Low Ambient Temperature Accelerates Short-Day Responses in Siberian Hamsters by Altering Responsiveness to Melatonin

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Abstract Exposure to low ambient temperatures (T_a) accelerates appearance of the winter phenotype in Siberian hamsters transferred from long to short day lengths. Because melatonin transduces the effects of day length on the neuroendocrine axis, the authors assessed whether low T_a promotes the transition to winterlike traits by accelerating the onset of increased nocturnal melatonin secretion or by enhancing responsiveness to melatonin in short day lengths. Male hamsters were transferred from 16L (16 h light/day) to 8L (8 h light/day) photoperiods and held at 5 °C or 22 °C. Locomotor activity was recorded continuously, and body mass, testis size, and pelage color were determined biweekly for 8 weeks. The duration of nocturnal locomotion (α), a reliable indicator of the duration of nocturnal melatonin secretion, lengthened significantly earlier in hamsters exposed to a T_a of 5 °C than 22 °C. Cold exposure increased the proportion of hamsters that were photoresponsive: gonadal regression in short days increased from 44% at 22 °C to 81% at 5 °C ($p < 0.05$); low T_a did not, however, accelerate testicular regression in animals that were photoresponsive. Nonphotoresponsive animals at 5 °C temporarily had longer αs during the first 4 weeks in short days and significant decreases in body mass and testicular size that were reversed during the ensuing weeks when α decreased. In a 2nd experiment, pinealectomized male hamsters infused for 10 h/day with melatonin for 2 weeks had significantly lower body and testes masses when maintained at 5 °C but not 22 °C. Low-ambient temperature appears to accelerate the appearance of the winter phenotype primarily by increasing target tissue responsiveness to melatonin and to a lesser extent by augmenting the rate at which the duration of nocturnal melatonin secretion increases in short day lengths.

Key words photoperiodism, seasonality, melatonin

Siberian hamsters (*Phodopus sungorus*) breed during the spring and summer and are reproductively quiescent in winter (Weiner, 1987). The transition from summer to winter day lengths is completed over the course of several months and is accompanied by gonadal regression, a decrease in body mass, molt to winter pelage, and initiation of daily torpor (Ruf et al., 1993). The duration of nocturnal pineal melatonin secretion is the principal endocrine representation of day length (Bartness et al., 1993; Illnerova et al., 1984).

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Melatonin is secreted during the scotophase in proportion to the duration of darkness; long (10 h) and short (5 h) daily infusions of melatonin in pinealectomized hamsters mimic effects of short-day (S-D) and long-day (L-D) photoperiods, respectively, on the neuroendocrine axis (Carter and Goldman, 1983). The duration of the nightly melatonin signal is considered to be the critical parameter that determines seasonal responses (Bartness et al., 1993).

Ambient temperature (T_a) modifies the effects of day length on seasonal traits. Exposure of male Siberian hamsters to low T_a (5 °C), in conjunction with short day lengths, accelerates decreases in testis size and body mass (Ruf et al., 1993; Stieglitz et al., 1994). T_a also modifies photoperiodic responses in deer mice (*Peromyscus maniculatus*) and Syrian hamsters (*Mesocricetus auratus*); low T_a (5 °C) accelerates and high T_a (30-32 °C) delays short-day responses (Desjardins and Lopez, 1980; Li et al., 1987; Millar and Gyug, 1981; Pevet et al., 1989; Ruf et al., 1997). Whether T_a affects photoperiodic responses by modifying secretion of melatonin or responsiveness of target tissues to this hormone is not known. Although cold exposure accelerates the appearance of short-day responses, hamsters held at 5 °C in long days maintain the typical long-day phenotype (Desjardins and Lopez, 1980; Tsutsui et al., 1988).

There is evidence that T_a affects melatonin production and release. Acute cold exposure to 5 °C prevents light inactivation of pineal N-acetyl transferase, an enzyme critical for melatonin synthesis, resulting in high melatonin concentrations in Siberian hamsters exposed to dim light in the cold; in sharp contrast, melatonin concentrations decrease precipitously in hamsters exposed to dim light at 22 °C (Steiglitz et al., 1991). The appearance of higher amplitude nocturnal melatonin signals in short days is accelerated in Siberian hamsters kept at low (5 °C) compared with moderate (22 °C) temperatures (Stieglitz et al., 1994). In contrast, high T_a (30 °C) decreases the maximal nocturnal pineal melatonin concentrations in European hamsters (*Cricetus cricetus*) (Vivien-Roels et al., 1997).

We sought to determine whether the accelerated appearance of the winter phenotype at low T_a reflects more rapid attainment of the short-day pattern of melatonin secretion. Exposure to low T_a may accelerate the transition from short-duration nocturnal melatonin secretion characteristic of long day lengths to the longer duration melatonin signals of short days. The phase and duration of nightly melatonin secretion is controlled by the circadian system (Illnerova, 1991). The expansion of the melatonin signal in the days after transfer from long to short photoperiods involves re-entrainment of the circadian system, of which the suprachiasmatic nucleus (SCN) is a major component (Illnerova, 1991). The duration of nocturnal locomotor activity (α), characterized by discrete onset and offset of activity each night, is driven by the same circadian oscillators as control melatonin secretion. Because phase and duration of nightly locomotor activity and melatonin secretion are highly correlated (Elliott and Tamarkin, 1994), α of the locomotor rhythm serves as a noninvasive marker of rate of expansion of melatonin secretion during the transition from long to short day lengths. Accordingly, we assessed rates of expansion of α in hamsters transferred from 16- to 8-h day lengths and held at either 22 or 5 °C and inferred that a more rapid expansion of α indicated a more rapid expansion of the nocturnal melatonin signal.

Alternatively, exposure to low T_as may accelerate appearance of the winter phenotype by increasing responsiveness of target tissues to melatonin, without necessarily affecting characteristics of the melatonin signal itself; that is, the same melatonin signal may exert greater or lesser effects on testes and body masses depending on the ambient temperature. To evaluate this hypothesis, pinealectomized hamsters were provided with subcutaneous infusions of physiological concentrations of melatonin for 10 h/day during maintenance in moderate (22 °C) and cold (5 °C) environments.

MATERIALS AND METHODS

Experiment 1: Ambient Temperature and Entrainment of Circadian Rhythms

Animals

Male Siberian hamsters (N = 36) were housed in polypropylene cages at 22 ± 1 °C in an L-D photoperiod (16 h light/day; lights on at 0200 h, Pacific Standard Time) from birth. Food (mouse chow No. 5015, Purina Mills, St. Louis, MO, USA) and tap water were available ad libitum. Hamsters were housed in groups of 2 to 5 individuals until 4 months of age (week –2), at which time their body mass and estimated testes volume (ETV) were measured (cf. Gorman and Zucker, 1995); thereafter, animals were housed individually. At week 0, hamsters were assigned to 1 of 2 groups, matched for body mass and age, and transferred to an

S-D photoperiod (8L:16D, lights on at 0800 h). One group (n = 18) was maintained at 5 ± 1 °C and the 2nd (n = 18) at 22 ± 1 °C. Previous work showed that preadaptation to low T_a (5 °C) did not significantly affect appearance of short-day responses in animals held at 5 °C in short days (Ruf et al., 1993).

Somatic, Gonadal, and Activity Measurements

Body mass, ETV, and pelage color were measured biweekly, starting 2 weeks prior to the temperature and photoperiod change (week –2). Body mass (± 0.1 g) was measured on weeks –2, 0, 2, 4, 6, and 8, and ETV on weeks –2, 2, 4, 6, and 8. To measure ETV, each animal was lightly anesthetized with methoxyflurane vapors (Metofane, Pitman Moore, St. Louis, MO, USA) and the length and width of the left testis measured externally. The product of testis width squared times length provides an ETV that is highly correlated with testis mass in this species (Gorman and Zucker, 1995). Paired testes mass was measured at week 8 and was highly correlated to ETV measured at week 8 on the previous day (R^2 = 0.96). Pelage was scored on a scale of 1 (gray, summer coat) to 4 (white, winter coat) (Duncan and Goldman, 1984).

From week 0 until the end of the study, locomotor activity was monitored with passive infrared motion detectors mounted on plastic hoods set on top of wire-cage lids. Movement in the cage across 3 or more of 27 zones activated a closed-contact relay to Dataquest III software (Data Sciences, St. Paul, MN, USA). From these data, nightly activity duration for each animal was determined using the Tau software package (Minimitter, Sunriver, OR, USA). Activity counts for 10-min intervals were averaged over 1 week to generate a 24-h histogram. Daily activity onset was defined as the time that activity levels first rose above the daily mean and remained above this value for ≥ 1 h. Activity offset was defined as the time that activity fell below the daily mean and stayed below this value for ≥ 1 h. Duration of the active phase (α) was calculated as the interval between activity onset and activity offset (Gorman and Zucker, 1997). Where more detailed measurements were required, α was measured in 2-day bins.

Hamsters were classified as photoresponsive (R) or nonphotoresponsive (NR) on the basis of testis size at the end of the experiment. R hamsters had regressed testes (< 160 mg and ETV < 330 mm^3). Three hamsters were excluded from the final analysis owing to arrhythmic or free-running activity patterns. Two animals at 5 °C were NR and behaviorally arrhythmic (Gorman and Zucker, 1997). One animal held in 22 °C had a circadian rhythm that free-ran with a period of 21 h throughout the 8 weeks in S-D. The 3 animals with abnormally short or disrupted circadian locomotor rhythms all failed to undergo gonadal regression.

Statistics

Differences in alpha, testis size, body mass, and mean daily activity as a function of T_a and responder status were assessed with two-factor repeated measures ANOVAs with T_a and responder status as the factors (Statview 5.0, SAS Institute, Cary, NC, USA). To determine whether T_a affected R and NR animals, one-factor repeated measures ANOVAs were run, separately for R and NR groups, with T_a as the factor. Where a significant effect or interaction was seen in the overall ANOVA, Fisher's PLSD (protected least significant difference) was used for pairwise, post-hoc comparisons at each time point. Chi-square test was used to compare the incidence of nonresponsiveness between T_as and the proportion of animals initiating molt at each T_a, with R and NR compared separately. Observed differences were considered significant if $p < 0.05$. Data are presented as mean ± SEM.

Experiment 2: Temperature and Responsiveness to Melatonin

Twenty-two adult male hamsters, age 3 months, from the long-day (16L:8D) colony, were pinealectomized under ketamine anesthesia (60 mg/kg i.p.) and allowed to recover for 2 weeks. Animals were given the analgesics acetaminophen and codeine in their drinking water (1% solution) for 3 days postoperatively. Hamsters were then implanted with subcutaneous catheters under methoxyflorane anesthesia and infused daily for 2 weeks with melatonin (100 ng/day over 10 h) or saline vehicle (0.9% NaCl over 10 h/day) as described in Prendergast et al. (1996). Hamsters were divided into 3 age- and body mass–matched groups for the infusions: 1 group (n = 8) was infused with melatonin daily while housed at T_a of 22 °C; the 2nd and 3rd groups were kept in a cold chamber (5 °C) and infused with melatonin (n = 9) or saline (n = 5). Daily infusions were initiated at onset of darkness and ended 2 h after light onset. Body mass and ETV were measured at the time the catheters were implanted, to verify that all animals were in full reproductive condition. All measurements were obtained

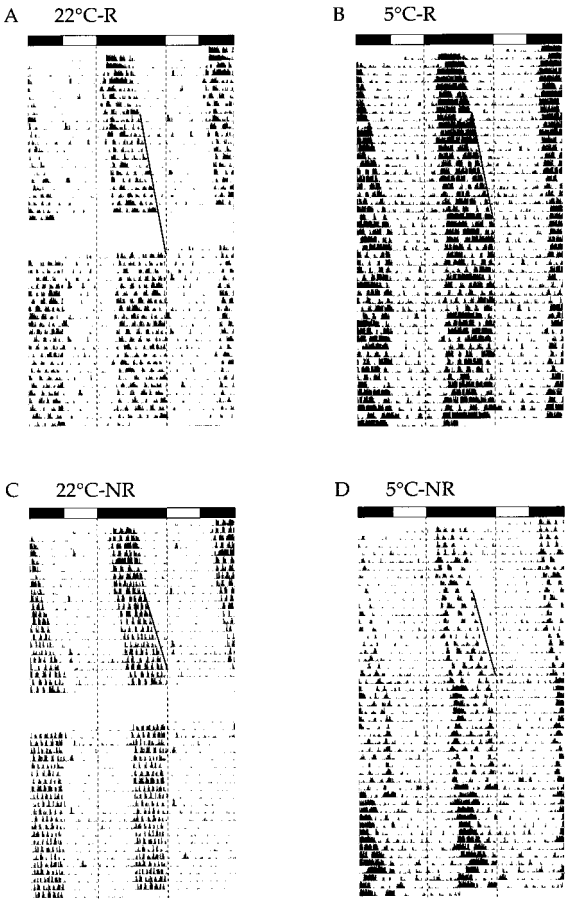

Figure 1. Double plot of locomotor activity during 8 weeks in short days. Each horizontal line represents 48 h with successive days plotted one beneath the other. The black bar at the top of each panel denotes the daily 16-h dark phase. Representative recordings are from photoresponsive hamsters held at 22 °C (A) or 5 °C (B) and nonresponsive hamsters held at 22 °C (C) or 5 °C (D). R = photoresponsive, NR = nonphotoresponsive. Diagonal lines join times of activity offset during re-entrainment to short days.

during the light phase. Treatments were terminated after 2 weeks of daily infusions, at which time body and paired testes masses were determined. Differences in body and testes masses were assessed by ANOVA. Where significant *F*-ratios were obtained ($p < 0.05$), pairwise comparisons between treatment groups were conducted using Fisher's PLSD test. Experimental procedures were approved by the Animal Care and Use Committee, University of California, Berkeley.

RESULTS

Experiment 1: T_a and Entrainment

Groups were equated with respect to baseline body mass (mean of 44.6 ± 0.6 g) and testis size (mean of 931 ± 27 mm^3) prior to differential treatment. The pattern of expansion of locomotor activity rhythm, α (Fig. 1 and see below), and testes size at the termination of the experiment permitted unequivocal categorization of hamsters as photoresponsive or nonphotoresponsive. Significantly more hamsters were photoresponsive in short days when maintained at 5 °C (81%) than 22 °C (44%) (Fig. 2).

During exposure to short days, the two-factor repeated measures ANOVAs indicated a significant effect of responder/nonresponder status on α and testis size, whereas ambient temperature (T_a) had a significant effect on body mass, testis size, and mean activity levels, but not α. There was a significant interaction effect with T_a for each of these 4 measures. Effects of T_a are described separately for photoresponsive and nonphotoresponsive hamsters.

Effect of T_a in Photoresponsive Hamsters

α lengthened in all R hamsters from 7.3 ± 0.2 h during the first 2 days in short days to 10.9 ± 0.4 h on

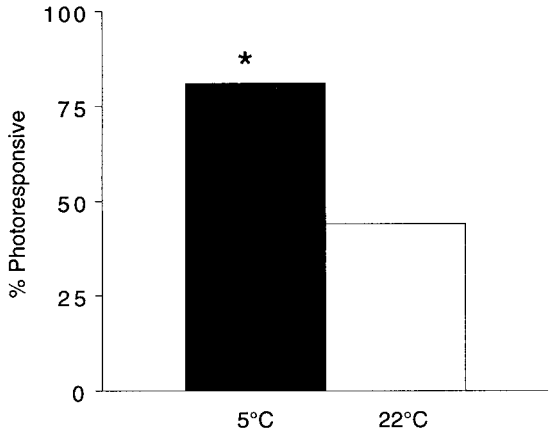

Figure 2. Percentage of hamsters that were photoresponsive, based on maintained testicular regression when transferred to short days at 5 °C and 22 °C. *N* = 16/group. **p* < 0.05, chi-square test.

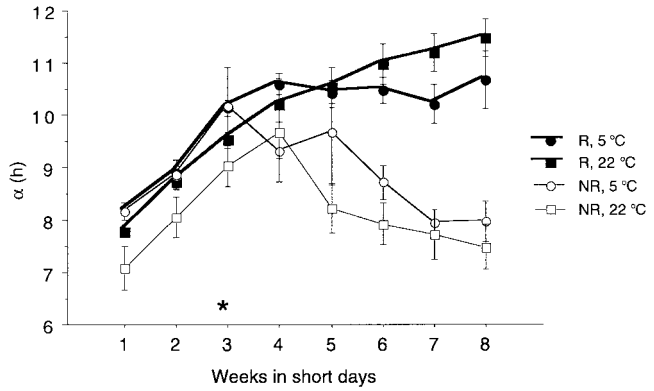

Figure 3. Mean (± SEM) activity duration (α) of hamsters held in short day lengths at 5 and 22 °C. *N* = 16 at each temperature. Photoresponsive (R) and nonphotoresponsive (NR) hamsters are indicated by filled symbols and open symbols, respectively. *α was significantly longer in hamsters kept at 5 °C than at 22 °C.

week 8. T_a did not have a significant effect in the one-way repeated measures ANOVA, but the interaction between time and T_a was significant ($p < 0.05$); α was significantly longer on week 3 in 5 °C than in 22 °C (Fig. 3), indicating that α expanded more rapidly in the cold-exposed animals. R hamsters at 5 °C attained αs of 9.0, 9.5, and 10.0 h 4 to 6 days earlier than those at 22 °C. α was first ≥ 10.0 h after 19.2 ± 1.5 days and 25.3 ± 2.4 days in 5 °C and 22 °C hamsters, respectively ($p < 0.05$).

T_a significantly affected body mass but not testis size or pelage color in R hamsters. Body mass decreased below baseline values sooner in 5 °C than in 22 °C R hamsters (Fig. 4A, week 2 versus week 6). Body mass was significantly lower in 5 °C than in 22 °C on weeks 2 and 4. In contrast, testis size did not differ as a function of T_a at any time point (Fig. 4B), nor did the proportion of hamsters that underwent a molt (4/13 at 5 °C and 3/7 at 22 °C, $p > 0.50$).

Mean activity level (counts/10 min/day) increased in all R hamsters during the 8 weeks of testing (Fig. 5); the most pronounced effect on activity level was exerted by T_a ($p < 0.05$ for factor T_a and for the interaction term). Activity in R hamsters increased by 52% at 22 °C and by 128% at 5 °C. By week 8, 5 °C R hamsters were twice as active as 22 °C R hamsters (17.2 ± 2.6 counts/10 min/day and 8.5 ± 1.7 counts/10 min/day, respectively).

Effect of T_a in Nonphotoresponsive Hamsters

As there were only 3 NR hamsters in the 5 °C group, statistical analyses were limited. In NR hamsters, α was 6.8 ± 0.5 h during the first 2 days in short days and 7.6 ± 0.3 h during week 8; α during the 1st and last

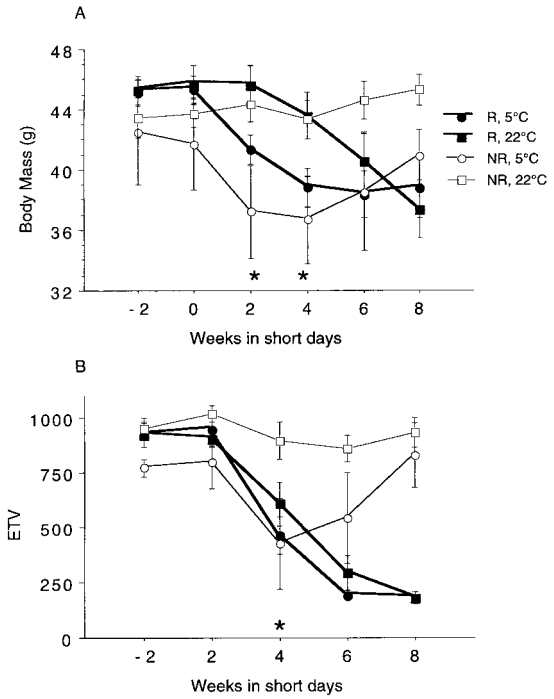

Figure 4. Means (± SEM) body mass (top) and estimated testis volume (bottom) in mm^3 for photoresponsive (R) and nonphotoresponsive (NR) hamsters at 5 and 22 °C beginning 2 weeks prior to transfer to short days (week 0). *significantly lower values in 5 °C than in 22 °C hamsters. ETV = estimated testes volume.

weeks of the study did not differ ($p > 0.30$). α temporarily expanded in all NR hamsters during the first 3 to 4 weeks in short days during re-entrainment to the short photoperiod (Figs. 1 and 3); for all NR hamsters, α peaked at 10.0 ± 0.4 h. α recompression was more rapid in 22 °C than in 5 °C (Fig. 3). In 22 °C, α recompressed rapidly beginning immediately after activity offset coincided with light onset, from 9.7 ± 0.5 h on week 4 to 8.2 ± 0.5 h on week 5 (Fig. 3). In contrast, in 5 °C, α remained expanded (α > 9 h) for 3 weeks (3-5) and only began recompressing on week 6. α recompressed more slowly at 5 °C, from 9.7 ± 1.0 h on week 5 to 8.7 ± 0.3 h on week 6. T_a was not a significant factor in the ANOVA for α ($p > 0.20$), but the interaction term approached significance ($p < 0.07$).

In contrast to the lack of an effect in R hamsters, T_a had a major impact on body mass, testis size, and proportion of animals undergoing molt in NR hamsters. Both body mass and testis size decreased temporarily in 5 °C NR hamsters in association with the temporary α expansion; values for these 2 measures did not change in 22 °C NR hamsters. T_a had a significant effect on body mass in NR hamsters. In 5 °C, body mass was significantly decreased during weeks 2 and 4, then increased through week 8; body mass in 22 °C remained constant throughout the experiment (Fig. 4A). Body mass was marginally higher during weeks 6 and 8 in 22 °C than in 5 °C ($p < 0.07$ and $p < 0.06$, respectively). Testis size was significantly lower in 5 °C than in 22 °C on weeks 2 and 4, and approached significance ($p < 0.07$) on week 6 as testes underwent recrudescence (Fig. 4B) in association with α recompression (Fig. 3). By week 8, testis size was equivalent at 5 °C and 22 °C. Whereas 2 out of 3 NR hamsters at 5 °C had initiated molt to winter pelage by week 8, none (0/9) of the 22 °C NR hamsters had done so ($p < 0.05$).

Mean activity level remained unchanged throughout the experiment in 22 °C NR hamsters, but it increased in 5 °C (Fig. 5). By week 8, hamsters in 5 °C were 3 times as active as those at 22 °C (19.7 ± 6.5 versus 6.4 ± 1.0 counts/10 min/day, respectively, $p < 0.001$). Whereas R hamsters in 22 °C significantly increased their mean activity level throughout the study, no increase was seen in 22 °C NR hamsters.

T_a and Locomotor Activity in Photoresponsive and Nonphotoresponsive Hamsters

During the first 4 weeks of treatment, R and NR hamsters held at 5 °C were indistinguishable and alike in manifesting rapid expansions of α, decreases in body mass and testis size, and increases in activity

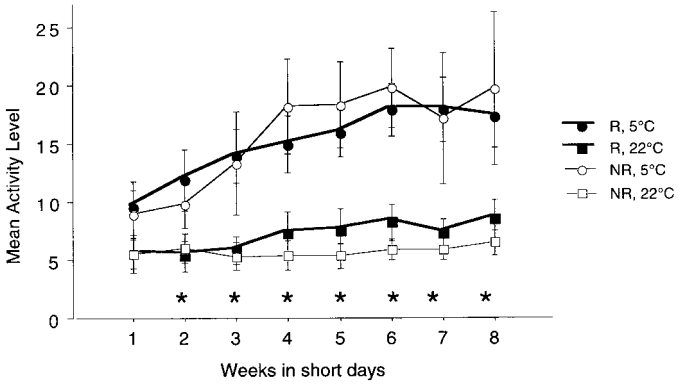

Figure 5. Weekly means of activity levels (counts/10 min/day) in photoresponsive (R) and nonphotoresponsive (NR) hamsters kept in short days at 5 and 22 °C. *significantly higher values in 5 °C than in 22 °C hamsters.

level and duration. The responder and nonresponder groups in 5 °C began to diverge on week 6, α shortened in NR animals, accompanied by increases in body mass and testis size. In contrast, although α expanded at a similar rate in R and NR hamsters at 22 °C during the first 4 weeks (Fig. 3), body mass and testis size remained high throughout the study in the NR animals and decreased on weeks 6 and 4, respectively, in R hamsters (Fig. 4).

Period Estimates of Activity Onset and Offset during Entrainment

The rate of α expansion during entrainment to short days depends on the periods of the putative evening and morning oscillators governing activity onset (τ_E) and activity offset (τ_M), respectively (Hoffmann and Illnerova, 1986; Pittendrigh, 1974). τ_E is < τ_M during re-entrainment, allowing α to expand (Gorman et al., 1997; Prendergast and Freeman, 1999). During the first 3 weeks in short days, when α is expanding, the pattern of activity superficially resembles a free-running activity rhythm (Fig. 1). Although the periods of τ_M and τ_E are best determined under conditions of constant illumination or constant darkness (LL or DD), period estimates of activity onset (τ_E') and offset (τ_M') were made during weeks 1 through 3 employing the methods of Prendergast and Freeman (1999). τ_E' was longer in NR than in R hamsters (24.17 ± 0.02 h vs. 24.11 ± 0.01 h, respectively, $p < 0.05$) but was unaffected by T_a ($p > 0.50$). Both T_a and responder status significantly impacted τ_M' ($p < 0.01$ and $p < 0.05$, respectively), but there was no significant interaction ($p > 0.40$). τ_M' was longer in NR than in R hamsters, as reflected in activity offsets that coincided with light onset 2 weeks sooner in NR than in R hamsters (week 4 versus week 6). Animals at 5 °C had longer τ_M' than those at 22 °C; hence, activity offset coincided with light onset sooner in 5 °C than in 22 °C. Because these effects were additive, 5 °C NR hamsters had the longest τ_M' (24.39 ± 0.02 h), 22 °C R hamsters the shortest τ_M' (24.24 ± 0.02 h), with 5 °C R and 22 °C NR hamsters generating intermediate values (24.33 ± 0.01 h).

Experiment 2: T_a and Infusions

The 3 treatment groups (22 °C melatonin, 5 °C melatonin, and 5 °C saline) had equivalent body masses and testis sizes when infusions began (ANOVA $p > 0.50$ in each case): body mass was 41.6 ± 1.2 g, and ETV was 956 ± 41 mm^3. After 2 weeks, body mass had not changed in 5 °C saline-infused animals (paired *t*-test, $p > 0.50$) and testes remained large (Fig. 6). Body and testes masses also remained high in the 22 °C group infused with melatonin (*t* test, $p = 0.60$); nightly 10-h melatonin infusions for 2 weeks were insufficient to produce decreases in these 2 measures. In contrast, the group held at 5 °C and infused with melatonin significantly decreased body and testes masses, from week 0 to week 2 (Fig. 6, $p < 0.05$ in each case). Cold exposure by itself did not affect body mass or testes size, as evidenced by the lack of effect in the group housed in 5 °C and infused with saline. In combination with daily 10-h melatonin signals, low T_a accelerated decreases in both measures.

DISCUSSION

Cold exposure accelerated appearance of winter traits in Siberian hamsters kept in short day lengths primarily by increasing responsiveness of target tis-

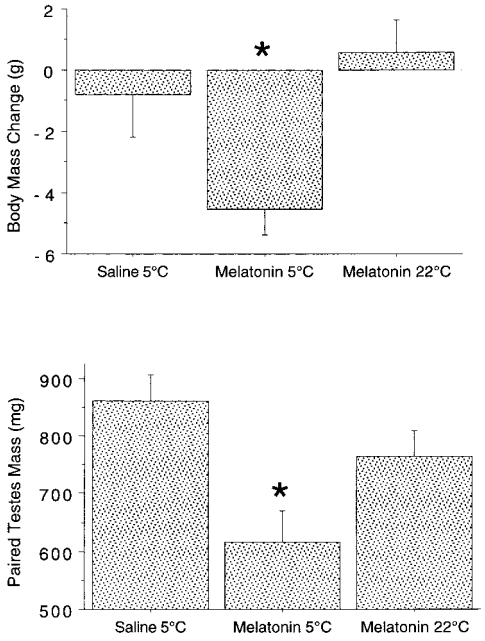

Figure 6. Mean (± SEM) changes in body and gonadal masses from baseline values after 2 weeks of daily infusions in pinealectomized male hamsters. *body and testes masses were significantly lower in melatonin-infused hamsters at 5 °C than either melatonin-infused hamsters at 22 °C or saline-infused hamsters at 5 °C.

sues to melatonin. Decreases in body and testes masses were initiated within 14 days in adult pinealectomized hamsters that received 10-h melatonin infusions at 5 °C, but not at 22 °C. Adult Siberian hamsters kept at 22 °C typically do not decrease their body and testes masses until they have been infused with 10-h melatonin signals for 4 weeks (Prendergast et al., 2000). The more rapid response of animals treated at 5 °C was not a generalized response to the cold as neither body nor testes mass declined in animals in 5 °C infused with saline. Previously, the role of T_a in modifying photoperiodic responses was assessed by injecting melatonin into intact hamsters to lengthen the duration of the endogenous melatonin signal (Li et al., 1987; Reiter et al., 1988). Because T_a affects endogenous melatonin production (Brainard et al., 1982; Stieglitz et al., 1994; Vivien-Roels et al., 1997), however, animals kept at different T_as may not experience equivalent melatonin signals. The timed infusion protocol avoided this potential confound by providing pinealectomized animals with identical daily melatonin signals within the physiological range. Consequently, differences in timing of S-D responses in 5 °C and 22 °C are attributable to temperature-induced changes in responsiveness to the melatonin signal.

Low T_a facilitated photoresponsiveness: Twice as many Siberian hamsters underwent gonadal regression when transferred to short days in 5 °C than in 22 °C. Previous studies on this species used colonies with a high incidence of photoresponsiveness at room temperature (Ruf et al., 1993; Stieglitz et al., 1994), so the augmenting effects of low T_a were not demonstrable. The high rate of nonphotoresponsiveness in our colony, for example (Prendergast et al., 2000), revealed the facilitatory action of low T_a on photoresponsiveness. Increased photoresponsiveness is also induced in Siberian hamsters that are provided with access to running wheels, which results in increased activity levels (Freeman and Goldman, 1997). The higher incidence of photoresponsiveness in 5 °C also may be a consequence of the doubling of activity levels in the cold.

Cold-induced acceleration of rates of testicular regression in photoresponsive Siberian hamsters was not observed in the present experiment. Our results and those from the 2 previous studies are in general agreement that the low T_a promotes appearance of the winter phenotype (Ruf et al., 1993; Stieglitz et al., 1994), even as there is substantial variability in the timing of S-D responses among the 3 studies. Ruf et al. (1993) reported longer latencies for appearance of S-D responses than those recorded in the present study; decreases in body mass were not observed before 4 weeks' exposure at 5 °C and 10 weeks at 23 °C in the earlier experiment. In contrast, Stieglitz et al. (1994) noted decreases in body mass and testis size after 2

weeks in 5 °C. Differences in experimental protocol or hamster stocks may contribute to variations in the timing of S-D responses as well as the magnitude of the temperature effects. Preacclimation to cold did not affect the response to low T_a; in hamsters held in short days at 5 °C, there was no difference in locomotor activity, testis size, or body mass between hamsters preacclimated or not preacclimated to 5 °C (Ruf et al., 1993).

Body and testes masses both showed significant decreases after 2 weeks of 10 h daily melatonin treatment at 5 °C, whereas in hamsters transferred from long to short days, body mass and testes mass changes occurred asynchronously. Body mass decreased 2 weeks prior to detectable decreases in testes size at 5 °C and 2 weeks after the gonadal change at 22 °C. Melatonin is required for the accelerated, cold-induced decline in body mass; pinealectomized animals held at 5 °C and infused with saline did not display this response. Whether melatonin is permissive or directly affects body mass is unknown. The greater lability of body mass than testis dimensions in response to cold exposure may reflect intrinsically shorter response latencies in substrates that control food intake and adipose tissue regulation than in those that mediate gonadal function.

Cold-induced changes to the rates of α expansion during entrainment to short day lengths may also contribute to the accelerated appearance of winter traits. α, and presumably the duration of nocturnal melatonin secretion, expanded more rapidly in hamsters held at 5 °C than at 22 °C. This more rapid expansion of α in 5 °C reflects lengthening of the period of activity offset (τ_M') without attendant change in the period of the oscillator that governs timing of activity onset (τ_E'). The magnitude of this effect was, however, small; in photoresponsive hamsters, the 0.1 h difference in τ_M' resulted in a 6-day advance in α expansion to ≥ 10 h at 5 °C compared to 22 °C after the abrupt photoperiod change from long to short day lengths.

Although we have not directly verified the correlation between melatonin and α at 5 °C, the results with the nonphotoresponsive hamsters suggest that α duration continues to be a useful indicator of melatonin duration in the cold. All nonresponder hamsters showed temporary α expansion during the 3 to 4 weeks of entrainment to short days. However, nonresponder hamsters in 5 °C maintained extended α (> 9 h) for 2 weeks longer than nonresponder hamsters at 22 °C and initiated short-day responses, which were subsequently reversed when α recompressed at 6 weeks. This correlation between α duration and short-day responses in nonresponder hamsters at 5 °C suggests that α is a reliable indicator of melatonin duration in the cold.

This relatively small effect of T_a on α expansion is unlikely to hasten appearance of S-D responses of Siberian hamsters in the field during exposure to gradually changing day lengths. Entrainment rate of circadian rhythms depends on the type of transition from long to short day lengths and is more labile with abrupt transitions of large magnitude (Gorman et al., 1997). The abrupt photoperiod transition used in the present study maximized opportunities for temperature to affect entrainment rates. A previous attempt to establish that cold exposure accelerated the onset of S-D melatonin signals was not definitive; infrequent sampling presumably precluded accurate assessment of the effect of T_a on the duration of the melatonin signal (Steiglitz et al., 1994). That study did, however, establish that cold exposure accelerated the attainment of melatonin signals of increased amplitude typical of short days. The role of signal amplitude in transducing effects of short day lengths is unknown; most studies point to the duration of the daily melatonin signal as the critical parameter in triggering photoresponses (Bartness et al., 1993).

Low T_a was equally effective in lengthening the period of activity offset τ_M' in hamsters eventually designated as nonphotoresponsive, as in photoresponsive animals; α expanded more rapidly and attained peak values a week earlier in the cold (Fig. 3). Low T_a, however, also delayed α recompression in NR hamsters, resulting in a 3-week interval during which α was ≥ 10 h in NR animals in the cold. As predicted (Prendergast and Freeman, 1999), this lengthened interval of α expansion in the 5 °C NR hamsters triggered the entire suite of short-day responses: decreased body and testes masses and initiation of molt to winter pelage. The much briefer α expansion in 22 °C NR animals resulted in α ≥ 10 h for less than 1 week, which was insufficient to trigger S-D responses. The delayed α recompression in 5 °C NR hamsters suggests that cold exposure affects the circadian system more than just by lengthening τ_M'.

Separate circadian oscillators have been proposed for control of activity onset and offset (Hoffmann and Illnerova, 1986; Pittendrigh, 1974). The inability of NR hamsters to permanently expand α when transferred to short days may reflect enhanced coupling between these 2 oscillators (Gorman et al., 1997; Prendergast and Freeman, 1999; Puchalski and Lynch, 1988). Our

findings suggest that low T_a, possibly mediated by the increased activity levels associated with cold exposure, affects the circadian system and may decrease coupling strength between morning and evening oscillators. The more rapid α expansion, increased photoresponsiveness (i.e., the higher percentage of animals that maintain an expanded α), and delayed α recompression in NR hamsters in the cold may all reflect decreased coupling strength between evening and morning oscillators in 5 °C.

In summary, our findings suggest that the accelerated S-D responses in hamsters challenged with low temperatures are mediated by increased responsiveness of target tissues to long duration melatonin signals. Cold exposure also affects the circadian system, possibly by weakening the coupling between the putative morning and evening oscillators (Hoffmann and Illnerova, 1986; Pittendrigh, 1974). Thus, in the cold, the duration of nocturnal melatonin secretion may have increased more rapidly, twice as many hamsters were photoresponsive, and α recompression and presumably shortened duration of melatonin secretion were delayed in nonphotoresponsive hamsters, resulting in transient expression of S-D responses.

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