under entrained conditions correspond to circadian time (CT)12–CT 22 (LD) and CT 10.4–CT 0.4 (SD). Graph is double-plotted to facilitate visual analysis. LD sample sizes (n) = 7, 8, 9, 11, 13, 9, 11, 6, 3, 7, 7, and 4 for CT 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 23, respectively. SD n = 8, 7, 10, 8, 10, 7, 4, 6, 4, 6, 5, and 6 for CT 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 23, respectively.

[photoperiod × CT $F(2,65) = 1.64$, $P > 0.2$]. As observed in *experiment 1*, NWR was more intense during subjective night than during subjective day (Tukey's HSD, $\alpha = 0.05$, $Q = 2.4$).

NWR levels during subjective day were more variable during *experiment 2* than at comparable timepoints in *experiment 1* [Fig. 6; Levene test, LD CT 01 $F(1,18) = 7.33$, LD CT 07 $F(1,19) = 6.09$, SD CT 01 $F(1,21) = 19.11$, $P < 0.05$ in all cases] with many animals discontinuing activity before the end of the pulse. Mean NWR activity at each CT, however, was similar to that displayed by animals in *experiment 1* [Fig. 6; $F(1,22) = 3.88$, $P > 0.06$ in all cases], with the one exception of LD levels at CT 01 [$F(1,18) = 7.64$, $P < 0.05$].

DISCUSSION

Amplitude of the NWR-induced PRC did not differ between hamsters entrained to LD and SD. In contrast, both light-induced phase delays and advances were significantly larger in SD animals. As reduction of pacemaker amplitude would be expected to increase phase shifts in response to all zeitgebers (13, 34), the differential effect of photoperiod on photic and nonphotic phase resetting argues against a reduction of overall pacemaker amplitude in SD animals. Rather, photoperiod may selectively modulate pacemaker inputs.

Ample precedent exists for photoperiodic modulation of central pacemaker function. In the SCN, rhythms of spontaneous neural firing and gene expression depend on prior entrainment conditions, as do various peripherally generated rhythms driven by the SCN (i.e., melatonin secretion and locomotor activity) (19, 20, 29, 38, 41). In all cases, markers of diurnal phase are expressed for longer after LD, whereas nocturnal functions lengthen under SD. Likewise, the circadian rhythm of light responsiveness in hamsters is altered by SD entrainment so that light induces phase shifts over a longer fraction of the circadian cycle, enhancing the magnitude of both advances and delays (31). In house sparrows, a similar SD-induced increase in phase delay magnitude augments overall photic PRC amplitude (3). Finally, the most definitive indicator of pacemaker function, endogenous period length, can be altered by prior entrainment conditions (32).

However, with the exception of SD-enhanced photic phase resetting, few of these changes implicate an effect of photoperiod on pacemaker amplitude. In complex oscillators, pacemaker amplitude and resetting magnitude may be inversely related (16). This relationship can be intuitively understood in terms of the swing of a pendulum: a given stimulus (i.e., zeitgeber) will induce a larger shift in the phase of a pendulum with a low-amplitude oscillation (i.e., dampened swing) than one with larger amplitude (i.e., a larger arc of swing). Studies of diverse taxa (e.g., *Neurospora*, *Culex*, *Kalanchoe*, and *Drosophila*) indicate that the circadian pacemaker is best characterized by both phase and amplitude, and strong (type 0) resetting can be elicited under states of reduced amplitude (8, 30, 34, 43). Although subject to debate, the mammalian circadian pacemaker may be characterized by phase and amplitude as well (5). While not contesting complex oscillator models,

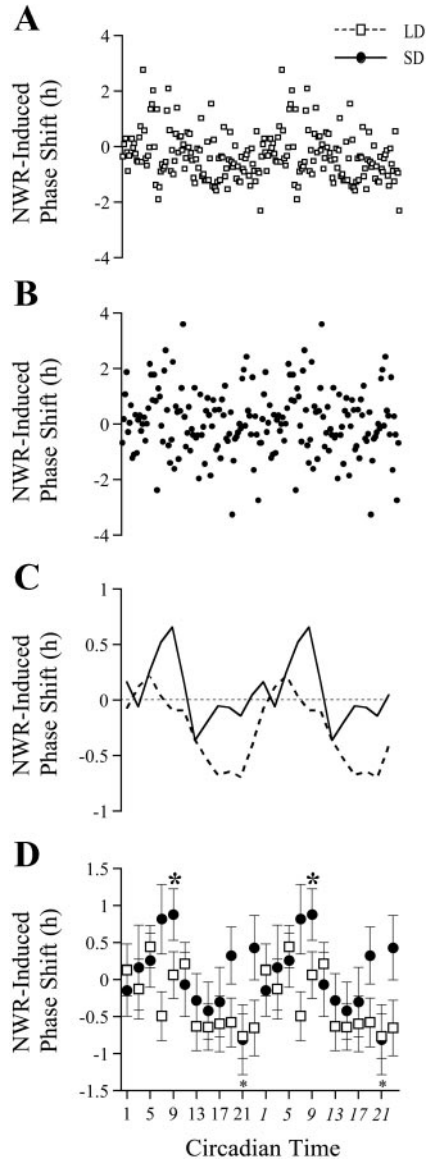

Fig. 3. NWR-induced resetting for LD and SD hamsters. *A* and *B*: plots of individual phase shifts for LD and SD animals. *C*: phase response curves (PRCs) for LD (dashed line) and SD animals (solid line), as drawn with a 5-h moving average. Stippled line at zero represents mean values of the control group for each photoperiod. *D*: mean phase shift magnitude (± SE) for each CT bin used in statistical analyses. *Values significantly larger than control values, $P < 0.05$. Conventions as in Fig. 2.

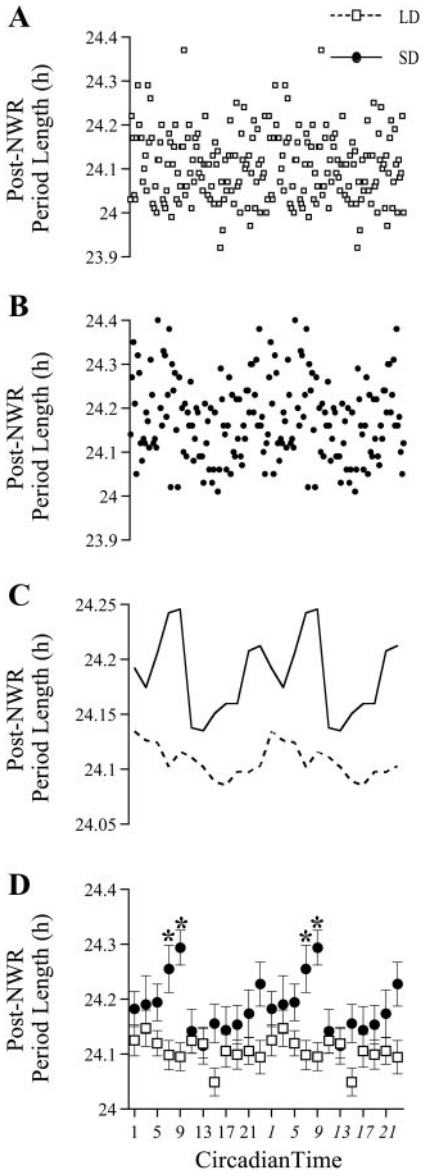

Fig. 4. NWR-induced τ responses for LD and SD animals. *A* and *B*: plots of individual phase shifts for LD and SD animals. *C*: τ-response curves (τRCs) for LD and SD groups, as drawn with a 5-h moving average. *D*: mean period length ($\pm$SE) for each CT bin used in statistical analyses. *Values significantly larger than control values, $P < 0.05$. Conventions as in Figs. 2 and 3.

our results do not support the hypothesis that changes in pacemaker amplitude underlie the SD enhancement of the photic PRC (31). Rather, this study suggests photic and nonphotic resetting are differentially modulated by photoperiod and that alterations of photic input pathways may be involved in the SD augmentation of the photic PRC. Potential sites of

Table 2. *Circadian period length (τ) for NWR-pulsed animals and controls*

	LD	*n*	SD	*n*	*P*
Pulsed animals	24.10±0.01	110	24.19±0.01	96	<0.001
Controls	24.11±0.02	17	24.15±0.02	15	>0.2

Values are means ± SE (h).

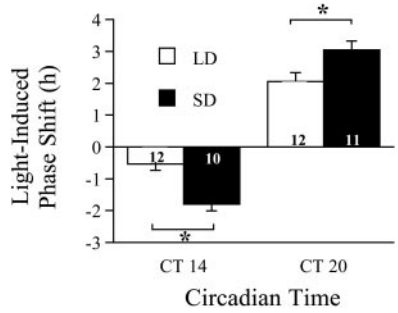

Fig. 5. Mean light-induced phase shifts ($\pm$SE) for LD and SD animals pulsed at either CT 14 or CT 20. Sample sizes are indicated at the base of each bar. *$P < 0.05$.

modulation include serotonergic and adenosinic receptors located on retinal afferents (36, 37), thalamic sources of photic input (9), and intercompartmental communication within the SCN itself (45).

Despite several methodological differences, our LD nonphotic PRC resembles those previously reported in several respects (e.g., 4, 28, 33). Across these NWR resetting curves, the end of subjective day invariably marks the transition between relative phase advances and delays, as verified here by the PRC bisection test. However, where previous studies describe large 2- to 3-h phase advances in subjective day, LD animals in the present study displayed only significant phase delays during subjective night. The behavior of control animals may help explain this discrepancy: after release into DD, nonpulsed LD animals showed several days of advancing transients (termed "knees," cf. Ref. 3), yielding an average "advance" of +0.45 h in controls. By plotting and analyzing our data relative to nonpulsed controls, we find significant delays rather than advances for LD animals. To our knowledge, this advancing pattern in LD controls has not been reported for hamsters with running wheels and its absence may reflect wheel running-induced feedback effects on the pacemaker (2). Without adjusting for control values, the LD NWR-induced PRC would be quite similar to those previously reported, albeit with smaller phase advances early in subjective day. Both the LD and SD PRCs collected in the current study were of lower

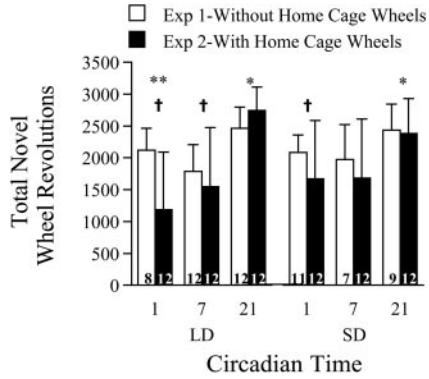

Fig. 6. NWR levels exhibited at CT 01, CT 07, and CT 21 by LD and SD animals with (*experiment 2*) and without (*experiment 1*) home cage wheels. Sample sizes are indicated at the base of each bar. *Higher NWR levels at CT 21 than either CT 01 or CT 07, $P < 0.05$ by Tukey's honestly significantly different test (HSD). **Difference in mean values between *experiments 1* and *2*, $P < 0.05$ by Tukey's HSD. †Difference in variance between *experiments 1* and *2*, $P < 0.05$ by Levene test.

amplitude (~1 h peak to trough) than previously reported (11, 28), although we note that nonphotic resetting magnitude is known to vary between labs (e.g., 17). Our lower amplitude PRCs may potentially reflect the lack of home cage wheels, castration, short NWR pulse duration, or other unanticipated factors that could have affected phase-shifting magnitude.

This is the first report to systematically characterize SD nonphotic resetting. Nonetheless, animals with SD-like behavioral phenotypes may have contributed to previously published Aschoff type I PRCs, where NWR pulses were given repeatedly to animals free-running in DD (28, 33). Like SD entrainment, extended exposure to DD promotes gonadal regression secondary to lengthening of α (6). Moreover, with extended time in DD, photic resetting is enhanced and triazolam-induced resetting is dampened (35, 39). Because we found no effect of photoperiod on NWR-induced PRC amplitude in castrated animals, dampening of nonphotic resetting under DD may not relate to changes in α but might instead reflect DD-induced decreases in testosterone and/or activity levels (23). However, the above factors clearly do not explain the photoperiod-induced change in photic resetting magnitude, as this effect remained after castration, used here to eliminate potential photoperiodic differences in NWR levels.

Wheel-naive animals run vigorously of their own accord when given a wheel for the first time (27), and animals in *experiment 1* were housed without wheels to encourage uniformly high NWR rates. Consistent with this objective, both photoperiod groups ran at levels within the range known to elicit nonasymptotic phase advances in gonadally intact LD animals (i.e., >1,001 revolutions/h; cf Ref. 4). Furthermore, both LD and SD NWR levels at each CT were normally, rather than bimodally, distributed, leading us to exclude no animals in the generation of our PRCs. This pattern contrasts with previous studies in which many animals, sluggards, run at subthreshold levels and are treated separately in the analysis of data (for review, see Ref. 24). The significantly more variable NWR in *experiment 2* suggests that the uniformly high NWR observed in *experiment 1* may have resulted from the lack of a home cage wheel. While animals were also older in *experiment 2*, they were not of an age at which induced activity levels generally decrease (26, 40).

Over a broad range of NWR intensity, activity-response curves are sigmoidal in nature (11). With the tightly clustered range of NWR levels in the present study, no statistical relationship was discernible between the level of NWR and the magnitude and direction of subsequent phase shifts (analyses not shown). There was a clear circadian rhythm in the number of NWR revolutions displayed by our hamsters but no evidence that differences in NWR levels within and between CTs were related to the strength or intensity of NWR as a phase-resetting stimulus per se. Thus, under the conditions of this study, it is unlikely that the circadian variation in NWR influenced the PRCs, a point further illustrated by noting that SD animals exhibited the largest phase shifts after pulses with the lowest NWR levels (i.e., CT 07 and CT 09, cf Figs. 2 and 3).

SD animals displayed a robust τ-response curve (τRC) and longer post-NWR pulse τs than LD animals. As significant photoperiodic aftereffects on τ were absent in nonpulsed controls, this suggests that SD animals are characterized by both augmented photic phase resetting and an enhanced sensitivity to the τ-lengthening effects of NWR pulses. Previous τRCs show longer post-NWR τs after pulses during subjective night, when relatively small phase delays are elicited (25). SD animals in the current study, however, had longer τs after NWR pulses during subjective day, a time marked by phase advances. Whereas light-induced phase delays and advances have been shown to be associated with longer and shorter τs, respectively (7), the same relationship appears not to hold for NWR under the present conditions.

The present study establishes that photic and nonphotic PRCs are differentially modulated by photoperiod. After SD entrainment, the amplitude of the photic PRC is markedly augmented while its general shape is conserved (31). In contrast, the amplitude of the NWR-induced PRC appears to be relatively resistant to photoperiodic modulation and instead photoperiod may alter the waveform of the nonphotic resetting curve. Examination of the LD and SD PRCs suggests photoperiodic differences in the phasing and distribution of phase-shift responses, which might be profitably examined by further study. Viewed in conjunction with the observed photoperiodic differences in post-NWR τ, the data suggest that photoperiod may affect nonphotic resetting in ways other than through changes in PRC amplitude.

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