

## Differential Effects of Multiple Short Day Lengths on Body Weights of Gonadectomized Siberian Hamsters

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### ABSTRACT

Siberian hamsters (*Phodopus sungorus*) maintained under simulated natural photoperiods exhibit marked reductions in body weight as day lengths decrease in summer and fall. This experiment assessed whether the component of the seasonal body weight rhythm that is independent of gonadal hormones exhibits a graded dependence on decreasing day lengths or whether the entire program of weight loss is triggered by the crossing of a single critical day length in late summer. Male hamsters born into a photoperiod with 13 h light and 11 h dark (i.e., 13L : 11D) were castrated and transferred to simulated natural photoperiod for early April at 40°N latitude. At the summer solstice (15L : 9D) some hamsters remained on that photoperiod whereas others experienced gradual decreases in day length. Three additional groups were moved to static photoperiods when day lengths had subsequently declined to 13L : 11D, 11L : 13D, or 9L : 15D, respectively. Day lengths decreasing to 13L : 11D were sufficient to suppress body weight but were less inhibitory than further decreases in day length. Hamsters identified as photoperiod nonresponsive on the basis of daily activity rhythms increased body weight monotonically. These results establish that steroid-independent modulation of body weight depends on photoperiod in a graded fashion.

### Introduction

Among many mammalian species, reproduction and body size fluctuate seasonally, presumably as adaptations to annual variations in ambient temperature and food availability. The Siberian hamster, *Phodopus sungorus*, is a model system for the study of the neuroendocrine mechanisms associated with changes in food intake and metabolism (Bartness and Wade

1985; Weiner 1987; Lerchl and Schlatt 1993; Goldman 1999). Attaining maximum size during the spring/summer breeding season, hamsters may reduce body weight by 30%–50% in autumn and winter, even in the presence of abundant resources and in the absence of energetic challenge (Hoffmann 1982; Steinlechner et al. 1983). In this and most other species studied, seasonal traits are largely regulated by changes in ambient photoperiod, or day length, and are readily induced in the laboratory by simulating the natural progression of day lengths or by acutely transferring animals between extreme summer and winter day lengths (Hoffmann 1982; Steinlechner and Heldmaier 1982). Exposure to long photoperiods (e.g., 16L : 8D) induces summer traits, whereas the winter phenotype develops after prolonged exposure to short day lengths (e.g., 8L : 18D). From these studies has emerged the concept of a critical day length (estimated to be 13L : 11D in this species) that categorically demarcates stimulatory and inhibitory photoperiods (Hoffmann 1982), although more detailed studies have defined different critical day lengths for various seasonal traits (Duncan et al. 1985). Day length is inversely correlated with the duration of elevated pineal melatonin secretion, and whether nightly melatonin signals are longer or shorter than a critical melatonin duration more proximally mediates photoperiodic responses (Goldman 1991).

Studies of gonadal function that have employed day lengths near the so-called critical value and naturalistic progressions of photoperiod have prompted revision of the critical day length model to incorporate not only the absolute length of photoperiodic signals but their temporal patterning as well (Horton 1984; Rivkees et al. 1988; Horton and Stetson 1992). Thus, equinox photoperiods can induce either gonadal growth or inhibition depending on whether prior day lengths were shorter or longer, respectively. The importance of photoperiodic change was similarly illustrated by the demonstration that annual reproductive rhythms are induced by artificial photocycles that mimic the natural pattern of day length change but which were unnaturally lengthened or shortened to never fall below the so-called critical value in one case or above it in another (Gorman and Zucker 1995). Finally, studies further indicate that the common laboratory practice of introducing abrupt, unecological shifts in photoperiodic signals may mask seasonal responses that are present under naturalistic conditions (Gorman and Zucker 1997b, 1998).

While a single value for the critical day length, independent of photoperiodic history, does not exist for hamsters, there has been little work to question whether a given seasonal response

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under natural conditions is indeed all-or-none as is posited in critical day-length models. The aim of this study was to assess, in the absence of abrupt, unecological changes in day length, whether the crossing of a critical day length in late summer or early fall was sufficient to trigger a complete seasonal transition of a photoperiodic trait. Alternatively, seasonal responses might exhibit a graded dependence on photoperiod with ongoing decreases in day length necessary to provoke the complete seasonal response. Body weight was chosen as a measure of seasonal responsiveness in hamsters because it can be measured continuously and with great accuracy. Day length influences body weight through photoperiod-driven changes in gonadal steroid concentrations and also through gonad-independent changes in prolactin (Dark and Zucker 1984; Wade and Bartness 1984; Vitale et al. 1985; Niklowitz and Hoffmann 1988). Furthermore, these two neuroendocrine mechanisms may be differentially regulated by photoperiod (Duncan et al. 1985; Donham et al. 1994; Shaw and Goldman 1995). To eliminate confounding effects on body weight of differential photoperiodic regulation of gonadal function, castrated hamsters were used to examine the steroid-independent seasonal decline in body weight. Contrary to the critical day length model, this single photoperiodic trait was shown to exhibit a graded dependence on decreasing photoperiods.

## Material and Methods

All hamsters were derived from a breeding colony founded on stock provided by Bruce Goldman (University of Connecticut, Storrs). Hamsters were housed in polypropylene cages (27cm × 16cm × 13 cm, 1–3/cage) on corncob bedding with food (Mouse Chow 5015, Purina Mills, St. Louis) and water available ad lib. Light intensity was 100–400 lux at the level of the cage floor. All procedures were previously approved by the University of California at San Diego Institutional Animal Care and Use Committee.

Male and female hamsters, 3–6 mo of age and maintained on 15 h of light/d (15L : 9D, lights on 0600 PST), were paired and transferred to 13L : 11D (lights on 0700 PST). Male offspring were weaned between 18 and 20 d of age and remained in 13L : 11D until 21–28 d of age, when they were gonadectomized under sodium pentobarbital anesthesia (50 mg/kg). Beginning at that point, hamsters were exposed to a simulated natural photoperiod corresponding to 40°N latitude with phase of year set to April 15 when day length was 13 h. Photoperiod simulations were generated by EC71ST Electronic SunTracker timers (Paragon Electric, Two Rivers, Wis.). The dates indicated hereafter reflect dates simulated by day length and not actual calendar dates, which did not coincide with those simulated. The latitude chosen was selected because in past studies it maximized the percentage of hamsters exhibiting seasonal cycles. Photoperiods of higher latitudes close to the origin of this laboratory strain increase the percentage of photononrespon-

sive hamsters (Gorman and Zucker 1995, 1997a). The annual rhythms of cycling hamsters, however, appear to be comparable under photoperiods of different latitudes (Gorman and Zucker 1995).

Under the chosen simulated photoperiod, day lengths increased gradually to a maximum solstice value of 15 h of light on June 22 and decreased to the minimum solstice value of 9 h of light on December 22. Gonadectomized hamsters were randomly assigned to one of four groups (Fig. 1). One group remained on the simulated natural photoperiod only until the summer solstice (June 22), at which point day length was fixed at 15 h (group 15L : 9D;  $n = 15$ ). Other groups were exposed to gradually decreasing day lengths past the solstice until they reached 13 h on August 31 (group 13L : 11D;  $n = 17$ ), 11 h on October 12 (group 11L : 13D;  $n = 15$ ), or 9 h on December 22 (group 9L : 15D;  $n = 16$ ) and were maintained on these respective photoperiods thereafter. Body weight was determined ( $\pm 0.1$  g) weekly during the light phase (1100–1500 PST) from May 23 until the end of the experiment approximately 9 mo later. Three waves of animals, initiated over a 2-mo span, were tested with each wave containing roughly equal numbers from each experimental group.

## Locomotor Activity Rhythms and Photoresponsiveness

Between 10 and 11 mo of age, hamsters were singly housed, and cages were equipped with externally mounted passive infrared motion detectors (Coral Plus, Visonic, Tel Aviv). While on their respective photoperiods, daily locomotor activity patterns were monitored using VitalView Data Acquisition System (Mini-mitter, Bend, Oreg.). Activity counts were recorded in 10-min bins, and daily 24-h histograms were prepared by averaging activity counts during each bin. Beginning 2 h before the light/dark transition, the first bin in the 24 h histogram

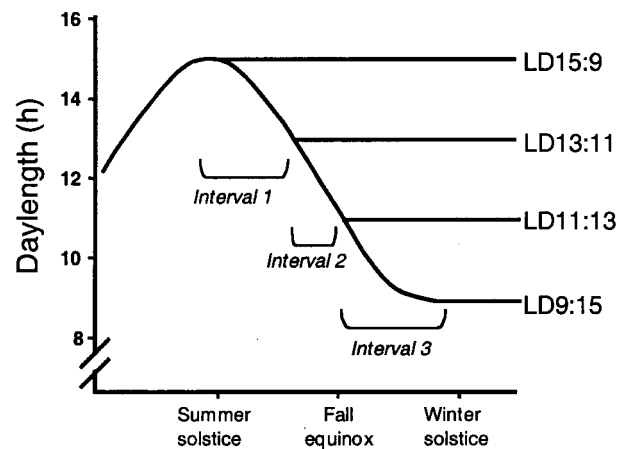


Figure 1. Schematic representation of experimental photoperiods and intervals of separate statistical analysis.

with a value that exceeded the daily mean by 50% and where equivalent activity was sustained at the same intensity for three of the next six intervals was defined as activity onset (Gorman et al. 1997).

Because photononresponsive hamsters do not entrain to short day lengths with activity onsets near dark onset as do photoresponsive hamsters (Puchalski and Lynch 1986), animals with activity onsets >2 h following the light : dark transition were classified as photoperiodic nonresponders. Entrainment patterns do not differentiate nonresponders and responders in longer day lengths (Gorman and Zucker 1997a), so all were classified as responders in 15L : 9D.

### Body Weight Analyses

For statistical analysis, body weight data were smoothed using 3-wk moving averages and transformed to percent of summer solstice values. For photoresponsive hamsters, repeated measures ANOVAs were conducted on these standardized body weight measures over several intervals during which groups were exposed to different photoperiodic conditions (Fig. 1). Interval 1 spanned from the week of the summer solstice (June 22) to August 29, when day lengths had decreased to approximately 13L : 11D. In interval 1, hamsters maintained in 15L : 9D were compared with all others to assess effects of day lengths decreasing from 15L : 9D to 13L : 11D. The influence of photoperiods between 13L : 11D and 11L : 13D was the focus of interval 2, which comprised the 5 wk between September 5 and October 10. In this interval, body weights of hamsters clamped on 13L : 11D were compared with those of hamsters experiencing further decreases to 11L : 13D. Interval 3 spanned from October 17 to the week of the winter solstice (December 26) and was the time frame for comparison of hamsters clamped in 11L : 13D with those experiencing further decreases. Because statistical power was reduced from an unexpectedly small sample of photoresponsive animals clamped on 11L : 13D and 9L : 15D and because these two groups never differed on any statistical test, a post hoc analysis was run to compare animals maintained in 13L : 11D with a composite group of hamsters exposed to shorter day lengths (groups 11L : 13D and 9L : 15D combined). Correspondingly, interval 2 + 3 ran from September 5 to the winter solstice (December 26). Body weight patterns following the winter solstice were not meaningfully predicted by earlier photoperiodic history. In the interests of brevity, these data are not presented.

Without exception, repeated measures ANOVAs revealed significant within-subjects main effects of time ( $P < 0.05$ ). As change in body weight over time is an expected result, these main effects are not further reported. Reported instead are main effects of treatment group, reflecting body weight differences averaged over the analysis interval, as well as treatment group  $\times$  time interactions that reflect group differences in the patterns of body weight change over the analysis interval.

For each photoresponsive hamster exposed to decreasing day lengths, the timing and degree of body weight suppression following the summer solstice was assessed on the basis of initial peak and subsequent trough body weights. For initial peak values, the smoothed data were examined for the latest point of monotonic increase in body weight. The subsequent trough value was defined as the lowest value following the peak. The difference between values, calculated as a percentage of peak weight, defined the percentage of body weight loss. This value and the simulated date of the body weight trough were analyzed with between-subjects ANOVA. For hamsters that exhibited monotonic increases in body weight, a value of zero was recorded for percentage of weight loss. The number of animals in each group exceeding a previously established threshold for photoperiod-induced weight loss (>12%; Gorman and Zucker 1995) was also assessed using nonparametric  $\chi^2$  analysis.

## Results

### Photoresponsiveness

Locomotor activity profiles varied markedly within treatment groups. Representative actograms of photoresponsive and non-responsive hamsters in 9L : 15D are illustrated in Figure 2. The incidence of nonresponsiveness did not differ among the three groups (13L : 11D, 11L : 13D, and 9L : 15D) in which it was assessed (Table 1;  $\chi^2(2) = 1.6$ ;  $P > 0.40$ ).

### Body Weights of Photoresponsive Hamsters

Between the summer solstice and August 29, photoresponsive hamsters exposed to day lengths that gradually decreased to 13L : 11D exhibited decelerated body weight trajectories compared to hamsters maintained on 15L : 9D following the solstice (Fig. 3A, 3B;  $F_{9,387} = 3.1$ ,  $P < 0.01$ ). Standardized body weights considered over the entire interval tended to be lower in hamsters exposed to decreasing day lengths ( $F_{1,387} = 3.8$ ,  $P < 0.06$ ). Hamsters exposed to further decreases in day length from 13L : 11D to 11L : 13D between September 5 and October 10 exhibited neither significantly different average body weights ( $F_{1,135} = 0.1$ ,  $P > 0.75$ ) nor different temporal patterns of body weight ( $F_{5,135} = 0.7$ ,  $P > 0.50$ ) compared to those left in 13L : 11D (Fig. 3A, 3C). Likewise, decreases after October 10 from 11L : 13D to 9L : 15D did not alter average body weight ( $F_{1,150} = 0.1$ ,  $P > 0.80$ ) or body weight trajectories ( $F_{10,150} = 1.3$ ,  $P > 0.20$ ) relative to that observed in hamsters maintained in 11L : 13D (Fig. 3D). Considered over a longer interval (interval 2 + 3), however, and more powerfully contrasted with all hamsters exposed to day lengths shorter than 13L : 11D, those maintained in 13L : 11D showed significantly different body weight patterns ( $F_{16,432} = 1.7$ ,  $P < 0.05$ ) compared to hamsters exposed to further photoperiod decreases (11L : 13D and 9L : 15D combined; Fig. 3C). The average body weight did not differ over this interval ( $F_{1,432} = 0.5$ ,  $P > 0.40$ ).

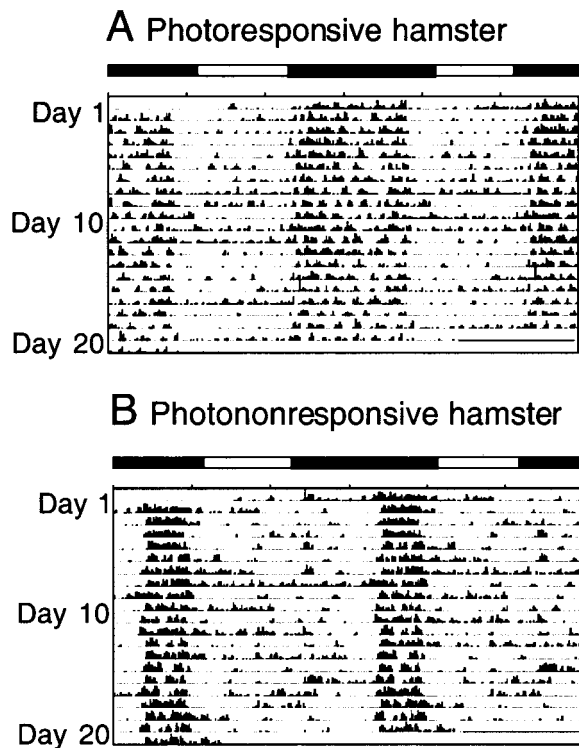


Figure 2. Representative double-plotted actograms of photoresponsive (A) and nonresponsive (B) hamsters maintained in 9L:15D. General locomotor activity was monitored by passive infrared motion detectors. Data are unclipped and are scaled from zero to maximum for that animal.

Peak weights differed significantly between groups (Table 1;  $F_{3,40} = 3.3$ ,  $P < 0.05$ ) with higher values in 15L:9D hamsters than in most groups experiencing decreasing day lengths ( $P < 0.05$ ). The latter also reached generally lower trough values (Table 1;  $F_{3,40} = 3.1$ ,  $P < 0.05$ ). The number of hamsters exceeding threshold body weight loss differed among treatment groups (Table 1;  $\chi^2(3) = 7.2$ ,  $P < 0.05$ ) with significantly higher incidences in 9L:15D and 11L:13D than in 15L:9D ( $P < 0.05$ ). Timing of the body weight trough also differed significantly between groups ( $F_{3,40} = 3.3$ ,  $P < 0.05$ ), with later values recorded in 11L:13D and 15L:9D than in 13L:11D (Table 1;  $P < 0.05$ ). The percentage of body weight lost, however, did not differ between groups (Table 1;  $F_{3,40} = 1.2$ ,  $P > 0.30$ ).

#### Body Weights of Nonresponsive versus Responsive Hamsters

Before the summer solstice, average body weight ( $F_{1,240} = 2.1$ ,  $P > 0.15$ ) and body weight trajectories ( $F_{4,240} = 1.2$ ,  $P > 0.30$ ) of nonresponders were not distinct from those of responsive hamsters. This result was obtained regardless of whether animals in 15L:9D were excluded or included as responders. Among animals exposed to decreasing day lengths in interval

1, nonresponders were heavier ( $F_{1,396} = 15.4$ ,  $P < 0.001$ ) and exhibited greater weight increases ( $F_{9,396} = 11.0$ ,  $P < 0.001$ ) compared to responders. Similarly, in interval 2, nonresponders maintained in 13L:11D weighed significantly more than did responders in 13L:11D ( $F_{1,70} = 11.4$ ,  $P < 0.01$ ), but the pattern of weight change did not differ between nonresponders and responders ( $F_{5,70} = 1.1$ ,  $P > 0.30$ ). Those responders exposed to further decreases during interval 2 weighed less ( $F_{1,140} = 10.3$ ,  $P < 0.01$ ) and lost more weight ( $F_{5,140} = 11.9$ ,  $P < 0.001$ ) than did nonresponders. In interval 3, responders of every photoperiod treatment group weighed less than nonresponders from the same group, but temporal patterning of body weight did not differ. Regardless of the interval of analysis, the type of decreasing day length (13L:11D, 11L:13D, or 9L:15D) had no effect on average body weights or patterns of weight change in nonresponders ( $P > 0.50$  for each; data not shown).

A post hoc analysis indicated that nonresponders considered as a whole differed from hamsters maintained in 15L:9D (Fig. 4): a trend towards accelerated weight increases appeared in interval 1 ( $F_{9,279} = 1.6$ ,  $P = 0.10$ ), and this difference reached statistical significance in interval 2 ( $F_{5,150} = 4.1$ ,  $P < 0.01$ ) and interval 3 ( $F_{10,300} = 2.1$ ,  $P < 0.05$ ). The main effect of photoresponsiveness was also significant over interval 3 ( $F_{1,300} = 5.0$ ,  $P < 0.05$ ).

#### Discussion

As previously reported for intact hamsters, gonadectomized male Siberian hamsters exposed to simulated natural photoperiods underwent gradual but substantial reductions in body weight as day lengths decreased in summer and fall (Steinlechner et al. 1983; Gorman and Zucker 1995). Two models might explain the incremental change in body weight over several months following the summer solstice. In one, body weight depends on day length in a graded fashion such that continued weight loss reflects the ongoing pattern of decreases in photoperiod, with animals responding proportionally or differentially to a range of day lengths. Alternatively, once triggered by a critical day length, an entire several-month program of body weight modulation is set in motion as long as the photoperiod remains below the critical value (cf. Steinlechner et al. 1983; Dark and Zucker 1985). The results establish that the gonadal hormone-independent component of the seasonal body weight response does not depend on a single critical value. Rather, this seasonal response depends on photoperiod in a graded fashion. At least two grades of inhibitory photoperiods were evident: day lengths decreasing from 15L:9D to 13L:11D suppressed body weights, but further decreases in day length elicited more complete weight loss trajectories and were necessary to sustain weight loss over a longer interval. As photoperiodic effects are generally mediated through patterns of pineal melatonin secretion, these results suggest that photoperiodic mechanisms are also differentially sensitive to mel-

Table 1: Incidence of photoresponsiveness and characteristics of weight loss trajectories of photoresponsive hamsters exposed to different regimens of decreasing day lengths

Group	15L : 9D	13L : 11D	11L : 13D	9L : 15D	<i>P</i>
Total <i>n</i> <sup>a</sup>	15	17	14	15	...
Responders <i>n</i>	15 <sup>b</sup>	12	8	9	NS
Nonresponders (%)	...	29.4	42.3	40.0	NS
Peak weight (% of summer solstice value)	114 ± 3.4	103 ± 1.7 <sup>c</sup>	108 ± 5.1	103 ± 1.4 <sup>c</sup>	<.05
Trough weight (% of summer solstice value)	103 ± 5.5	88 ± 2.6 <sup>c</sup>	89 ± 8.1	88 ± 4.5 <sup>c</sup>	<.05
Percentage decrease	10.3 ± 2.6	14.5 ± 2.6	18.5 ± 4.1	14.7 ± 3.7	NS
Trough date	Nov. 6 ± 11 <sup>d</sup>	Sept. 26 ± 11	Nov. 21 ± 13 <sup>d</sup>	Oct. 19 ± 19	<.05
Hamsters exceeding threshold weight loss	3/15	5/12	6/8	5/9	<.05

<sup>a</sup>Lower sample size than noted in text reflects the death of two hamsters before the end of the study.

<sup>b</sup>15L : 9D hamsters were not included in ANOVA because designation of all as responders was arbitrary.

<sup>c</sup>Group differs significantly ( $P < 0.05$ ) from 15L : 9D hamsters.

<sup>d</sup>Group differs from 13L : 11D hamsters.

atonin signals of continuously varying duration (Gorman and Zucker 1997b).

Under highly unecological conditions, discrimination of multiple short day lengths has been previously reported in several measures related to reproduction as evidenced by differential responses to various inhibitory photoperiods: male Siberian hamsters raised from birth in 10L : 14D versus 12L : 12D underwent pubertal development at different ages (Gorman and Zucker 1995). Adult male Syrian hamsters more rapidly regressed their gonads after abrupt transfer from 16L : 8D to 6L : 18D versus 11.5L : 12.5D (Vitaterna and Turek 1993; Powers et al. 1997). Timed infusions of melatonin for 12 h similarly induced more rapid gonadal inhibition than did 8.5-h infusions in Syrian hamsters housed in 16L : 8D (Powers et al. 1997). The existence of multiple critical day lengths has also been established for separate photoperiodic traits such as gonadal regression versus winter molt (Duncan et al. 1985). Several other traits including photononresponsiveness, triggering of the interval timer underlying refractoriness to gonadally inhibitory photoperiods, and breaking of photorefractoriness all appear to be triggered at different day lengths (Gorman and Zucker 1998). The current results complement and extend these earlier findings by demonstrating the discrimination of multiple inhibitory day lengths in a single trait monitored under more naturalistic conditions than commonly used.

The avoidance of abrupt photoperiodic shifts may be critical for this demonstration as the conclusion that body weight exhibits a graded dependence on photoperiod differs from that of Steinlechner et al. (1983), who transferred gonadally intact hamsters directly to 8L : 16D from natural photoperiods at different times in summer or fall. These transfers failed to accelerate loss of body weight relative to that of controls left under the natural photoperiods, leading the authors to suggest that

8L : 16D is equivalent to natural photoperiods once the critical day length is reached. Because hamsters remained in 8L : 16D for only 8 wk, and entrainment to abrupt photoperiod transfers may require several weeks or months (Gorman et al. 1997), the circadian systems of these animals may not have had sufficient time to fully entrain to the altered photoperiodic conditions, thereby precluding any type of differential response based on altered melatonin secretion patterns. Gonadally intact female Syrian hamsters also exhibit larger photoperiodic body weight responses following abrupt transfer from 16L : 8D to 8L : 16D versus 10L : 14D (Wade et al. 1986). The possibility that these responses reflect group differences in entrainment rates or changes in gonadal function, however, cannot be ruled out.

A program of body weight loss that unfolds gradually has also been suggested by studies of food restriction in voles and hamsters. Moderate food restriction (e.g., to approximately 60% of prior ad lib. consumption) accelerates the pattern of weight loss of hamsters exposed to naturally decreasing day lengths and of voles transferred abruptly from 14L : 10D to 10L : 14D. Rather than maintaining their accelerated weight loss, a return to ad lib. feeding induces both species to increase body weight to match that of unrestricted subjects, only to lose weight again under the ongoing inhibitory day lengths (Steinlechner et al. 1983; Dark and Zucker 1985). These results are consistent with a temporally unfolding change in weight "set point." Alternatively, both results may reflect separate mechanisms mediating food-related and photoperiod-induced changes in body weight (e.g., Mercer et al. 1995). Regardless, the current demonstration of a graded dependence does not imply that photoperiodically induced body weight losses are not manifest gradually over time. Rather, these results argue

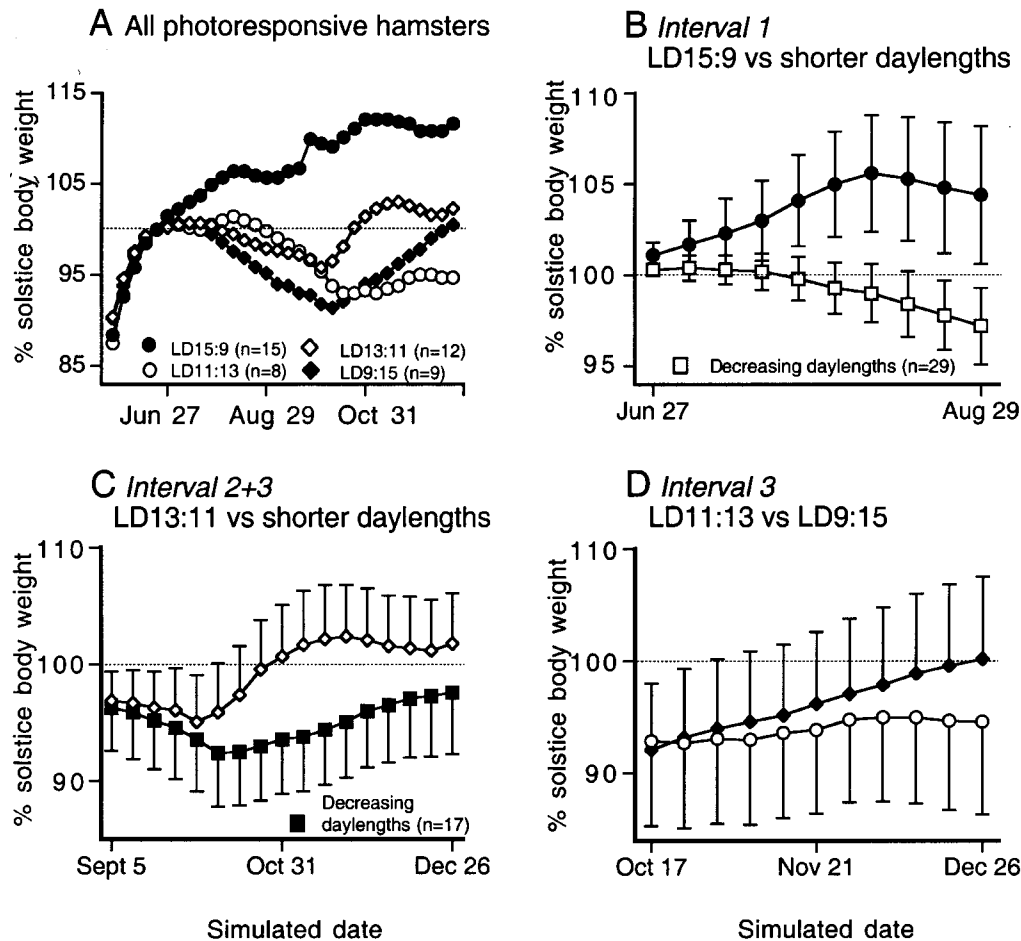


Figure 3. A, Mean body weights expressed as a percent of the summer solstice weight of photoresponsive hamsters assigned to various treatment groups. One hamster from 15L:9D died before December 26 and is omitted from analysis present in A. B–D, Mean  $\pm$  SEM body weights are contrasted between animals clamped on fixed photoperiods versus those experiencing further decreases in day length. B, 15L:9D ( $n = 15$ ) versus gradual decreases to 13L:11D ( $n = 29$ ); C, 13L:11D ( $n = 12$ ) versus further decreases ( $n = 17$ ); D, 11L:13D ( $n = 8$ ) versus decreases to 9L:15D ( $n = 9$ ). Dashed horizontal line corresponds to solstice body weight.

that a single photoperiodic trigger is insufficient to account for the entire program.

The assessment of effects of day lengths decreasing gradually from 15L:9D to 13L:11D exploited the high statistical power that resulted from combining treatment groups (13L:11D, 11L:13D, and 9L:15D) for the 10 wk following the solstice when these three groups still received identical treatment. By considering only these 10 wk, however, this analysis illustrates only relatively rapid responses to day lengths decreasing to 13L:11D and does not address those that might be apparent several weeks after the inducing photoperiodic stimulus. Delayed effects can be assessed only in hamsters clamped on 13L:11D because in other groups they are confounded with further reductions from 13L:11D. In 13L:11D, however, the minimum body weight was reached fully 6 wk after day lengths stopped decreasing. Comparison of hamsters in 15L:9D versus

13L:11D over a longer interval after the summer solstice led to the identical conclusion that 13L:11D induced a photoperiodic weight loss (analyses not shown). Other conclusions were also robust with respect to the time frames over which the groups were contrasted.

The selection of a simulated natural photoperiod of 40°N latitude did not prevent a high incidence of photoresponsiveness as it did in another study (Gorman and Zucker 1995). Although nonresponsiveness has a strong genetic component (Kliman and Lynch 1992), its expression depends in large part upon exposure to long day lengths, which prevent the circadian system from entraining to short photoperiods with an activity duration that tracks photoperiod (Freeman and Goldman 1997; Gorman and Zucker 1997a; Goldman et al. 2000). Hamsters from nonresponsive lineages never exposed to long day lengths are able to entrain in the typical short day fashion (Stanfield

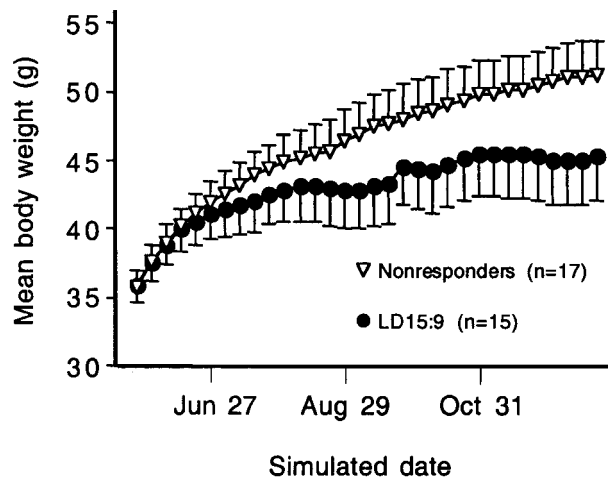


Figure 4. Mean  $\pm$  SEM body weights of photononresponsive hamsters from 13L : 11D, 11L : 13D, and 9L : 15D groups combined and all hamsters maintained in 15L : 9D following the summer solstice.

and Horton 1996). Selection experiments for short day non-responsiveness in adulthood, moreover, altered the threshold long photoperiod sufficient to induce this status (Goldman et al. 2000). Differences in the incidence of nonresponsiveness between experiments, therefore, might likewise reflect colony differences in the response to various long day lengths, due either to inadvertent selection or genetic drift.

As would be expected of hamsters not entraining to inhibitory short day lengths with expansion of activity duration and melatonin secretion (Puchalski and Lynch 1986), non-responders were heavier than responders from groups exposed to decreasing day lengths. Surprisingly, a post hoc analysis revealed that they were also heavier than hamsters maintained in 15L : 9D following the solstice—a treatment that precludes assessment of photoresponsiveness by entrainment to short photoperiods. In contrast to hamsters in 15L : 9D, which exhibited subthreshold and transient declines in body weight several weeks following the solstice, body weight increased absolutely monotonically in nonresponders (Fig. 4). If non-responsiveness is simply a failure to adopt a short-day entrainment pattern, this difference would not be explained as neither nonresponders nor animals in 15L : 9D would experience lengthening melatonin signals following the solstice. Prior studies demonstrate developmental differences between responders and nonresponders that are independent of their entrainment state (Stanfield and Horton 1996): in 12L : 12D, young from photoresponsive hamster lineages weighed less than those from nonresponsive lines, whereas nonresponder young, but not responder young, in 16L : 8D underwent transient but substantial reductions in gonad size at 10 wk of age. These results suggest that regulatory processes independent

of the circadian system also differentiate responsive and non-responsive hamsters under more naturalistic conditions.

Most investigations of mammalian photoperiodism have examined how extremely long and short day lengths corresponding to conditions in early summer and winter induce physiological and behavioral adaptations appropriate for those seasons. Research subjects are commonly maintained in these extreme day lengths for months at a time and subsequently transferred between conditions on a single day. While use of this strategy has allowed investigators to sketch the outlines of photoperiodic time measurement systems, it has simultaneously obscured a rich phenomenology that may only emerge under more naturalistic conditions. Specifically, the introduction of ecologically uninterpretable jumps in day length and the standard elimination of predictive information inherent in the pattern of gradually changing day lengths have led to incomplete characterization of seasonality mechanisms as they function in nature. The current paradigm, in contrast, reveals a graded response of the gonadal hormone-independent regulation of body weight by decreasing summer and fall photoperiods.

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