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Evidence that the Circadian System Mediates Photoperiodic Nonresponsiveness in Siberian Hamsters: The Effect of Running Wheel Access on Photoperiodic Responsiveness

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Abstract Juvenile male Siberian hamsters from a line of hamsters selected for nonresponsiveness to short photoperiod (PNRj) and animals from the general colony (UNS) were separated at weaning into two groups. Group 1 males were moved into short days (10 h light:14 h dark [10L:14D]) with free access to running wheels (RW). Group 2 animals were the male siblings of Group 1 hamsters; they were moved at the same time into the same room, but were housed in cages without access to RW. Group 2 hamsters only had access to RW for the final week of short-day exposure (Week 8). Animals were blood sampled at the time of sacrifice for analysis of serum prolactin (PRL) and follicle-stimulating hormone (FSH) concentrations. At sacrifice, paired testis weights were obtained and pelage color was scored. Animals from the UNS line showed the expected declines in testis weight, body weight, and serum concentrations of both PRL and FSH, regardless of the presence or absence of RW. These animals also exhibited a high proportion of individuals molting to winter-type pelage. By contrast, a marked difference was noted between siblings from the PNRj line depending on whether RW access was provided at the time of weaning. Animals with access to RW exhibited identical responses to those of the UNS responder animals, whereas PNRj animals without access to RW showed no adjustments to short days (i.e., testis regression, pelage molt, expansion of alpha). In a second experiment, PNRj and UNS males were placed in constant darkness (DD), with or without RW access. The results of this experiment indicated that PNRj animals respond to DD regardless of the presence or absence of RW. In DD, PNRj hamsters also exhibited significantly longer free-running period lengths (taus) than did UNS hamsters; all the PNRj hamsters had taus > 24 h, whereas none of the UNS hamsters had a tau > 24 h. These results indicate that PNRj hamsters retain the proper neural pathways for responding to short day lengths and establish a role for locomotor activity feedback in modulating the circadian system and, subsequently, photoperiodic responsiveness in PNRj hamsters.

Key words photoperiod nonresponsiveness, circadian rhythms, photoperiod time measurement, Siberian hamster, locomotor activity, seasonal reproduction

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INTRODUCTION

Siberian hamsters (*Phodopus sungorus*) acclimate in a variety of ways to seasonal environmental changes. Like many other seasonal organisms, hamsters use the annual cycle of photoperiod variations to cue these adaptive responses. Under tightly controlled laboratory conditions, hamsters transferred from a long to a short photoperiod exhibit a range of adjustments including body weight loss, increased thermogenic capacity, daily torpor, and gonadal regression (Heldmaier et al., 1981; Hoffmann, 1973; Steinlechner and Heldmaier, 1982).

In laboratory colonies of Siberian hamsters, some individuals fail to exhibit the typical responses to short photoperiod just listed; these individuals have been termed *nonresponders* (Puchalski and Lynch, 1986). Short-day nonresponsive hamsters exhibit certain differences in circadian characteristics as compared to responsive individuals. Most notably, when exposed to short days, nonresponsive hamsters typically show a 4- to 6-h more negative phase angle of activity onset as compared to responsive animals (Puchalski and Lynch, 1986, 1991a). The difference in phase angles of entrainment may be explained by the longer taus of the short-day nonresponders (Puchalski and Lynch, 1988).

There is a genetic basis for the variation in short-day responsiveness among individual Siberian hamsters (Lynch and Lynch, 1986; Puchalski and Lynch, 1991b). We have reported that there also is a relationship between age and the probability that an individual Siberian hamster will fail to respond to short days (Freeman and Goldman, 1997). In the present article, we describe effects of a nonphotic stimulus (wheel-running activity) on responsiveness to short photoperiod. Nonphotic stimuli, including induced wheel running, triazolam injections, and social cues, have been shown to be capable of phase shifting or entraining circadian rhythms of locomotor activity in Syrian hamsters (Reebs and Mrosovsky, 1989; Turek and Losee-Olson, 1986). The effects of triazolam on the circadian system appear to be mediated by an acute increase in locomotor activity; hamsters that do not exhibit a large increase in activity following injection of the drug also fail to show a phase shift (Mrosovsky and Salmon, 1990). Intense locomotor activity may have additional effects on the circadian system besides entraining or phase shifting rhythms; thus free access to running wheels (RW) shortened taus in mice housed in continuous darkness (Edgar et al., 1991).

The neural mechanisms mediating these circadian effects are not well understood. However, ablation of

the intergeniculate nuclei of the thalamus (IGL) prevents the phase-shifting effect of induced locomotor activity (Wickland and Turek, 1994) and triazolam injections (Johnson et al., 1988), suggesting an involvement of the IGL in the effects of these nonphotic stimuli on the circadian system. It has been firmly established that the circadian system is intimately involved in the process of photoperiodic time measurement in mammals (Elliott, 1976). Yet, although numerous experiments have demonstrated that nonphotic stimuli can affect the circadian system, there have been few attempts to explore possible effects of nonphotic stimuli on photoperiodic responsiveness. In Syrian hamsters, RW access delayed testicular regression in short days (Elliott, 1974) and stimulated ovarian activity in short-day exposed, photoregressed females (Borer et al., 1983). A similar effect of RW access has been reported for meadow voles (Kerbeshian et al., 1994). In voles, the wheel-running effect appeared to be specific to photoperiod modulation of the reproductive axis, as no effect was seen on photoperiod-induced changes in body weight.

The present experiments were designed following our observation that RW access, if initiated during juvenile life, could reverse short-day nonresponsiveness in Siberian hamsters of a breeding line that had been subjected to artificial selection to increase the incidence of the nonresponsive trait. We report that RW access can lead to the expression of typical short-day responses in juveniles of an otherwise nonresponsive strain of hamsters.

MATERIALS AND METHODS

Animals and Housing

Photoperiod nonresponder (PNRj) and general colony (UNS) breeding pairs of hamsters were used to provide offspring for use in this study. The UNS colony was derived from animals originally supplied by Klaus Hoffmann. The PNRj colony was derived from a subset of the UNS hamsters and refined through several generations of artificial selection for the photoperiod nonresponsive phenotype (Freeman and Goldman, 1997). This resulted in a breeding line (PNRj) in which approximately 80% of the individuals fail to exhibit the typical short-day responses when exposed to short photoperiod beginning at 17-19 days after birth. All animals were housed in a 16 h light:8 h dark (16L:8D) photoperiod (lights on 0200-1800 h).

Food (Agway Prolab 3500 RMH) and water were available ad libitum. Offspring were weaned at 17-18 days of age.

Experiment 1

Juvenile male littermates from the UNS and PNRj lines were assigned on the day of weaning into either of two treatments for 8 weeks: (a) 10L:14D (lights on 0500-1500 h) with access to RW for the 8th week only (referred to as the groups "without RW" in later sections; $n = 11$ PNRj, $n = 8$ UNS) or (b) 10L:14D in cages equipped with RW for the entire 8 weeks ($n = 25$ PNRj, $n = 11$ UNS). A control group consisted of PNRj and UNS animals that were left in 16L:8D for comparison to the 10L groups. Activity rhythm data were collected continuously using the Datacol 3 system (Mini-Mitter Co., Sunriver, OR). Data obtained from Datacol 3 were analyzed using the Tau program (Mini-Mitter Co.), which plots activity data in the form of actograms. The duration of locomotor activity (alpha) was determined by drawing eye-fitted lines through the onset and offset of activity for at least 5 consecutive days. The phase angle of entrainment (psi) was calculated using an eye-fitted line drawn through activity onset for at least 5 consecutive days. The difference between the times of lights off and of activity onset was determined in hours (e.g., a phase angle of entrainment of -2 h indicates that the animal became active 2 h after lights off). Eye fitting and determination of tau were accomplished with the aid of graphics generated via the Tau software.

After 8 weeks in 10L, each animal was autopsied, paired testis weights (PTWs) were obtained, and pelage condition was scored via the pelage index described by Duncan and Goldman (1984). In a prior study (Freeman and Goldman, 1997), it was shown that a small proportion (around 20% to 30%) of males from the PNRj line did respond to 10L with testis regression. In the present experiment, 3 PNRj animals without access to RW responded to 10L (i.e., PTW less than the 16L control mean PTW minus 2 standard deviations [421.6 mg]); these 3 hamsters and their 4 siblings with RW were discarded from the final analyses, because it was possible that the siblings would have responded even without RW access.

Hormone Concentrations

Blood samples (250 μ l) were obtained by retroorbital sinus puncture at 0900-1000 h from a subset of

animals ($n =$ at least 8 per group) prior to sacrifice. Serum was recovered after centrifugation and stored at -50°C until determination of serum prolactin (PRL) and follicle-stimulating hormone (FSH) concentrations via double-antibody radioimmunoassay (RIA). A control group of long-day housed animals also was blood sampled for comparison to the short-day groups.

Follicle-stimulating hormone. Serum FSH was measured by use of the NIAMDD rat FSH RIA kit, using anti-rat FSH-S-10 as the primary antibody (1:17,600 final dilution). This method has been validated for use in Siberian hamsters. Displacement curves for Siberian hamster serum and pituitary gland extracts are parallel to the displacement curve for the rat pituitary reference standard that was employed in this assay (Yellon, 1982; Yellon and Goldman, 1984). Goat anti-rabbit gamma globulin (1:96 final dilution) was used as second antibody. Intraassay coefficients of variance for 20% and 50% binding were 8.2% and 6.3%, respectively. The range of detectable values (10%-90% total binding) was 0.8-21.3 ng/ml serum. Samples outside the 10%-90% binding limits were assigned the appropriate value for 10% or 90% binding.

Prolactin. Anti-hamster PRL (1:120,000 final dilution), generously donated by Katerina Borer (University of Michigan), was used as the primary antibody. This antibody previously has been validated for the measurement of Siberian hamster PRL (Duncan et al., 1985). Goat anti-rabbit gamma globulin (1:96 final dilution) was used as second antibody. Purified Syrian hamster PRL provided by Frank Talamantes (University of California, Santa Cruz) was iodinated using the chloramine-T method to serve as trace. The standard was purified Syrian hamster PRL supplied by Albert Parlow (Harbor-UCLA Medical Center, Lot No. AFP10302E). This PRL assay has been appropriately validated for Siberian hamsters in our laboratory (Duncan et al., 1985; Yellon and Goldman, 1984). The range of the PRL RIA (10%-90% total binding) was 0.3 to 61.8 ng/ml serum. Samples outside the 10%-90% binding limit were assigned the appropriate value for 10% or 90% binding. The intraassay coefficients of variance at 15% and 60% bound were 5.8% and 11.6%, respectively.

Experiment 2

Male hamsters (3-12 weeks of age) from the UNS and PNRj breeding lines were transferred from a

Table 1. Effect of running wheel access on responsiveness to short days in responsive and nonresponsive phenotypes.

Phenotype	Treatment	Percentage Failing to Exhibit Testicular Inhibition	Percentage Failing to Exhibit Molt	Pelage Score ^a
UNS (8)	10L	0	5.3	2.9 ± 0.16
UNS (11)	10L/RW	0	0	
PNRj (11)	10L	73*	100*	1 ± 0*
PNRj (25)	10L/RW	0	0	2.6 ± 0.17

NOTE: Numbers in parentheses are *ns*. UNS = general colony animals. PNRj = photoperiod nonresponder animals. RW = running wheel access for entire 8 weeks.

a. Combined pelage score for all short-day exposed UNS animals (10L and 10L/RW).

*Significantly different from all other groups ($p < .001$).

16L:8D photoperiod to constant darkness (DD). Some animals were housed in cages equipped with RW ($n = 19$ PNRj, $n = 12$ UNS), whereas their siblings were housed in cages without RW ($n = 20$ PNRj, $n = 5$ UNS). After 8 weeks of DD exposure, testis weights and pelage condition were recorded. Animals were classified as nonresponders using the same criteria as for Experiment 1 (i.e., PTW > 421.6 mg). Determination of tau was accomplished with the aid of graphics generated via the Tau software. Tau was measured over at least a 7-day period. No attempt was made to adjust measurements of tau in relation to expansion of alpha.

Statistics

Differences in PTWs, alphas, psi's, and FSH and PRL concentrations were analyzed by one-way analysis of variance. Group means were compared using Fisher's protected least significant difference. Differences in taus were compared by two-tailed Student's *t* test. The proportion of animals exhibiting taus > 24 h were compared by Fisher's exact probability. Proportions of nonresponders were compared using chi-square test of proportions or Fisher's exact probability where appropriate. Differences were considered significant if $p < .05$.

RESULTS

Experiment 1

Percentage nonresponders. All of the UNS animals responded to short photoperiod regardless of the presence or absence of RW. All 25 PNRj hamsters with RW

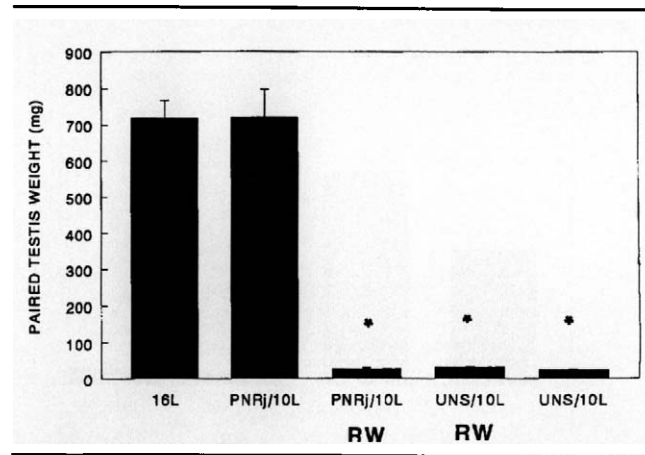


Figure 1. Mean paired testis weights (PTWs) of animals in 10L with or without running wheel access. All groups were in 10L except the 16L control group. RW designates groups that had running wheel access for 8 weeks; the other 10L groups had RW access only for the final week of the experiment. The 16L and photoperiod nonresponder (PNRj) without RW groups exhibited mean PTWs significantly greater than any other 10L group. Asterisk (*) indicates significantly less than 16L control and PNRj/10L without RW groups mean PTW ($p < .05$). UNS = general colony animals.

access responded, whereas only 27% (3/11) of the PNRj animals without RW responded. The 3 PNRj animals that responded without RW were discarded from later analyses, as were their siblings that had RW access (Table 1).

Paired testis weight. Analysis of PTWs revealed a significant effect of treatment on PTW ($F_{4,50} = 136$, $p < .00001$). No difference was seen between the 16L control group and the PNRj short-day group. The mean PTWs of the PNRj were significantly greater than those of their siblings with RW access. Furthermore, the PNRj and 16L control animals had significantly greater PTWs than did any short-day UNS group. There were no significant differences between the PTWs of the PNRj/RW and UNS short-day groups (Fig. 1). Because no differences were observed between the UNS 10L groups with or without access to RW, data from these two groups were combined for later analyses.

Prolactin. Analysis of serum PRL concentrations indicated a significant effect of treatment on PRL concentrations ($p < .0001$). Mean serum PRL concentrations were significantly greater in the 16L control and PNRj animals without RW (i.e., RW access only for Week 8) than in any other group, whereas no difference was

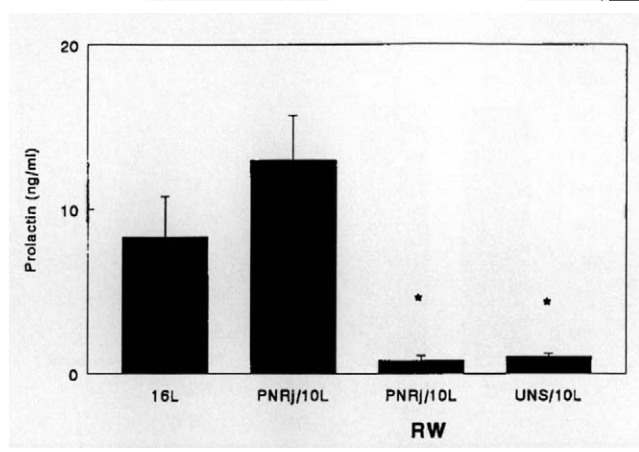


Figure 2. Mean serum prolactin (PRL) concentrations of general colony (UNS) and photoperiod nonresponder (PNRj) animals in a short photoperiod with or without running wheel (RW) access. The UNS short-day groups (with or without RW access) were combined because no difference existed between them. Both short-day UNS groups and the PNRj group in 10L with RW access exhibited significantly lower serum PRL concentrations than did the 16L control group and the PNRj animals in 10L without RW access (* $p < .05$). All groups except the 16L group were exposed to 10L.

evident between PNRj animals without RW and 16L controls (Fig. 2).

Follicle-stimulating hormone. The PNRj without RW and 16L control animals had significantly higher serum FSH concentrations than did the short-day PNRj/RW animals or the short-day UNS hamsters ($p < .0001$). Serum FSH concentrations exhibited by PNRj short-day animals without RW did not differ significantly from those exhibited by 16L UNS controls (Fig. 3).

Pelage. The PNRj group without RW had a mean pelage score of 1 (all had summer pelage), and this differed significantly from all other short-day groups ($p < .0001$). No difference existed between the PNRj/RW and UNS short-day groups. All of the PNRj/RW animals molted, whereas none of the PNRj/10L animals molted; this difference was significant ($p < .001$). Approximately 5.3% (1/19) of the UNS animals failed to molt, which was not significantly different from the PNRj/RW group (Table 1).

Alpha. Representative examples of wheel-running activity records can be found in Fig. 4. PNRj animals without access to RW exhibited alphas that were two- to threefold shorter than those of any other short-day group ($p < .0001$).

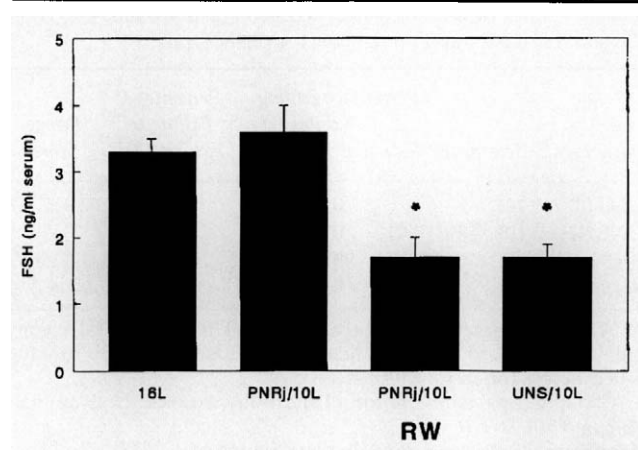


Figure 3. Mean serum follicle-stimulating hormone (FSH) concentrations of general colony (UNS) and photoperiod nonresponder (PNRj) animals in a short photoperiod with or without running wheel (RW) access. The UNS groups were combined as in Fig. 2. The UNS and PNRj/RW groups exhibited significantly lower serum concentrations of FSH as compared to 16L controls and the PNRj/10L without RW groups (* $p < .05$).

Phase angle of entrainment. PNRj animals without access to RW had a mean phase angle of entrainment that was significantly more negative than the mean of any other short-day group ($p < .0001$) (Fig. 4).

Experiment 2

Percentage nonresponders. All UNS and PNRj animals housed in DD for 8 weeks exhibited regressed testes, independent of the presence or absence of RW (Table 2).

Tau. Representative RW records can be found in Fig. 5. Significant differences were evident in the free-running period between the PNRj and UNS groups that had RW access. All PNRj animals exhibited taus greater than 24 h, whereas all UNS animals exhibited taus less than 24 h (Table 2).

DISCUSSION

Laboratory populations of Siberian hamsters have been found to exhibit at least two distinct phenotypes with respect to the expression of a variety of presumably adaptive, winter-type responses following exposure to short-day photoperiods. Although most individuals undergo gonadal regression, molt to winter pelage, and show a reduction of body fat stores, a

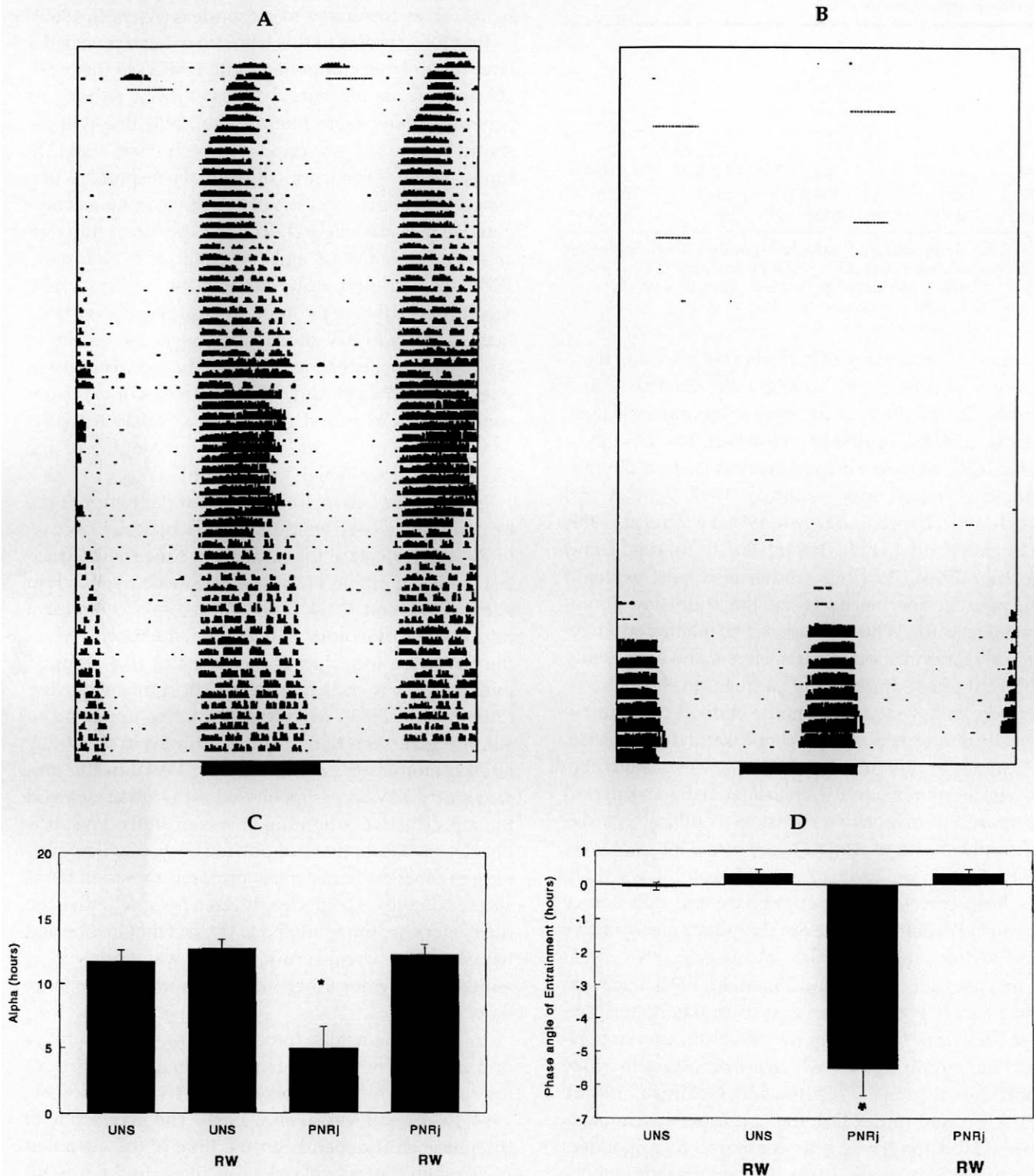


Figure 4. Double-plotted actograms from photoperiod nonresponder (PNRj) hamsters transferred to 10L with running wheel (RW) access during the entire short-day exposure (A) and for only the final week of short-day exposure (B). The dark bar at the bottom of each actogram represents the dark phase of the light:dark (LD) cycle. RW access for the entire experiment led to a significant increase in the duration of locomotor activity (alpha) in PNRj hamsters (C). The PNRj animals without RW access exhibited a shorter alpha than did any other group ($*p < .05$). The PNRj hamsters without RW access exhibited a significant delay in the onset of activity as compared to every other group ($*p < .05$). Note: 0 = Time of lights off. (D). All groups were exposed to a 10L photoperiod.

Table 2. Determination of photoperiod responses and tau of each phenotype after exposure to DD.

Phenotype	Treatment	n	Percentage Exhibiting Paired Testis Weight (mg)	Tau	t > 24 h
UNS	DD/RW	12	39.3 ± 1.7	23.8 ± 0.05	0
PNRj	DD/RW	19	24.9 ± 1.7	24.5 ± 0.06*	100*
UNS	DD	5	32.8 ± 7.0	N/A	N/A
PNRj	DD	20	26.3 ± 4.7	N/A	N/A

NOTE: RW designates groups that had running wheel access for the entire 8-week experiment. DD = constant darkness. UNS = general colony animals. PNRj = photoperiod nonresponder animals.

*Significantly greater than UNS ($p < .001$).

substantial percentage of hamsters fail to exhibit these changes (Kliman and Lynch, 1992; Puchalski and Lynch, 1986). Studies in several laboratories have demonstrated a heritable component for these individual differences in responsiveness to short photoperiods (Freeman and Goldman, 1997; Kliman and Lynch, 1992; Lynch and Lynch, 1986; Lynch et al., 1989; Puchalski and Lynch, 1991a, 1991b; Stanfield and Horton, 1996). Previous studies also have explored the neuroendocrine basis for the short-day nonresponsive trait. When compared to photoperiod responders, nonresponsive hamsters show a markedly different phase angle of entrainment to short photoperiods, as assessed by the time of onset of nocturnal wheel-running activity (Puchalski and Lynch, 1986, 1991a). Short-day nonresponsive hamsters begin nocturnal locomotor activity much later in the dark period compared to responsive hamsters, resulting in a significantly shorter duration of nocturnal locomotor activity. The phase and duration of locomotor activity has been correlated with the phase and duration of nocturnal melatonin release in short-day nonresponsive Siberian hamsters (Puchalski and Lynch, 1986) and in Syrian hamsters (Elliott and Tamarkin, 1994). Puchalski and Lynch (1986) confirm that short-day nonresponsive hamsters fail to generate long-duration (short-day) melatonin signals, which are necessary to evoke short-day responses (Carter and Goldman, 1983a). When housed under DD, the nonresponsive animals also exhibit longer taus as compared to responders (Puchalski and Lynch, 1988). Because the taus of those nonresponders examined in the present study all were > 24 h whereas those of responders generally are < 24 h, this difference in free-running period lengths could explain why nonresponder hamsters entrain to short

day lengths with a delayed phase angle (relative to lights off) as compared to responders (Aschoff, 1965).

Previous studies in this laboratory have revealed a large age-related component with respect to the probability that an individual hamster from either our general colony (UNS line) or the PNRj line will respond to short photoperiod. For both lines, a significantly larger proportion of animals respond when exposed to short days shortly after weaning as compared to the case where short-day exposure is initiated around 3 months of age (Freeman and Goldman, 1997). The present study reveals the ability of RW access to modify the photoperiod nonresponsive trait; that is, access to RW shortly after weaning results in rapid and consistent reversal of the nonresponsive phenotype. Interestingly, several months of exposure generally are required for a similar effect to occur when RW access is not begun until 3 months of age (Freeman, unpublished observation).

Interactions between RW access and photoperiodic response have been reported in other species. Access to RW may increase the amount of time required for testicular regression in short-day housed male Syrian hamsters (Elliott, 1974), and RW access accelerated a return to the ovulatory state in the same species when the RW were introduced after females had stopped ovulating as a consequence of short-day exposure (Borer et al., 1983). In voles, RW access appeared to stimulate the secretion of gonadotropins in short-day housed animals (Kerbeshian et al., 1994). In the present study, RW access facilitated rather than delayed the reproductive inhibiting effects of short days. It is not clear whether these opposite effects of wheel running in Siberian hamsters as opposed to Syrian hamsters and voles is a species difference, or whether the differences are more related to the fact that in Siberian hamsters the wheel-running effect was observed in animals that were otherwise unresponsive to short days.

In Syrian hamsters, forced running-wheel activity or access to a novel RW can result in a phase shift of the circadian rhythm of locomotor activity (Janik et al., 1994; Reebs and Mrosovsky, 1989). The magnitude of the phase shift depends on the time of the circadian cycle when the "wheel pulse" is given. In the present study, hamsters were allowed free access to RW at all times, and this resulted in an advance in the onset of the phase of heightened locomotor activity and an increase in the duration of locomotor activity. This

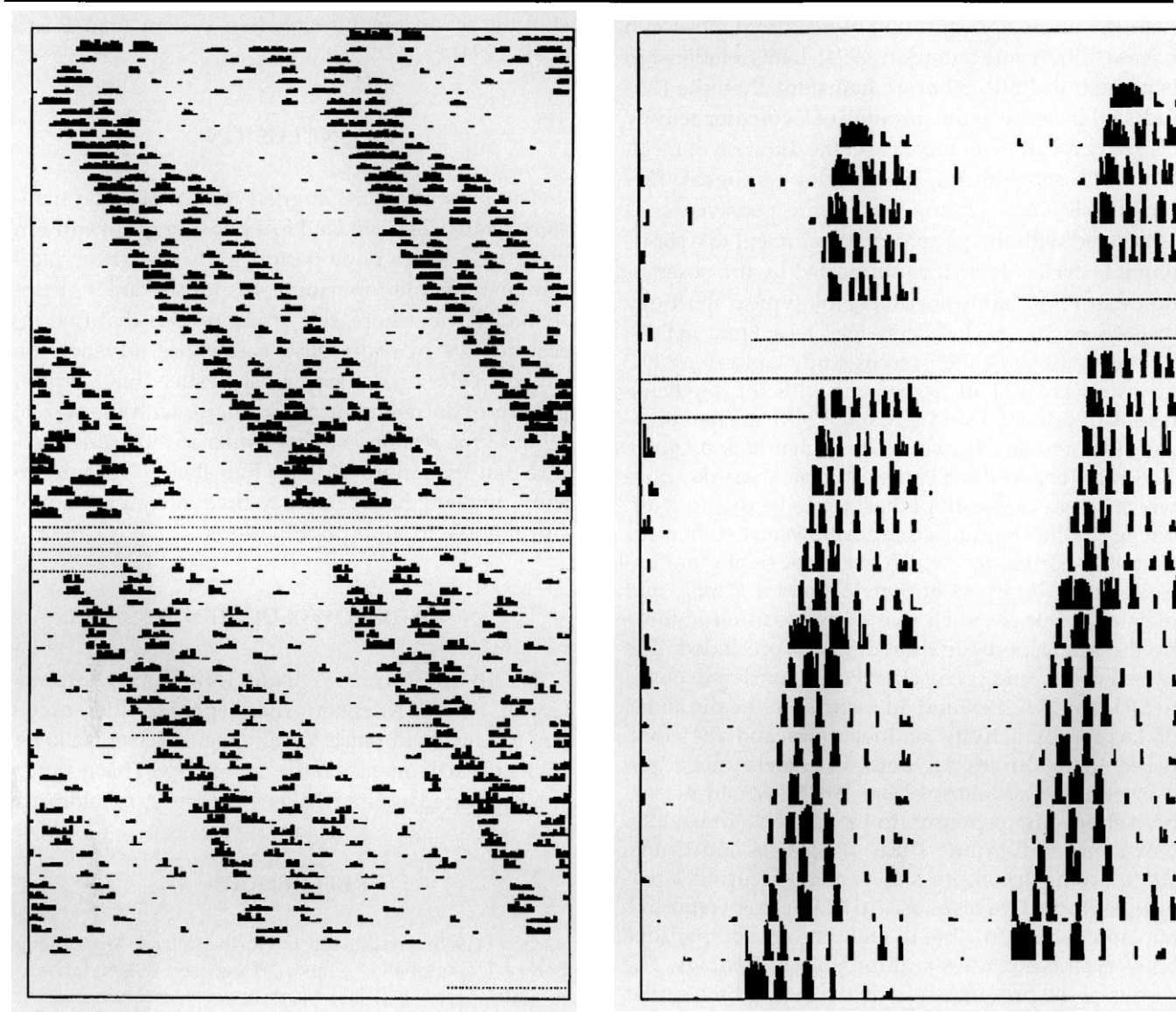


Figure 5. Representative actograms from photoperiod nonresponder (PNRj) (left) and general colony (UNS) hamsters (right) in constant darkness. Note that τ is > 24 h for the PNRj animal, whereas τ is < 24 h for the UNS animal. Dotted lines in middle of records represent lost data due to hardware failure.

advance occurred within a few days when RW were introduced shortly after weaning but required at least 8-10 weeks if RW were not available until 3-4 months of age (Freeman and Goldman, unpublished observation). It is unknown whether the circadian phase of wheel running is important for this effect, but this presumably could be tested by allowing hamsters only limited access to RW.

It is likely that the altered phase angle of entrainment to the short photoperiod that is seen in PNRj hamsters following access to RW is responsible for the

short-day responses (i.e., inhibition of FSH and PRL, testis regression, and pelage molt) that occur in this paradigm. The advance in the phase angle of the entrained rhythm relative to the light:dark (L:D) cycle would be expected to result in "dawn" illumination falling later during the circadian cycle, perhaps leaving a critical portion of the subjective night unexposed to light. This may result in a longer duration of elevated nocturnal melatonin release, thereby resulting in short-day responses. In Syrian hamsters, the phase and duration of locomotor activity has been correlated

with the phase and duration of nocturnal melatonin release (Elliott and Tamarkin, 1994). If this relationship is present in PNRj Siberian hamsters, then the RW-induced increase in the duration of locomotor activity also may result in an increase in the duration of melatonin release, resulting in short-day responses. This hypothesis—that photoperiod nonresponsiveness is associated with the pattern of entrainment to a short-day L:D cycle—is further supported by the observation that PNRj hamsters do exhibit typical short-day responses when no light cues are present (i.e., in DD).

The findings of the present study concerning DD exposure are not in agreement with the predicted results of previous studies using PNRj Siberian hamsters (Kliman and Lynch, 1991; Puchalski and Lynch, 1991a, 1991b). In these earlier studies, short-day nonresponsiveness was hypothesized to be due to a difference in the coupling strength between two or more circadian oscillators, which are responsible for controlling the durations of both locomotor activity and melatonin release, such that decompression of these rhythms in response to short days was precluded. This hypothesis would predict that PNRj hamsters exposed to DD would not exhibit an increase in the durations of locomotor activity or melatonin and therefore, based on the duration hypothesis of melatonin action (Carter and Goldman, 1983a, 1983b), would not exhibit short-day responses. In these two studies, alpha was measured; in one study (Puchalski and Lynch, 1991a), some decompression of alpha occurred. However, reproductive responses to DD were not reported, and so it is not possible to make strong comparisons between these studies and the present findings. The results of the present study do not support the earlier hypothesis (i.e., that short-day nonresponsiveness is attributable strictly to increased coupling strength between two component oscillators); rather, the present findings suggest that short-day nonresponsiveness in Siberian hamsters is the result of an altered phase angle of entrainment to a short photoperiod.

Pharmacological manipulations that lead to altered patterns of entrainment have been demonstrated to alter the response to short photoperiods. Administration of deuterium oxide in the drinking water resulted in a lengthening of tau and a change in the phase relationship between the circadian rhythm of wheel-running activity and the L:D cycle in Syrian hamsters; these effects of deuterium oxide were associated with the prevention of the testicular regression that normally occurs in short days (Eskes and Zucker, 1978). Also, daily injections of triazolam induced testicular recrudescence in short-day housed Syrian hamsters, presumably as a result of the benzodiazepine's phase-

shifting action on the circadian system (van Reeth et al., 1994).

CONCLUSION

The present results suggest that in Siberian hamsters, a tau > 24 h can lead to a phase angle of entrainment to a short photoperiod such that the typical winter-type photoperiodic responses are not observed. Furthermore, this study reveals that free access to RW in short days can phase advance the circadian clocks of these hamsters such that a typical pattern of entrainment occurs along with the typical winter-type responses. The results of this study indicate that the failure of PNRj hamsters to respond to short day lengths is related to their altered pattern of entrainment to short photoperiods.

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