

Triggering of Neuroendocrine Refractoriness to Short-Day Patterns of Melatonin in Siberian Hamsters

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Abstract

Short day lengths induce gonadal regression within 6 weeks in Siberian hamsters, but this inhibitory effect eventually wanes and reproductive competence is regained after 4–6 months in short days. These experiments were undertaken to determine whether continuous exposure to short days for several months is necessary to induce neuroendocrine refractoriness, or if a few weeks of short days are sufficient to trigger processes that culminate in refractoriness several months later. Adult male Siberian hamsters transferred from their natal long-day photoperiod of 15L (15 h light/day) to a short-day photoperiod of 10L were pinealectomized (PINx) after 0, 6, 12, 21 or 40 weeks of exposure to 10L. Intact hamsters kept in 10L manifested gonadal regression by week 6 and completed gonadal recrudescence by week 40, at which time they too were PINx. Beginning at week 40 all hamsters were infused s.c. with melatonin for 10 h/day for 6 consecutive weeks. This treatment induces gonadal regression in photosensitive hamsters. At the end of melatonin treatment, maximal gonadal regression was recorded for hamsters PINx at week 0 and those PINx after 40 weeks in long days. Hamsters PINx after 40 week of short day treatment were completely unresponsive to melatonin whereas those PINx after 6 and 12 weeks had intermediate responses. The percentage of hamsters whose reproductive apparatus was refractory to melatonin at week 40 increased with increasing duration of exposure to short days prior to PINx. Refractoriness was induced by relatively few weeks of short days in some hamsters, whereas others required much more extensive exposure. Induction of refractoriness is triggered by a fraction of the short days that hamsters experience in nature but may not be manifested until many weeks later when it coincides with gonadal recrudescence. In nature all hamsters are exposed to short days for at least 25 weeks, ensuring refractoriness in most individuals.

In mammals, seasonal reproduction is often timed so that parturition and lactation coincide with annual peaks in food availability; this reproductive strategy is generally conceded to increase fitness in a seasonally fluctuating environment (1). Seasonal changes in day length can function as proximate cues for phasing annual patterns of mammalian breeding (2, 3). Information provided by day length is transduced into the neuroendocrine axis via daily and seasonal changes in melatonin secretion by the pineal gland. Melatonin is secreted almost exclusively at night, and variations in day length entrain an endogenous rhythm of secretion in which elevated melatonin production is directly proportional to the duration

of the dark phase (4). Nightly melatonin secretion is therefore much longer under short days than long days (typically 10–13 h/night vs 4–6 h/night, respectively (5, 6)), and provides a seasonally changing endocrine representation of ambient day length.

In several rodent species, long ($\geq 14L$) day lengths stimulate and short ($\leq 12L$) day lengths inhibit reproductive activity (7). Likewise, exogenous treatment with short- and long-duration melatonin stimulates and inhibits reproductive physiology, respectively (8). Regression of the testes, decreases in circulating androgens, and increases in negative-feedback sensitivity of gonadotropin secretion characterize

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the response to short days observed in males of most long-day breeding rodent species (9). After prolonged (>16 weeks) exposure to inhibitory photoperiods, hamsters, in common with voles and mice (10, 11), spontaneously revert to a reproductively competent (i.e. long-day) phenotype (12, 13). This recovery is termed spontaneous recrudescence, as it occurs despite continued maintenance in photoperiods which were previously non-stimulatory (14). Unless first exposed to 10–20 weeks of long days, hamsters do not exhibit a second bout of gonadal regression in short photoperiods (or in response to a short-day melatonin treatment regime) after spontaneous recrudescence (15, 16). The reproductive system is considered refractory to short days when a short-day photoperiod previously sufficient to sustain regression of the gonads no longer does so (17); reproductive refractoriness to short-day photoperiods appears equivalent to refractoriness to long-duration melatonin signals (15).

In the wild, long-day breeders undergo testicular regression in response to the short days of late summer and autumn; gonadal and reproductive quiescence is maintained through autumn and much of winter, but resumption of reproductive function begins in mid-to-late winter, while prevailing photoperiods are still too short to directly stimulate reproductive development. Thus, refractoriness and spontaneous recrudescence are of considerable functional significance, responsible for initiating the recovery of reproductive function that permits early spring breeding (18, 19). Nonetheless, formal descriptions relating refractoriness to spontaneous recrudescence, as well as mechanistic insights into the biological basis of these phenomena remain cursory (20). For example, gonadal recrudescence occurs after continued maintenance in a short photoperiod, but it remains unknown whether the neuroendocrine axis requires continuous exposure to short days throughout this interval, or whether only a few weeks of short days trigger an interval timer that provokes gonadal recrudescence many weeks later. Indeed, it is not even clear that spontaneous recrudescence is caused by the onset of the refractory state, or whether the central state of refractoriness follows spontaneous recrudescence—common products of exposure to short days, but causally unrelated events. Though unlikely, such a relationship between spontaneous recrudescence and refractoriness is not impossible, and if demonstrated would force a redefinition of the concept of refractoriness. There are some indicators that in Syrian hamsters refractoriness may be induced in advance of spontaneous recrudescence (17); but the confounding introduction of long-day treatments to provoke premature recrudescence in these experiments and an earlier one by Zucker and Morin (21) do not support unequivocal conclusions.

In the present study, we sought to determine the minimum number of weeks of exposure to short days sufficient to induce refractoriness to melatonin in male Siberian hamsters. By removing the hamsters' pineal glands after different durations of exposure to a short-day photoperiod, we manipulated the number of short-day melatonin signals received by individuals. After short-day treatments were complete, pinealectomized (PINx) hamsters were challenged for 6 consecutive weeks with daily long-duration melatonin infusions, a potent short-day stimulus (8, 22). If the number of short-day melatonin signals necessary to cause refractoriness is equivalent

to that sufficient to elicit spontaneous recrudescence, then refractoriness would only be manifested by hamsters that remained pineal-intact through the onset of recrudescence. If, on the other hand, the number of short day melatonin signals sufficient to induce refractoriness is considerably lower than that necessary to cause spontaneous recrudescence, then hamsters that had been PINx weeks in advance of spontaneous recrudescence would nevertheless be refractory to melatonin several weeks later.

Materials and methods

Animals

Adult male Siberian hamsters (*Phodopus sungorus*) were housed in polypropylene cages from birth at an ambient temperature of $21 \pm 2^\circ\text{C}$ in a room illuminated for 15 h/day with fluorescent light (15L, lights on 03.00 h, Pacific Standard Time). Food (mouse chow no. 5015, Purina Mills, St Louis, MO, USA) and tap water were provided *ad lib*. Hamsters were group-housed (3–5/cage) until they underwent surgery (see below), after which they were individually housed.

Photoc and surgical manipulations

At 9–12 weeks of age (=week 0), hamsters were transferred to a 10L photoperiod (lights on 08.00). Hamsters were PINx at week 0 (immediately prior to transfer to 10L; Px0; n=17), week 6 (Px6; n=16), week 12 (Px12; n=15), week 21 (Px21; n=10) or week 40 (Px40; n=15). Only hamsters that manifested testicular regression (Estimated Testis Volume <400, indicative of photoresponsiveness to 10L) by week 6 were included in the latter four groups. Hamsters remained in 10L until week 40, at which time they were transferred to 15L, fitted with s.c. polyethylene catheters, and infused with melatonin for 10 h/day for 6 consecutive weeks (see below). An additional long-day control group of hamsters (LDc; n=9) that remained in 15L from weeks 0–40, was PINx at week 40, and received 10 h melatonin infusions from weeks 40–46, identical to those given to 10L-treated hamsters. All hamsters were killed on week 46.

Somatic and reproductive measures

Hamsters were weighed (± 0.1 g) at predetermined intervals beginning at week 0, and body weight (BW) was determined weekly beginning at week 6. Testis size was estimated in hamsters lightly anaesthetized under methoxyflurane vapors (Metofane; Pitman-Moore, St Louis, MO, USA) by externally measuring (± 0.1 mm) the length and width of the left testis. The product of testis width squared times testis length provided a measure of estimated testis volume (ETV) that is highly correlated with testis weight in this species (16). ETV was determined for each hamster at weeks 0 and 6, and at 3-week intervals thereafter until week 36. ETV was also determined on weeks 40 and 46. Paired testis weights (PTW) were determined (± 0.1 mg) at autopsy on week 46.

Pinealectomy

Pinealectomies were performed under ketamine cocktail anaesthesia according to the procedure of Bartness and Goldman (23). Briefly, anaesthetized hamsters were secured in a stereotaxic apparatus. A hole (≤ 2 mm diameter) was drilled in the skull circumscribing bregma, and the pineal gland was removed with a pair of microdissecting forceps. Hamsters were given the analgesics acetaminophen and codeine in their drinking water (1% solution in water) for 3 days postoperatively.

Cannulation/infusion

At week 40, hamsters were implanted s.c. with a polyethylene catheter as described elsewhere (24) and transferred to a 15L photoperiod. Catheters were attached to a swivel mounted inside the cage lid, which permitted unrestricted locomotion and burrowing. Infusions were delivered by a variable-flow pump (flow rate=0.017 ml/h; Razel Scientific Instruments, Stamford, CT, USA) loaded with 1 ml syringes and controlled by a digital timer (Intermatic, Spring Grove, IL, USA). From weeks 40 to 46, all hamsters were provided with single daily 10-h melatonin (Sigma) infusions (100 ng/infusion, in saline vehicle) from 17.30 to 03.30 h.

All surgical and non-surgical animal care procedures received prior approval

from the Animal Care and Use Committee of the University of California at Berkeley.

Determination of refractoriness to melatonin

At week 40, hamsters were provided with long-duration melatonin infusions for 6 consecutive weeks to assess photoresponsiveness. This treatment produces gonadal regression in photoresponsive hamsters (8, 22), defined as a decrease of 48% in ETV between weeks 40–46 (16), as validated previously (20, 25). Hamsters that failed to meet or exceed this criterion were classified as unresponsive, or refractory, to melatonin.

Data analysis and statistics

At a limited number of time points, BW or ETV data were unavailable for individual hamsters. In cases of missing data the arithmetic mean of the value from the preceding and following weeks was interpolated and used in statistical comparisons at that time point. Interpolated values for ETV ($n=16$) and BW ($n=176$) constituted 1.4% and 5.6%, respectively, of the total number of data points for each measure.

Differences in ETV and BW were analysed separately by repeated measures ANOVA. Where significant F -ratios were obtained, pairwise comparisons between treatment groups on a given week were conducted using Fisher's protected least significant difference test (PLSD). Within-group changes in BW and ETV over successive weeks were analysed by paired t -tests. Week 46 PTW and BW values were analysed separately by between-subjects ANOVA, and pairwise differences were assessed with Fisher's PLSD. The incidence of non-responsiveness to melatonin (indicative of refractoriness to melatonin) was assessed with either χ^2 or Fisher's exact test (Statview 4.1, Abacus Concepts, Berkeley, CA, USA), where appropriate. Differences were considered significant if $P < 0.05$. To protect against Type I error, the level of statistical significance was set at $P < 0.01$ for repeated pairwise comparisons of weekly BW values.

Results

Weeks 0–40

Gonadal responses

One hundred and eleven hamsters that exhibited testicular regression (ETV < 400) within 6 weeks after transfer to 10L were assigned to treatment groups. Additional groups of hamsters were PINx at the time of transfer to 10L, or were kept in long days. Testis sizes differed among treatment groups during the 40 weeks in the 10L photoperiod ($F=19.8$; d.f. = 48,816; $P < 0.001$; Fig. 1A). Px0 hamsters did not undergo gonadal regression in 10L. Px6 hamsters exhibited substantial gonadal regression by week 6 ($t=7.84$; d.f. = 15; $P < 0.001$ vs week 0 value) which was sustained for several weeks, but by week 15 gonadal recrudescence was evident and ETV was elevated relative to week 6 values ($t=4.30$; d.f. = 15; $P < 0.001$). At week 21 ETV for this group was indistinguishable from that of Px0 hamsters ($P > 0.10$), indicating that recrudescence had been completed. Px12 hamsters had regressed gonads at the time of pinealectomy, and regression was sustained for ≤ 6 weeks after pinealectomy; significant increases in ETV were detectable by week 18 ($t=2.97$; d.f. = 14; $P=0.01$), and recrudescence was complete by week 24 ($P > 0.20$ vs Px0). Px21 hamsters sustained gonadal involution until subjected to pinealectomy at week 21, with significant gonadal recrudescence detectable at the next measurement 3 weeks later (week 24: $t=2.62$; d.f. = 9; $P < 0.05$); recrudescence was complete by week 36 ($P > 0.05$ vs Px0). Gonads of pineal-intact hamsters (Px40) remained photoregressed until approximately week 18, the first week at which ETV significantly exceeded the previous week's value ($t=2.59$; d.f. = 14; $P < 0.05$). Although gonadal growth in Px40

hamsters had reached a plateau by week 33 ($t=0.88$; d.f. = 14; $P=0.40$ vs week 30), ETV remained slightly lower than that of Px0 hamsters through week 40 ($P < 0.05$).

Body weight responses

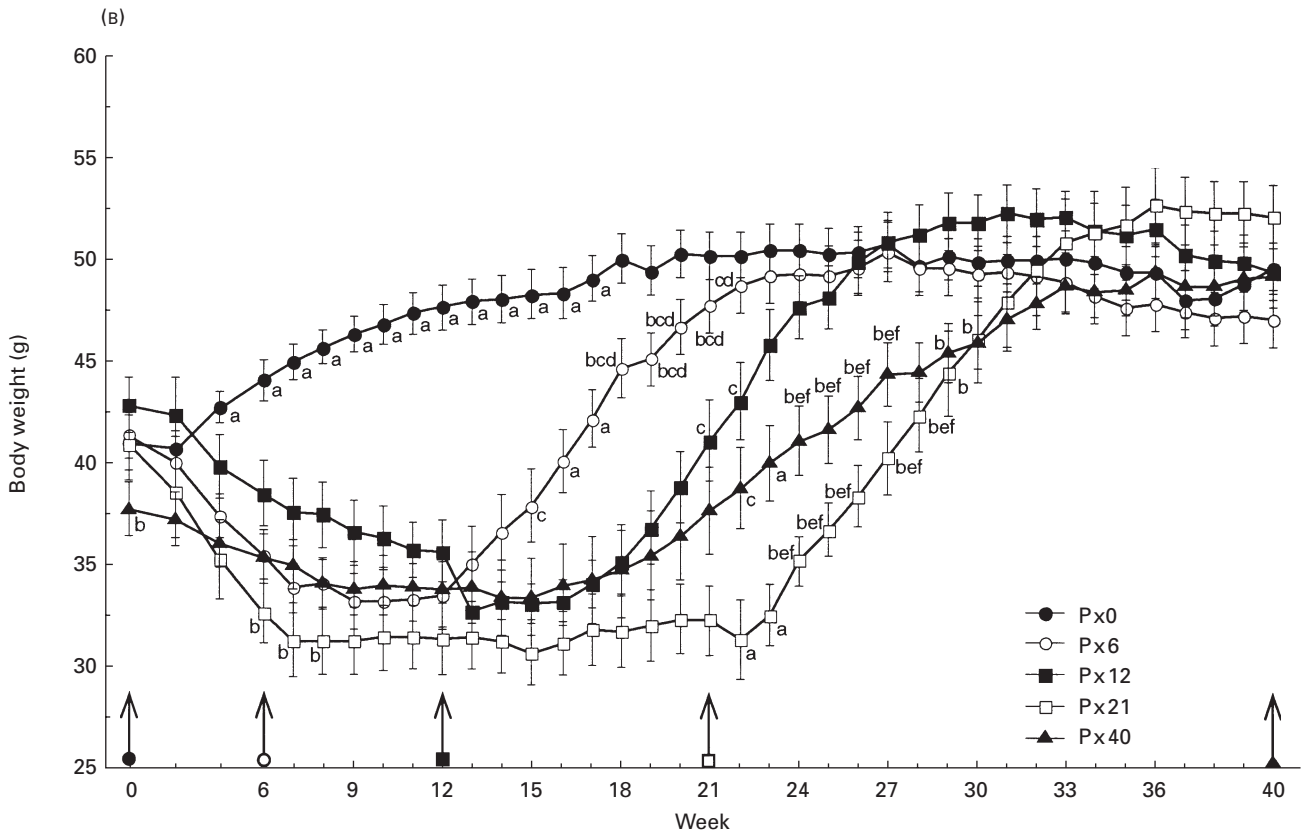
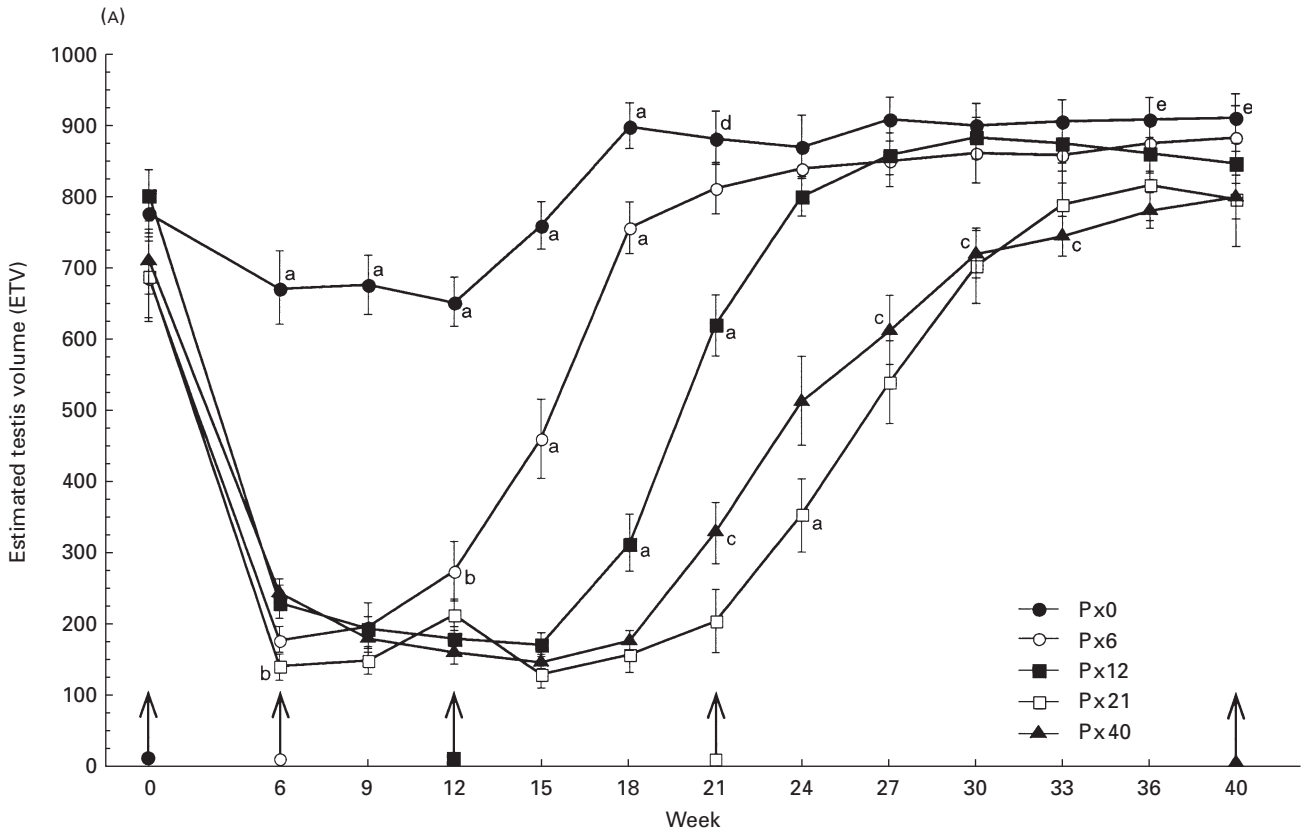
The pattern of changes in BW in response to short days and pinealectomy was similar to that obtained for ETV measures. Pineal-intact hamsters underwent significant decreases in BW within 4 weeks after transfer to 10L. The pattern of change in BW differed significantly among treatment groups over weeks 0–40 ($F=15.3$; d.f. = 148,2516; $P < 0.001$; Fig. 1B). The BW of Px6 hamsters was still decreasing at the time of pinealectomy, and reached a nadir value on week 9. A significant increase in BW relative to the minimum value was first detectable on week 14 ($t=3.26$; d.f. = 15; $P < 0.01$), and BW of Px6 hamsters was indistinguishable from that of Px0 hamsters (hamsters that did not exhibit photoperiodic BW decreases in 10L) by week 19, indicating recovery was complete at this time. Px12 hamsters achieved nadir BW values on week 13, and a significant increase in BW by week 19 ($t=3.17$; d.f. = 14; $P < 0.01$). Recrudescence of BW in Px12 hamsters was complete by week 24 ($P > 0.10$ vs Px0). Px21 hamsters exhibited reduced BW from approximately week 7 through week 22, and gained weight beginning shortly after PINx. BW was significantly elevated by week 24 ($t=2.29$; d.f. = 9; $P < 0.05$ vs week 21), and indistinguishable from that of Px0 hamsters by week 30 ($P > 0.05$). Px40 hamsters reached a BW minimum on week 15. Increases in BW from this value were significant by week 16 ($t=3.32$; d.f. = 14; $P < 0.005$), and recovery was complete by week 31 ($P > 0.10$ vs Px0).

Weeks 40–46

Gonadal responses

All hamsters had completed gonadal recrudescence (ETV > 400) by week 40 (ETV mean \pm SEM = 851.6 ± 16.1). Significant differences in paired testis weights were detected after 6 weeks of daily 10 h melatonin infusions ($F=10.4$; d.f. = 5,76; $P < 0.001$). As expected, Px40 hamsters did not undergo testicular regression in response to melatonin treatment (Fig. 2A): testis weights among these hamsters exceeded 600 mg after 6 weeks of infusions. In contrast, Px0 and long-day control hamsters sustained substantial decreases in testicular weights to ≈ 200 mg. Among hamsters PINx after 6, 12 or 21 weeks in 10L, PTW was directly proportional to the number of weeks of short-day exposure they had received prior to pinealectomy (Fig. 2A).

Slight differences in ETV were detected between treatment groups after recrudescence was complete (on week 40): ETV of Px0 hamsters exceeded that of Px21 and Px40 hamsters ($P < 0.05$, each comparison). To take into account individual differences in testis sizes before melatonin treatment, we computed individual changes in ETV (Δ ETV) between weeks 40 and 46. The pattern obtained across groups was similar to that for PTW values ($F=26.2$; d.f. = 5,76; $P < 0.001$; Fig. 2B). Hamsters that were pineal-intact through 40 weeks of 10L exposure (Px40) did not exhibit testicular regression in response to melatonin infusions, whereas hamsters PINx before exposure to 10L (Px0) exhibited significant reductions in ETV, as did long-day hamsters never exposed to 10L



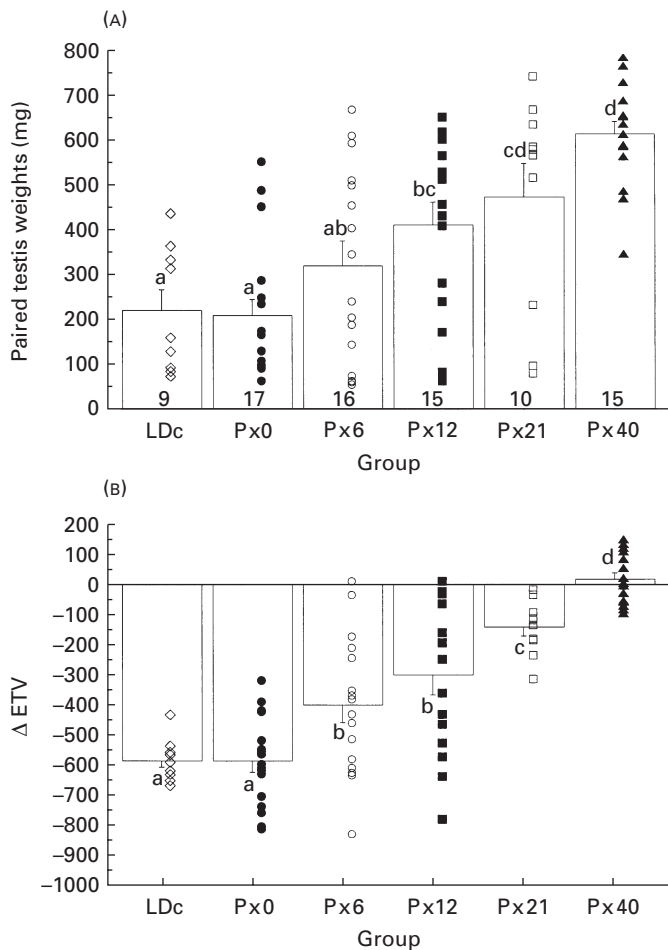


FIG. 2. (A) Mean (\pm SEM) paired testis weights (PTW) at week 46 of hamsters infused with melatonin for 10 h/day from weeks 40–46 and (B) mean (\pm SEM) change in estimated testis volumes (Δ ETV) between weeks 40 and 46. Abbreviations as in Fig. 1. Sample sizes appear within bars. Values with similar letters do not differ significantly ($P > 0.05$).

(LDc; $P > 0.90$ vs Px0). Px6 and Px12 hamsters manifested modest decreases in ETV that were significantly smaller than those observed in the Px0 group, but over twice the magnitude of the decrease observed in Px21 hamsters ($P < 0.05$, all comparisons). As a group, Px21 hamsters exhibited a slight decrease in ETV which was significant relative to the increase in ETV observed in Px40 hamsters ($P < 0.05$).

Body weight responses

The pattern of BW responses was similar to that observed for gonadal measures; individual body weights at week 46 varied considerably ($F = 3.75$; d.f. = 5,76; $P < 0.005$; not illustrated). Px40 and Px21 hamsters were heaviest; Px0 and LDc hamsters weighed significantly less than Px40 hamsters after

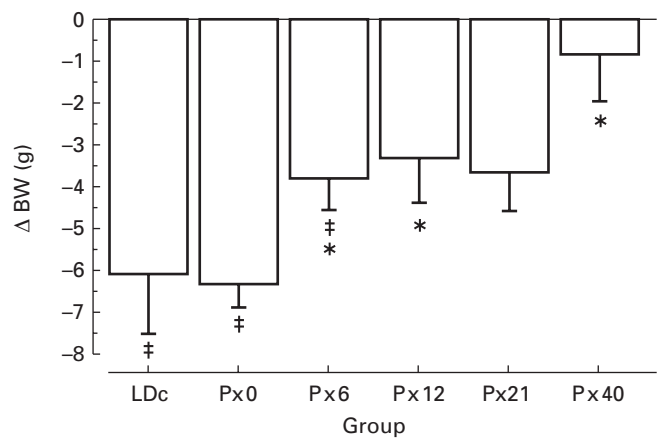


FIG. 3. Mean (\pm SEM) change in body weight (Δ BW) between weeks 40 and 46 of hamsters infused with melatonin for 10 h/day. Abbreviations as in Fig. 1. Symbols indicate group means that differ significantly (* $P < 0.05$ vs Px0, ‡ $P < 0.05$ vs Px40).

infusions were completed ($P < 0.05$, all comparisons). Over the course of melatonin treatments, the pattern of changes in BW differed among treatment groups ($F = 4.61$; d.f. = 5,76; $P < 0.001$; Fig. 3): LDc and Px0 animals exhibited the largest decreases in BW. Relative to week 40 values, all groups weighed less at week 46 (paired t -tests, $P < 0.05$, all comparisons) except Px40 ($t = 0.75$; d.f. = 14; $P > 0.40$).

Refractoriness to melatonin

Measures of central tendencies of reproductive responsiveness to long-duration melatonin infusions may obscure individual responses to treatment. We therefore determined the proportion of individuals in each treatment group that failed to undergo testicular regression in response to the long-duration melatonin treatment, a defining characteristic of refractoriness in this species (13). Idiographic analyses take into account individual variance in testis size at week 40.

All 15 Px40 hamsters failed to exhibit gonadal regression and were classified as refractory to melatonin, as were 90% (9/10) of Px21 hamsters (Fig. 4); these proportions did not differ significantly ($\chi^2 = 1.6$; d.f. = 1; $P > 0.20$). In contrast, 0/9 LDc hamsters and 3/17 (18%) Px0 hamsters were refractory to melatonin treatments; these proportions likewise did not differ from each other ($\chi^2 = 1.8$; d.f. = 1; $P > 0.10$). Half (8/16) of the Px6 hamsters failed to undergo gonadal regression in response to melatonin infusions, a significantly higher incidence of refractoriness than observed among both Px0 ($\chi^2 = 3.9$; d.f. = 1; $P < 0.05$) and Px40 ($\chi^2 = 10.1$; d.f. = 1; $P < 0.005$) hamsters, but indistinguishable from the incidence of refractoriness in Px12 hamsters (9/15; 60%; $\chi^2 = 0.3$; d.f. = 1; $P > 0.50$). Ninety per cent (9/10) of Px21 hamsters were refractory to long-duration melatonin infusions, a frequency

FIG. 1. (A) Mean (\pm SEM) estimated testis volumes (ETV) and (B) body weights (BW) of hamsters transferred from long to short day lengths at week 0 and pinealectomized at week 0 (Px0), week 6 (Px6), week 12 (Px12), week 21 (Px21), or week 40 (Px40), as indicated by arrows on the abscissa. Letters indicate group means that differ significantly from those of other groups; in Panel A (a= $P < 0.05$ vs all other groups, b= $P < 0.05$ vs Px40, c= $P < 0.05$ vs all other groups except Px21, d= $P < 0.05$ vs all other groups except Px6, e= $P < 0.05$ vs Px40) and Panel B (a= $P < 0.01$ vs all other groups, b= $P < 0.01$ vs Px12, c= $P < 0.01$ vs Px21, d= $P < 0.01$ vs Px40, e= $P < 0.01$ vs Px0, f= $P < 0.01$ vs Px6).

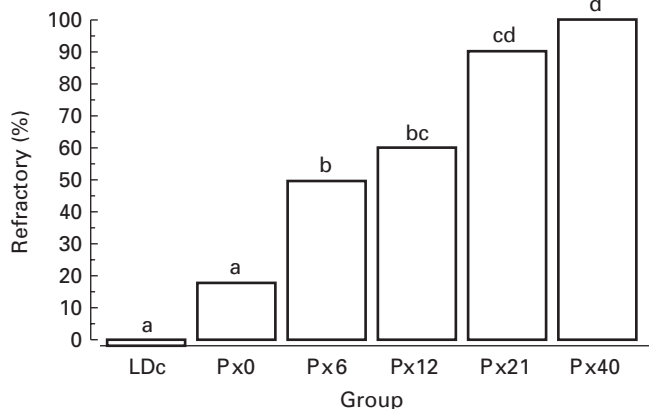


FIG. 4. Percentages of hamsters with testes refractory to melatonin infusions. Abbreviations as in Fig. 1. Values with similar letters do not differ significantly ($P > 0.05$).

comparable to that observed in Px40 hamsters, and significantly greater than that of Px6 hamsters ($\chi^2 = 4.4$; d.f. = 1; $P < 0.05$), but not different that of Px12 hamsters ($\chi^2 = 2.7$; d.f. = 1; $P = 0.10$).

Discussion

Siberian hamsters PINx after ≥ 6 weeks in a short-day photoperiod failed to exhibit testicular regression or decreases in body weight when challenged with long-duration melatonin infusions many weeks later. This suggests that as few as 6 weeks of short days are sufficient to render hamsters refractory to short-day patterns of melatonin many weeks later. In contrast, hamsters PINx before transfer to short days (Px0), like those never exposed to short days (LDc), manifested substantial gonadal regression when challenged with 10 h melatonin infusions. Chronic exposure to short days elicits gonadal regression, followed by spontaneous gonadal recrudescence and subsequent refractoriness to short days, the latter phenomenon inferred from a failure to manifest additional bouts of regression under the same short photoperiod (13, 14). The pineal gland is necessary for short days to induce refractoriness, as refractoriness was not observed in PINx hamsters exposed to short days for 40 weeks, consistent with the theory that it is the short-day pattern of pineal melatonin secretion that renders the brain refractory to melatonin (17).

The incidence of refractoriness to melatonin increased in a non-linear manner with increasing duration of pineal-intact exposure to short days. As few as 6 weeks of exposure to short days was sufficient to render half of the hamsters refractory to melatonin 34 weeks later, yet an additional 6 weeks of short-photoperiod treatment did not significantly increase the incidence of refractoriness. Exposure to short days for 21 weeks before PINx, however, rendered 90% of hamsters refractory, and this rate did not differ from the proportion that was refractory after 40 weeks in short days. It is unlikely that group differences in the melatonin-free intervals (following PINx) contributed in any meaningful way to the observed incidence of refractoriness, as the absence of

melatonin neither alleviates (26, 27) nor induces (present data) refractoriness in hamsters. We infer that the first few weeks of exposure to short days in the late summer trigger processes that eventually render a substantial proportion of individuals refractory to short days later in life, and that there are marked individual differences in the photic requirements for induction of refractoriness; exposure to short-day patterns of melatonin secretion throughout the winter appears to ensure refractoriness in all individuals.

This is the first unconfounded demonstration that processes leading to refractoriness can be triggered by short-day exposure minimally sufficient to cause gonadal collapse. Previous attempts (17, 21, 28) allowed Syrian hamsters to undergo testicular regression and, after variable intervals in short photoperiods, premature recrudescence was induced with long-day treatments. Long-day exposure is known to undo, or break, refractoriness in hamsters (29), and it is therefore likely that treatment of photoregressed hamsters with long photoperiods in previous studies functioned to break refractoriness in an unsystematic manner. Thus the actual incidence of refractoriness induced by the short-day treatments was probably under-reported in these earlier studies. For example, when followed by ≤ 11 weeks of long days, 6 weeks of short days were ineffective in inducing refractoriness in Syrian hamsters (17, 21), yet here we report 8/16 hamsters were refractory after 6 weeks of short-day melatonin. Though this may reflect a species difference, we propose that a proportion of hamsters in previous studies was rendered refractory by short-day exposure minimally sufficient to cause gonadal collapse, only to have refractoriness broken by subsequent long-day treatments. It seems unlikely, though, that gonadal collapse is causal in the induction of refractoriness, as hamsters with access to running wheels (which retards gonadal regression in short photoperiods) still exhibited refractoriness (17), and hamsters in which testicular regression is masked (via NMDA injections) exhibit spontaneous recrudescence and become refractory to short days on an appropriate time course (30). Nonetheless, the mere cessation of fertility was associated with a profound incidence of refractoriness. The neuroendocrine signal requirements for rendering hamsters refractory to melatonin are achieved well in advance (≥ 3 months) of the neuroendocrine events that initiate recrudescence.

Early induction of refractoriness was not associated with heightened responsiveness to short days. BW and ETV minima before pinealectomy did not predict whether an individual would later be refractory to long-duration melatonin infusions. BW after recrudescence induced by pinealectomy was higher at a few time points in refractory Px6 animals, but the opposite relation obtained in Px12 hamsters, and no such relation was observed between refractory and responsive hamsters from other groups at similar time intervals (data not illustrated). Neither individual changes in testis sizes in response to pinealectomy, nor testis sizes before melatonin treatments, was predictive of refractoriness. Across groups, only the number of weeks of exposure to short days prior to pinealectomy was positively associated with refractoriness to melatonin.

The extent to which variability in the induction of refractoriness by short days was influenced by the abrupt transition from a long to a short photoperiod remains unknown. On one hand, expansion in the duration of nightly melatonin

secretion probably occurs more rapidly in hamsters transferred abruptly from 15L to 10L than in hamsters exposed to a natural progression of decreasing day lengths (31). The resulting greater number of long-duration melatonin signals would presumably be more effective in inducing refractoriness. If, however, information contained in the rate of change in day length and melatonin secretion contributes to the induction of refractoriness, then exposure to a naturally decreasing photoperiod could be more effective than a static short day length in triggering refractoriness. Although the rate and direction of change in day length and melatonin secretion affect reproductive status (25, 32), the extent to which such photic information influences the induction of refractoriness is presently unspecified.

Many individuals that had not yet exhibited spontaneous gonadal recrudescence prior to pinealectomy (in Px6, Px12, and Px21 groups) were nevertheless rendered refractory to melatonin by such short-day exposure. Indeed, Px6 hamsters were PINx \approx 12 weeks before undergoing gonadal recrudescence (as estimated by the onset of recrudescence in Px40 hamsters), yet fully 50% had already received a sufficient number of melatonin signals to incur refractoriness to melatonin. Several Px21 hamsters had initiated spontaneous gonadal recrudescence before undergoing the pinealectomy procedure (as had all Px40 hamsters), the overwhelming majority of which was refractory to the melatonin treatments at week 40; however, one Px21 hamster (no. 1795) had initiated testicular growth well in advance of week 21, yet was responsive to melatonin at week 40 (Fig. 5). That most hamsters which had initiated spontaneous recrudescence were refractory to melatonin constitutes circumstantial evidence for a causal role of refractoriness to melatonin in spontaneous recrudescence, but does not force such a conclusion. The observation that one individual which had initiated spontaneous recrudescence was not refractory to melatonin does not compel rejection of the hypothesis that refractoriness to melatonin causes spontaneous recrudescence, but accounting for this individual, in terms other than as an existence-proof that spontaneous recrudescence is not caused by refractoriness to melatonin, is problematic. In Siberian and Syrian hamsters, refractoriness to a particular short photoperiod is not necessarily accompanied by refractoriness to shorter photoperiods (16, 33). If this hamster regarded the 10 h melatonin infusions as equivalent to a photoperiod substantially shorter than 10L, then refractoriness (to 10L only) might have caused spontaneous recrudescence while preserving responsiveness to much shorter photoperiodic stimuli. This *post-hoc* account is weakened, however, by evidence that the onset of spontaneous recrudescence in 10L is not postponed by transfer to 8L; photoperiods equal to or less than 10L are regarded as equivalents by photoperiodic time measurement mechanisms responsible for inducing refractoriness (16). Instead, these data suggest a role for short-day melatonin signals experienced after the onset of spontaneous recrudescence in the eventual induction of refractoriness. Melatonin signals received during the regression phase (first 6 weeks of short-day exposure) and the quiescent phase (weeks 6–20) both contribute, perhaps additively, to induction of refractoriness.

Although refractoriness may be the cause of spontaneous recrudescence, caution is recommended in equating spontan-

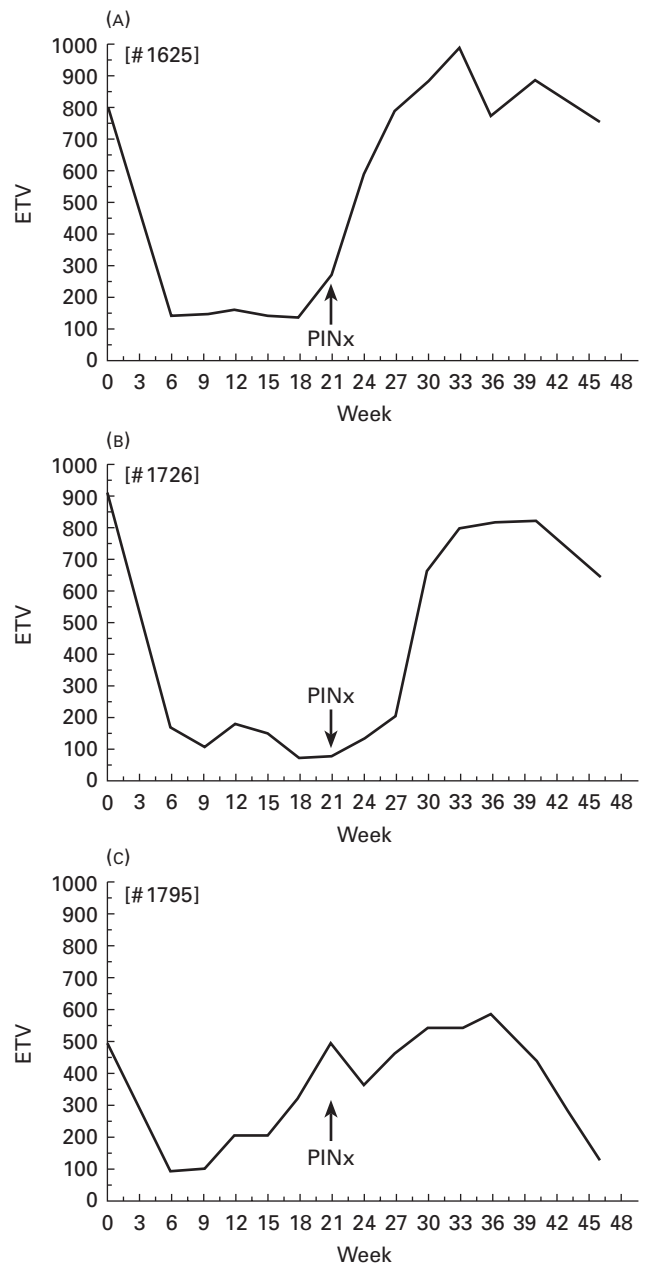


FIG. 5. Estimated testis volume (ETV) of hamsters transferred to 10L at week 0 and pinealectomized (PINx) at week 21. Hamsters were housed in 10L until week 40, then transferred to 15L and infused daily with 10 h melatonin for 6 consecutive weeks. (A) This hamster had initiated spontaneous gonadal recrudescence prior to PINx and was refractory to melatonin infusions. (B) This hamster had not initiated gonadal recrudescence prior to PINx but was refractory to melatonin infusions. (C) This hamster had initiated spontaneous gonadal recrudescence prior to PINx but was not refractory to melatonin infusions.

eous recrudescence with refractoriness. Clearly, many individuals had already met the photic requirements for eventual refractoriness by week 6, but had not yet initiated spontaneous recrudescence. The interval between photic triggering of the refractory state and gonadal growth is not attributable to intrinsic time lag in the activation of gonadotropin secretion, which occurs within a few days in hamsters transferred

from short to long days (34–36). Rather, spontaneous recrudescence may represent a programmed consequence of short-day exposure first manifested long after the central state of refractoriness has been triggered. Thus gonadal recrudescence (and general attainment of the long-day phenotype) does not definitively establish when refractoriness was induced. To determine when a trait (e.g. testis size) is refractory (after some interval of short-day exposure) would require exposing hamsters to a short photoperiod for some interval (e.g. 6 weeks), then rapidly provoking gonadal recrudescence with stimuli not associated with the breaking of refractoriness (e.g. peripheral administration of follicle stimulating hormone (FSH)), and then challenging such hamsters with a short photoperiod after discontinuation of FSH treatment. When the central state of refractoriness is achieved, relative to when refractoriness is manifest, remains unknown.

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