Social environment modulates photoperiodic immune and reproductive responses in adult male white-footed mice (*Peromyscus leucopus*)

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Pyter, Leah M., Gretchen N. Neigh, and Randy J. Nelson. Social environment modulates photoperiodic immune and reproductive responses in adult male white-footed mice (*Peromyscus leucopus*). *Am J Physiol Regul Integr Comp Physiol* 288: R891–R896, 2005. First published November 18, 2004; doi:10.1152/ajpregu.00680.2004.—Social cues may interact with photoperiod to regulate seasonal adaptations in photoperiod-responsive rodents. Specifically, photoperiod-induced adjustments (e.g., reproduction and immune function) may differ among individuals in heterosexual pairs, same-sex pairs, or isolation. Heterosexual cues may be more influential, based on their potential fitness value, than same-sex cues or no social cues. The present study examined the effects of pair (with a male or female) or individual housing on reproductive and immune responses in male white-footed mice (*Peromyscus leucopus*) maintained in long or short photoperiods. Female pairing did not affect reproductive responses in short-day males. In long days, however, the presence of a female increased both testosterone concentrations and testes mass compared with individually housed and male-paired mice, respectively. Short-day, individually housed males enhanced delayed-type hypersensitivity (DTH) responses compared with single-housed mice in long days, but all paired groups decreased DTH responses regardless of photoperiod. The lack of enhanced DTH response in male mice paired with females coincided with reduced circulating corticosterone concentrations in both photoperiod treatments. Together, these results suggest that social environment may have important modulatory effects on photoperiod-regulated immune responses in male white-footed mice.

Photoperiod-responsive rodents undergo annual changes in reproductive and immune functions in response to photoperiod (day length; Refs. 24, 26). Generally, rodents maintained in short days for several weeks inhibit reproductive function and enhance some immune responses compared with rodents maintained in long days. It has been hypothesized that the energetic savings attained by seasonal suspension of reproduction liberates energy for immune function, thereby enhancing immune responses in short-day animals (7). Although additional environmental cues (e.g., temperature, food availability, precipitation, and social cues) have also been studied separately or in conjunction with photoperiod manipulation (8, 10, 20, 28), photoperiod generally appears to be the most influential cue for seasonal adaptations.

The vast majority of studies on seasonally changing traits have been conducted in single-housed animals. However, one “secondary” environmental cue, social environment, may be significant for animals living in the tropics where seasonal photoperiod changes minimally. For example, male mice from low latitudes (*Peromyscus azteca*) enlarge reproductive tract size and function in response to a conspecific female but not in response to photoperiod (10). In nontropical rodents that respond reproductively to photoperiod, social factors may also influence reproduction. For example, male adult Siberian hamsters (*Phodopus sungorus*) exposed to short days while cohabitating with an unrelated female failed to regress their reproductive tract (14). Male hamsters paired with males or housed alone, on the other hand, inhibited reproductive system size and function (14). Similarly, reproductive development was stimulated in short-day male juvenile deer mice (*P. maniculatus*) by the presence of an adult female (35). Pairing juvenile male deer mice with a male inhibited reproductive maturation, whereas pairing with a female slightly enhanced reproductive tract mass, even in breeding (long day) conditions (2, 35). These studies suggest that social environment modulates the effects of photoperiod on the reproductive system under certain conditions.

The effects of social environment on photoperiod-induced changes in immune response are uncommon. Previous studies have focused on photoperiodic effects on immune function or modulation of immune function in breeding rodents (reviewed in Refs. 19, 26). These studies demonstrated that sex differences in cell-mediated and humoral immune function are enhanced by social housing in polygynous voles (18, 20). Therefore, in general, short days enhance immune responses (25, 31), and nonagonistic social relationships facilitate recovery from immune challenges (5, 11, 17).

The present study examines the effects of pair housing (with a sibling male or nonsibling female) vs. single housing on both reproductive and immune responses in male white-footed mice housed in either long or short photoperiods. White-footed mice are generally considered polygynous (37), and males are primarily solitary during the breeding season but huddle in communal nests during the winter (23, 38). We predicted that the social stimulation provided by housing females with males would override the inhibitory and stimulatory effects of short days on reproduction and immune response, respectively. We also predicted that housing males with males in short days would not affect the already regressed reproductive parameters, whereas male-male pairings in long days would result in...
slightly reduced reproductive parameters. On the other hand, we expected that heterosexual pairing would subtly enhance the reproductive parameters of long-day males. Finally, we hypothesized that social stimulation, regardless of the sex of the stimulus mouse, would alter immune responses in long-day males.

**EXPERIMENTAL PROCEDURES**

**Animals**

Seventy-two male and twenty-four female adult (>55 days of age) white-footed mice (P. leucopus) from a breeding colony maintained at Ohio State University were used in this study. Breeder mice were originally obtained from the Peromyscus Genetic Stock Center at the University of South Carolina (Columbia, SC). Mice were housed in polypropylene cages (27.8 × 7.5 × 13 cm) with a constant temperature and humidity of 21 ± 5°C and 50 ± 5%, respectively, and ad libitum access to food (Harlan Teklad 8640 rodent diet, Indianapolis, IN) and filtered tap water. Mice were housed in either long photoperiods (LD: n = 30 males) with a reverse 16:8-h light-dark cycle (lights on at 2300 EST) or in short photoperiods (SD: n = 42 males) with an 8:16-h light-dark cycle (lights on at 0700 EST). Within these photoperiod treatments, male mice were housed under one of three social conditions: 1) individually (single group; LD: n = 10; SD: n = 13), 2) with a male sibling (+male group; LD: n = 10; SD: n = 14), or 3) with a nonsibling, ovariectomized female (+female group; LD: n = 10; SD: n = 14). Male siblings were used for the male–male pairs to reduce fighting. The photoperiod and social conditions were maintained for the duration of the 14-wk study. Animals were left undisturbed except for routine cage changing. All studies were conducted with approval of the Ohio State Institutional Animal Care and Use Committee and were conducted in compliance with all US federal animal welfare requirements.

**Delayed-type Hypersensitivity**

After 12 wk of exposure to the designated photoperiod and social conditions, immune responsiveness was assessed via delayed-type hypersensitivity (DTH) by sensitizing the mice to 2,4-dinitro-1-fluorobenzene (DNFB; Sigma, St. Louis, MO) (29). All DTH procedures occurred between 0900 and 1100. For sensitization, mice were anesthetized with isoflurane vapor (Minrad, Bethlehem, PA), fur on the animal’s dorsum was shaved, and 50 μl of DNFB [0.5% (wt/vol) in 4:1 acetone/olive oil] was applied to the skin. Sensitization was repeated the next day. Eight days later, after light anesthetization, baseline thickness of both pinnae was measured with a constant-loading dial micrometer (Mitutoyo America, Aurora, IL), and DNBF immune response was challenged by applying 20 μl of DNFB [0.3% (wt/vol) in 4:1 acetone/olive oil] to the skin of the dorsal surface of the right pinnae. Left pinnae were treated with vehicle. Measurement of pinna thickness was repeated every day under light anesthetization for 1 wk. All females were also treated with DNFB to control for possible effects of DNFB treatment on social behavior. Mice that were pair-housed were anesthetized simultaneously to control for the potential stressor of disturbing their cage multiple times.

**Tissue Collection**

Male mice were rapidly decapitated after final ear measurements were made and trunk blood was collected. Blood was allowed to clot at room temperature for at least 30 min, clots were removed, blood was spun at 2500 rpm for 30 min at 4°C, and serum was stored at −70°C until testosterone and corticosterone concentration assessments. Paired testes and spleens were removed and weighed. The average testes mass for single-LD male mice was determined, and single-SD male with testes mass two standard deviations below this mean were considered reproductively responsive to short days. One single-SD mouse failed to meet this criterion and was dropped from the study. Responsiveness was not determined in any of the pair-housed groups because of the possible effects of social environment on testes mass (14).

**RIA Procedures**

Serum testosterone and corticosterone concentrations were determined using 125I kits purchased from ICN Biomedicals (Costa Mesa, CA). Each sample was assessed in duplicate in a single assay according to the manufacturer’s protocol with one exception. Because corticosterone concentrations in Peromyscus are elevated relative to Mus musculus and Rattus rattus, serum was diluted 5.2-fold more than recommended for other rodents, and two additional standard dilutions were added to the low end of the standard curve. Cross-reactivity with other steroid hormones is <3.5% for testosterone and <0.5% for corticosterone. Intra-assay variance was <10% for both assays, with minimum detection levels of 0.1 ng/ml for testosterone and 5 ng/ml for corticosterone.

**Statistical Analyses**

Three-by-two ANOVA tests were used to compare housing treatment by photoperiod groups. A repeated-measures ANOVA was used to compare DTH data across days. Within days, multiple pairwise comparisons were planned a priori in the analysis models and were conducted with Student’s t-tests (16). Data with unequal variances were compared with the use of nonparametric tests; Kruskal-Wallis for housing comparisons and Mann-Whitney for photoperiod comparisons were also used. All comparisons were considered statistically significant at P < 0.05. StatView software was used for all analyses (version 5.0.1; Cary, NC).

**RESULTS**

**Tissue Mass**

There was a main effect of photoperiod such that short days decreased paired testes mass in all mice, regardless of social environment (Fig. 1A; F1,62 = 39.24; P < 0.001). LD mice housed with females had larger testes than mice housed with males (P < 0.05). Testes mass did not differ among social groups in SD (P > 0.05). Although there were no main effects, within SD, mice housed with females had larger spleens than mice housed alone (Fig. 1B; P < 0.05). Spleen mass did not differ among any other groups (P > 0.05). All differences remained the same after analyzing with body mass as a covariate.

**DTH**

There was a main effect of housing (F2,59 = 6.045; P < 0.005) such that single-housed mice exhibited an enhanced DTH response compared with mice housed with females (P < 0.05). Consistent with previous studies, there was an effect of photoperiod on single-housed animals such that short days enhanced the DTH response (Fig. 2A; P < 0.05) but not in either the pair-housed groups (P > 0.05).

**Long days.** Within LD, there was a simple main effect of housing (F2,229 = 2.891; P < 0.05). In LD, mice housed with females displayed lower DTH responses compared with single-housed mice on day 4 post-DNFB challenge and compared with both single-housed and male-paired mice on days 5 and 6 (Fig. 2B; P < 0.05 in all cases). On day 7 post-DNFB challenge, both pair-housed groups exhibited a lower DTH response compared with single-housed mice (P < 0.05).
Short days. Within SD, there was a simple main effect of housing ($F_{2,316} = 5.487; P < 0.005$). Paired groups did not differ ($P > 0.05$); therefore, data were combined for analyses. Pair-housed mice displayed a decreased DTH response compared with single-housed males on days 1, 2, and 7 post-DNFB challenge (Fig. 2C; $P < 0.05$).

Serum Hormone Concentrations

Testosterone. There were main effects of photoperiod ($F_{1,71} = 21.353; P < 0.001$) and housing condition ($F_{2,71} = 4.854; P < 0.05$) on testosterone concentrations. Short days decreased serum testosterone concentrations in all mice, regardless of social environment ($P < 0.05$). All mice housed with females had higher testosterone concentrations than either single-housed or male-paired mice, regardless of photoperiod ($P < 0.05$). Of the mice housed in LD, those paired with females had higher testosterone concentrations than those housed alone (Fig. 3A; $P < 0.05$). In SD, differences in
Because short-day males continued to regress their reproductive tracts in the presence of a female, it appears that reproduction in *P. leucopus* is influenced more by photoperiod than by social environment. *P. aztecs*, on the other hand, which resides at low latitudes and displays a greater enhancement of reproductive parameters in response to female-pairing than photoperiod treatment, appears to be more sensitive to social cues than to photoperiod (10). Reproductive involution of short-day white-footed mice, despite the presence of a female, contradicts previous results in male short-day Siberian hamsters paired with a female (14); male hamsters did not respond to inhibitory photoperiods. Taken together, it seems plausible that species differences exist. Given the lack of field data on Siberian hamsters, we can only speculate that species differences in social influence may represent differences in life history strategies. Also, pairing with reproductively intact (as opposed to ovariectomized) females may be necessary to block reproductive regression in short days as it has been demonstrated in Siberian hamsters and juvenile *P. maniculatus* (14, 35). This seems unlikely, however, because exposure to short days inhibits female *P. leucopus* reproductive status, behavior, and fecundity (1, 34) and reduces reproductive tract mass and estradiol concentrations in *P. maniculatus* (deer mice; an ecologically similar related species; Refs. 9, 36), resulting in functional ovariectomy of females. Also, acute exposure of male mice to a female after weeks of long- or short-photoperiod exposure may affect reproductive and immune parameters differently than chronic exposure and remains to be tested.

Our results also appear to contrast with data on reproductive development of male deer mice exposed to females; reproductive development of juvenile males paired with adult conspecific females is stimulated in short days (35). To our knowledge, similar studies have not been conducted in adults of this species, and the influence of social environment may differ between adolescence and adulthood. Perhaps precocious reproductive maturity is less energetically costly than maintaining breeding condition out of season as an adult.

Considering that in field studies *P. leucopus* have been observed to exhibit intraspecific huddling with the opposite sex during the winter (23, 38), the lack of effect of social housing on reproductive status may be adaptive. Huddling enhances energetic conservation in this species and is triggered by short photoperiods (21). If social housing attenuated reproductive regression, then mice might be stimulated to breed year round with potentially negative fitness consequences (30).

The presence of a female increased serum testosterone concentrations and testes mass in LD mice. These data support previous findings that the presence of a female cagemate increases initial testosterone concentrations (1 h to 2 wk after cohabitation with a female) in *Mus musculus* and *P. californicus* (22, 33). Suppression of reproductive parameters, on the other hand, by male-male pairings was evident in the present study but only statistically significant for testosterone concentrations of LD mice. Inhibitory effects of male-male pairings have been previously described in deer mice (2). The present study suggests that reproductive status can only be modified by social environment during the breeding season in *P. leucopus*. The functional significance of elevated testosterone concentrations and testes mass in LD males paired with females remains to be determined.

**DISCUSSION**

In the present study, the presence of a female did not override the inhibitory effects of short days on male reproductive responses. In long days, however, the presence of a female increased both testosterone concentrations and testes mass compared with single-housed and male-paired mice, respectively. Additionally, although single-housed males in short days displayed a more robust immune response than those in long days, male or female pairing decreased immune response in both photoperiods. The blunted immune response in males paired with females correlated with low circulating corticosterone concentrations in both photoperiod treatments.

**Corticosterone.** There was a main effect of photoperiod on corticosterone concentrations (*F*<sub>1,71</sub> = 13.288; *P* < 0.001). Short days decreased serum corticosterone concentrations in all mice (*P* < 0.05; Fig. 3C). All mice housed with females had lower corticosterone concentrations than either single-housed or male-paired mice, regardless of photoperiod (*P* < 0.05). SD mice that were single housed or male paired had lower corticosterone concentrations compared with their respective LD groups (*P* < 0.05); however, this comparison was not statistically significant between the female-paired groups (*P* = 0.06).

**Testosterone.** There was a main effect of photoperiod on testosterone concentrations (*F*<sub>1,71</sub> = 13.288; *P* < 0.001). Long days increased testosterone concentrations compared with single-housed and male-paired mice, respectively. Additionally, although single-housed males in short days displayed a more robust immune response than those in long days, male or female pairing decreased immune response in both photoperiods. The blunted immune response in males paired with females correlated with low circulating corticosterone concentrations in both photoperiod treatments.
The initial enhanced DTH response in SD single-housed mice relative to LD mice was similar to that reported in Siberian hamsters (3, 4). We also observed that, in the present study, the presence of a cagemate (regardless of sex) decreased immune responses on the last day of pinna measurement in both photoperiods. Therefore, pair housing (regardless of sex) appears to modulate immune response in white-footed mice, results that are consistent with the immunomodulatory effects in previous studies on humans, Siberian hamsters, and Mus musculus (11, 13, 15, 17).

Coincident with altered immune responses, corticosterone concentrations decreased in female-paired mice in both photoperiods. Corticosterone concentrations are considered a physiological marker of a “stress response” (32), suggesting that male isolation or housing with another male triggers a higher stress response than males housed with females. Short photoperiods also decreased corticosterone concentrations in white-footed mice compared with long days, similar to collared lemmings (Dicrostonyx groenlandicus) but opposite to Siberian hamsters and prairie voles (Microtus ochrogaster; Refs. 4, 27). DTH response has been correlated both positively and negatively with corticosterone concentrations, and these discrepancies have been postulated to be associated with the duration of a stressor (i.e., acute or chronic; Ref. 12). In our photoperiodic model, it is possible that a stressor (i.e., social condition or photoperiod treatment) may exist; however, given the role of corticosterone in energy mobilization, corticosterone concentrations may reflect seasonal metabolic strategies. Specifically, corticosterone stimulates food intake (6). Decreased corticosterone concentrations in short days may decrease food intake and therefore mediate the metabolic deceleration thought to promote winter survival. This hypothesis is supported by the uncoupling of corticosterone and DTH responses in pair-housed mice in the present study. However, in male Siberian hamsters, corticosterone concentrations positively correlated with DTH response following a restraint stressor (Ref. 3; but see females, Ref. 4).

Similar to the sex-dependent housing effect apparent in our reproductive observations, a female cagemate curtailed immune response in short days on more consecutive days postimmunotherapy challenge than a male cagemate. Spleen mass, a potential indicator of immune activity, however, tended to decrease with increasing DTH responses. Similar to those in SD, LD males paired with females displayed decreased DTH responses compared with those with either a male cagemate or no cagemate. Overall, our results suggest that having a cagemate (particularly of the opposite sex) decreases DTH responses and corticosterone concentrations. These results suggest that reproductive responsiveness to photoperiod is less plastic than immune responsiveness to photoperiod in white-footed mice. Field studies are necessary to support the ecological significance of these results, and comparative studies in females might reveal potential sex differences.

The differential effects of social environment on immune and reproductive measures indicate that social influences vary based on individual photoperiodic traits. These differences may represent the cost of plasticity (i.e., capacity to change based on season) of particular photoperiodic traits. The lack of influence of cohabitation (regardless of sex) on reproduction in short days may represent the resilience of reproductive inhibition in the winter. Therefore, the cost of maintaining reproductive readiness in short days may be greater than the benefit. Previous studies suggest that some traits of an individual can be “nonresponsive” to the effects of short days, whereas other traits are responsive (30). The ability of social stimulation to suppress immune response and corticosterone secretion suggests that the cost of immune plasticity may be less expensive than the cost of reproductive plasticity.

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