Intermediate-duration day lengths unmask reproductive responses to nonphotic environmental cues

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Paul MJ, Galang J, Schwartz WJ, Prendergast BJ. Intermediate-duration day lengths unmask reproductive responses to nonphotic environmental cues. Am J Physiol Regul Integr Comp Physiol 296: R1613–R1619, 2009. First published February 18, 2009; doi:10.1152/ajpregu.91047.2008.—Many animals time their breeding to the seasons, using the changing day length to forecast those months when environmental conditions favor reproductive success; in Siberian hamsters (Phodopus sungorus), long summer days stimulate, whereas short winter days inhibit, reproductive physiology and behavior. Nonphotic environmental cues are also thought to influence the timing of breeding, but typically their effects on reproduction are minor and more variable under categorically long and short photoperiods. We hypothesized that the influence of nonphotic cues might be more prominent during intermediate photoperiods (early spring and late summer), when day length is an unreliable predictor of year-to-year fluctuations in food availability. In hamsters housed in an intermediate photoperiod (13.5 h light/day), two nonphotic seasonal cues, mild food restriction and same-sex social housing, induced gonadal regression, amplified photoperiod history-dependent reproductive responses to decreasing day lengths, and prevented pubertal development indefinitely. These cues were entirely without effect in hamsters maintained under a long photoperiod (16 h light/day). Thus intermediate photoperiods reveal a heightened responsiveness of the reproductive axis to nonphotic cues. This photoperiod-dependent efficacy of nonphotic cues may explain how animals integrate long-term photic and short-term nonphotic cues in nature: intermediate day lengths open a seasonal window of increased reproductive responsiveness to nonphotic cues at a time when such cues may be of singular relevance, thereby allowing for precise synchronization of the onset and offset of the breeding season to local conditions.

Paradoxically, compared with photoperiod, the nonphotoperiodic environmental cues (e.g., food, humidity, and temperature) that actually determine energy availability appear to exert relatively minor and more variable effects on reproductive physiology (13, 17, 24, 32, 36). Hamsters housed in long days typical of early summer do not exhibit reproductive inhibition when challenged with decreases in ambient temperature or moderate food restriction (19, 31), whereas short photoperiods typical of winter induce gonadal collapse in adults and delay puberty in weanlings, even in the face of moderate laboratory temperatures and abundant food (15, 16). Control of the mammalian reproductive system by photoperiod, rather than by nonphotic environmental cues, has been presumed to be adaptive, because photoperiod allows anticipation of environmental conditions, rather than requiring animals to engage in costly reproductive responses to episodic, potentially transient events. In this view, nonphotic cues act to modulate reproductive responses to photoperiod by hastening or retarding photoperiod-induced transitions between spring and winter phenotypes (reviewed in Ref. 23). How this complex integration of long-term (day length) and short-term (nonphotic) environmental cues is accomplished by seasonally breeding animals is not understood (1).

Importantly, the role of nonphotic cues in the regulation of mammalian seasonal breeding has only been investigated using categorically long early summer (>14 h light/day) and short winter (<12 h light/day) photoperiods. Intermediate-duration photoperiods (12–14 h light/day), characteristic of the early spring and late summer, have not been well studied. Notably, review of available field data indicates that vernal and autumnal day lengths are times of considerable year-to-year variability in food availability in northern habitats. In Fig. 1, we have analyzed the interannual coefficients of variation in mean values of aboveground plant biomass over the course of the year, as measured at six agricultural research stations located between 47° and 52°N latitude. These data show that long photoperiods reliably signal a time of year when the interannual variance in food availability is relatively low, whereas intermediate photoperiods are associated with annual maxima in environmental unpredictability. Intermediate photoperiods alone are thus poor predictors of the onset and offset of favorable breeding conditions, raising the possibility that responsiveness to local nonphotic cues might be enhanced under such day lengths.

In this study we evaluated the ability of nonphotic cues (food availability and social interactions) to influence the hamster reproductive system under an intermediate photoperiod (13.5 h light/day) that in nature coincides with conditions of high interannual environmental variability, i.e., unpredictability. The results demonstrate that nonphotic
cues robustly control the reproductive axis under intermediate, but not long, day lengths, suggesting a novel mechanism for the integration of long-term (photoperiodic) and short-term (nonphotic) stimuli, whereby day length gates the access of nonphotic cues to the hypothalamic-pituitary-gonadal (HPG) axis.

MATERIALS AND METHODS

Agricultural data analysis. Aboveground plant biomass (AGPB) data were obtained from the Net Primary Productivity database at the Oak Ridge National Laboratory Distributed Active Archive Center for Biogeochemical Dynamics. Complete details of the data collection are available at http://daac.ornl.gov. In brief, plant matter was clipped manually to 0.1 cm of the ground at regular intervals from terrestrial grassland field sites. Clippings were oven-dried to constant weight, which was then recorded (as g/m²). Six field sites fit the inclusion criteria of (1) >3 yr of data, (2) 47°–52°N latitude, corresponding to the approximate latitudinal distribution of Siberian hamsters (29), and (3) reporting information on long-term management practices, including fertilization, burning, irrigation, and grazing. Data were binned at 10-day intervals for a maximum of 3 data points per month. These sites correspond to Modified Bailey ecoregions 252 and 332 (prairie) and 330, 331, and 333 (steppe).

Animals and housing conditions. Siberian hamsters (P. sungorus) were obtained from 13.5 h light/day (135L) or 16L breeding colonies maintained at the University of Chicago. Hamster pups were weaned at 21 days of age and housed one to four animals per cage with same-sex conspecifics from different litters in polypropylene cages (28 × 17 × 12 cm) with wood shavings (Harlan Sani-Chips, Harlan, Indianapolis, IN) in 13.5L (lights off at 12:30 central standard time; intermediate-duration day length) or 16L (lights off at 12:30 central standard time; long-duration day length). In all experiments, the time of dark onset remained the same for all photoperiods used. Ambient temperature of the experimental rooms was 20 ± 0.5°C, and relative humidity was maintained at 53 ± 2%. Food (Teklad Rodent Diet 8604; Harlan) and filtered tap water were provided ad libitum, except where otherwise indicated. All procedures conformed to the U.S. Department of Agriculture “Guidelines for the Care and Use of Laboratory Animals” and were approved by the Institutional Animal Care and Use Committee of the University of Chicago.

Reproductive and somatic measures. In males, testis size was estimated by measuring the length and width of the left testis (mm) through the abdominal skin while the animal was under light isoflurane anesthesia. This estimated testis volume (ETV) is correlated with testis weight, circulating testosterone, and spermatogenesis (9, 30). Testes were considered regressed if they were <503 mm³, a threshold value of 2 SD below the mean of ETV measures from 80 male hamsters housed in long days, spanning two recent experiments in our laboratory (8, 34). Epididymal white adipose tissue was dissected and weighed (g).

In females, vaginal patency was recorded in animals under light isoflurane anesthesia. The onset of vaginal patency can be used as a marker for reproductive development in female rodents (Ref. 11), and hamsters with photoperiod-induced nonpatent vaginas exhibit lower uterine and ovarian masses and fewer primordial follicles relative to those with patent vaginas (25).

Body masses (g) were recorded at the time of reproductive measurements.

Food restriction. Food restriction (FR) was accomplished by administering rodent chow to each hamster in a single daily ration.
Differences in categorical variables were compared between groups using least significant difference tests and ANOVA was used to measure variation among experimental groups. Hamsters had free access to food throughout the study.

**RESULTS**

Intermediate photoperiods unmask reproductive responses to mild FR. To determine whether intermediate day lengths render the reproductive axis more responsive to food-related cues, we examined the effects of mild FR on reproductive physiology in adult male hamsters (>4 mo) housed in long (16L) or intermediate (13.5L) photoperiods. FR did not affect testis size (Fig. 2A) in hamsters housed in 16L (P > 0.05 vs. AdLib, all comparisons). FR likewise failed to affect body mass, epididymal white adipose tissue, plasma cortisol, or plasma leptin levels in 16L hamsters (P > 0.05, all comparisons; Fig. 2, B–E), indicating that this FR did not deplete energy stores and did not constitute a metabolic stressor. In marked contrast, FR induced rapid and sustained decreases in testis size and body mass among hamsters housed in 13.5L (P < 0.05, all comparisons; Fig. 2, A and B). Neither cortisol, the dominant glucocorticoid in Siberian hamsters (28), nor leptin concentrations was affected by FR in either 13.5L- or 16L-housed hamsters (P > 0.05, all comparisons; Fig. 2, D and E).

Intermediate photoperiods unmask reproductive responses to social cues. To examine the generality of increased reproductive responsiveness under intermediate day lengths, we investigated the effects of a different nonphotic cue, same-sex social housing. Beginning on the day of weaning, male (n = 186) and female (n = 173) hamsters reared in an intermediate day length (13.5L) were housed at a population density of 1.86 animals/cage (male: n = 24 cages; female: n = 24 cages) or three to four animals per cage (male: n = 44 cages; female: n = 40 cages). Testis volume and vaginal patency were recorded in a cross-sectional manner, and hamsters were categorized as <3, 3–6, or 6+ mo of age, based on ages at the time of measurement (range = 2.1–7.4 mo of age). Among hamsters raised in social isolation in 13.5L from weaning (1 animal/cage), >90% of males and >85% of...
females exhibited adult reproductive status (ETV > 503 mm³ or patent vaginas; Fig. 3), indicating that 13.5L was interpreted as a reproductively stimulatory photoperiod by singly housed hamsters. In contrast, among group-housed hamsters, only 37% of males and 60% of females were in adult reproductive condition (P < 0.0001 vs. isolates, both comparisons; Fig. 3). The proportion of individuals with undeveloped testes or non-patent vaginas did not vary significantly among the three age cohorts (P > 0.05). Although our cross-sectional data prevent definitive conclusions regarding reproductive development, the most parsimonious explanation for these findings is that group housing blocked pubertal development. We further assessed the maintenance of social inhibition in a subset of male (n = 10) and female (n = 8) group-housed hamsters that were followed longitudinally for 10 wk, beginning at 3–6 mo of age. In these hamsters, 100% (10/10) of males and 75% (6/8) of females remained reproductively inhibited over the 10-wk interval [data not shown; adult gonadal status in this species is achieved by 40–60 days of age under a similar photoperiod of 14L (22)]; thus for most individuals, social inhibition of puberty is likely maintained indefinitely.

To confirm the inhibitory effects of the social environment on the reproductive axis and to determine whether this inhibition is dependent on intermediate day lengths, we separated group-housed adult (age = 5.0 ± 1.3 mo; 3–4 animals/cage) male and female hamsters into single housing conditions in the same 13.5L photoperiod (13.5L-Isolate), or we maintained them in group housing conditions and transferred the cages from 13.5L to 16L (16L-Group). Control hamsters remained group housed in 13.5L (13.5L-Group). Separation of 13.5L hamsters from group to single housing conditions (13.5L-Isolate) induced rapid testicular growth in males (P < 0.005; Fig. 4A), vaginal opening in females (P < 0.005; Fig. 4B), and parallel body mass gains in both sexes (P < 0.05; data not shown), even in the absence of any changes in photoperiod. Likewise, transfer of group-housed reproductively inhibited hamsters from 13.5L to 16L (16L-Group) triggered rapid and complete reproductive development (P < 0.05; Fig. 4, A and B). Evidently, social inhibition of the reproductive axis is obtained in intermediate, but not long, day lengths. Testis development induced by social isolation or 16L exposure was accompanied by increases in circulating testosterone concentrations (P < 0.05, both comparisons; Fig. 4C). Cortisol was significantly elevated in hamsters group housed in 13.5L relative to their socially isolated controls and relative to group-housed 16L hamsters (P < 0.05, both comparisons; Fig. 4D).

In addition, to determine whether same-sex social housing alone could induce gonadal regression in adult hamsters, socially isolated males with fully developed testes in 13.5L were returned to their original group housing conditions. Absent any changes in photoperiod, the reinstatement of group housing conditions induced substantial gonadal regression compared with hamsters that remained singly housed in 13.5L (ETV = 520 ± 76 and 820 ± 56 mm³, respectively; n = 11–14 animals/group).

Autumnal gonadal regression is accelerated by increased population density. In nature, animals track changes in photoperiod rather than absolute day length per se: increasing day lengths stimulate, and decreasing day lengths inhibit, reproductive physiology (10). If increased reproductive responsiveness under intermediate day lengths is used by hamsters in the wild, nonphotic cues must potentiate the response to changing day lengths of intermediate duration. We tested this hypothesis using a photoperiodic history design. Adult male and female hamsters that had been group housed (3–4 animals/cage) in 16L for 10 wk were transferred to 13.5L or remained in 16L. Also, on the day of transfer, hamsters from each photoperiod were either singly housed (1 animal/cage) or remained group housed. Transfer from 16L to 13.5L induced only modest and transient decreases in testis size if transferred hamsters were singly housed (P < 0.05 on wk 9 only; Fig. 5A). In contrast, if transferred hamsters remained group housed, they exhibited substantially greater decreases in testis size, which were evident within 3 wk of transfer and were sustained for >4 mo (P < 0.005, all comparisons; Fig. 5A). Similar data were obtained in females: 13.5L failed to cause vaginal closing in singly housed hamsters but was effective in more than half of the group-housed females (P < 0.05; Fig. 5B).

![Fig. 3. Social housing suppresses reproductive development in intermediate day lengths. A: estimated testis volume (ETV) of individual male Siberian hamsters (n = 169) gestated and raised in intermediate day lengths in cages containing 3–4 hamsters/cage (open symbols) or 1 hamster/cage (filled symbols). Hamsters were categorized as <3, 3–6, or 6+ mo of age, based on ages at the time of measurement (range = 2.1–7.4 mo of age). Dashed line indicates criterion (503 mm³ ETV) for testicular development. B and C: percentages of hamsters in each age group with developed testes (B) or patent vaginas (C). *P < 0.05; #P < 0.005 vs. hamsters housed 1/cage.](http://ajpregu.physiology.org/doi/10.1152/ajpregu.00532.2008)
DISCUSSION

The present data demonstrate robust effects of nonphotic environmental stimuli (food availability and population density) on the reproductive physiology of a photoperiodic mammal. Nonphotic cues induced substantial, albeit incomplete, gonadal regression in hamsters housed in a static intermediate day length, amplified reproductive involution in response to a late summer decrease in day length, and prevented pubertal development indefinitely in hamsters maintained in the intermediate day length. These nonphotic manipulations were entirely without effect on hamsters housed in a long day length (cf. Ref. 19; present findings). These manipulations can be considered, at most, to reflect only mild social and energetic perturbations; the FR used did not alter cortisol or leptin concentrations and did not deplete fat stores. Thus intermediate photoperiods unmask reproductive responses to nonphotic stimuli, revealing a photoperiod-regulated responsiveness of the reproductive axis not previously described in mammals.

Categorical reproductive responses to FR and social housing in intermediate photoperiods (absent photoperiod manipulations) permitted evaluation of potential neuroendocrine mechanisms by which photic and nonphotic cues induce changes in reproductive physiology. Cortisol mirrored reproductive responses to social manipulations in 13.5L, raising the possibility that increased cortisol might be sufficient to mediate effects of social cues on the HPG axis. However, FR sufficient to induce gonadal regression in 13.5L failed to elevate cortisol concentrations above those of ad libitum-fed hamsters, indicating that elevated cortisol is not necessary for FR to inhibit the reproductive system. Leptin and changes in body fat stores also did not appear to act as signals of decreased food availability. Thus, if changes in glucocorticoid production participate in reproductive responses to nonphotic cues, then they may do so in a cue-specific manner. It remains to be determined whether the increased cortisol in socially housed 13.5L hamsters is the cause, rather than a consequence, of reproductive inhibition. The model described should prove useful in specifying neuroendocrine mediators of photoperiod and nonphotic information onto the HPG axis (e.g., kisspeptin, gonadotropin-inhibitory hormone, and thyroid hormone and its catabolism enzymes).

The nature of the social cue(s) responsible for reproductive inhibition in group-housed hamsters is not presently known. Suppression of the reproductive axis has been reported in other mammalian species housed in larger group sizes than those
used in the present experiment (for reviews, see Ref. 4), even in the face of ad libitum food (e.g., Ref. 3). Aggressive interactions, social rank, and pheromonal communication each can mediate the inhibitory effects of such “crowding” on reproduction, in part through activation of the hypothalamic-pituitary-adrenal axis (4, 18). In the present experiments, no obvious wounds were observed in socially housed hamsters, suggesting that severe fighting was not prominent; other agonistic behaviors, however, cannot be ruled out. Furthermore, the proportions of reproductively inhibited and developed hamsters within each cage did not match the typical dominant-subordinate ratios expected in populations of this size in other species (e.g., 1 dominant, reproductively competent and 2–3 subordinate, reproductively inhibited individuals; Ref. 26); in fact, often all animals in a cage were either inhibited or developed.

A substantial literature documents nonphotic regulation of the reproductive cycle of nonmammalian vertebrates, the magnitude of which can vary by species, habitat, and sex (2, 12, 35). The present findings implicate photoperiod as an additional factor that modifies the efficacy of nonphotic cues and as a potential source of experimental variability. In reproductively photoperiodic canaries (Serinus canarius) and white-crowned sparrows (Zonotrichia leucophrys), the stimulatory effects of conspecific bird song on nest building and ovarian growth, respectively, are substantially potentiated under intermediate, as opposed to absolutely long or short, photoperiods (14, 20). The constraints imposed by photoperiod on responsiveness to nonphotic cues may be widespread among presumed-obligate photoperiodic vertebrates. Historically, the reproductive impact of nonphotic cues may have been underestimated due to the common practice of testing animals in categorically long or short photoperiods.

Perspectives and Significance

In nature, the long and short photoperiods of summer and winter, respectively, are sufficient to predict environmental conditions, and photoperiod alone engages seasonally appropriate reproductive responses at these times of year. Yet, in early spring and late summer when day lengths are intermediate, the onset of favorable energetic conditions for breeding varies from year to year. Intermediate day lengths may open a window of increased reproductive responsiveness to nonphotic exteroceptive cues. This would permit sampling of multiple environmental factors at a time when they may be of singular relevance, thereby allowing precise timing of the onset and termination of the breeding season to match local conditions.

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REFERENCES


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