J Comp Physiol B (1998) 168: 419–426

© Springer-Verlag 1998

ORIGINAL PAPER

G. E. Demas · R. J. Nelson

Short-day enhancement of immune function is independent of steroid hormones in deer mice (*Peromyscus maniculatus*)

Accepted: 17 April 1998

Abstract The effects of photoperiod and steroid hormones on immune function were assessed in male and female deer mice (*Peromyscus maniculatus*). In experiment 1, male deer mice were castrated, castrated and given testosterone replacement, or sham-operated. Half of each experimental group were subsequently housed in either long (LD 16:8) or short days (LD 8:16) for 10 weeks. Short-day deer mice underwent reproductive regression and displayed elevated lymphocyte proliferation in response to the T-cell mitogen concanavalin A, as compared to long-day mice. In experiment 2, female deer mice were ovariectomized, ovariectomized and given estrogen replacement, or sham-operated. Animals from each of these experimental groups were subsequently housed in either LD 16:8 or LD 8:16 for 10 weeks. Short-day deer mice underwent reproductive regression and displayed reduced serum estradiol concentrations and elevated lymphocyte proliferation in response to concanavalin A, as compared to long-day mice. Surgical manipulation had no effect on lymphocyte proliferation in either male or female deer mice. Neither photoperiod nor surgical manipulation affected serum corticosterone concentrations. These results confirm that both male and female deer mice housed in short days enhance immune function relative to long-day animals. Additionally, short-day elevation in splenocyte proliferation appears to be independent of the influence of steroid hormones in this species.

Key words Testosterone · Estrogen · Seasonal · Melatonin · Lymphocyte

Abbreviations *Con A* concanavalin A · *EST* Eastern Standard Time · *EWAT* epididymal white adipose tissue · *MTS* 3-(4,5-demethylthiazole-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium · *OD* optical density · *PMS* phenazine methosulphate · *RIA* radioimmunoassay

G.E. Demas (✉) · R.J. Nelson
Department of Psychology, The Johns Hopkins University, Baltimore, MD 21218, USA
e-mail: demas@ren.psy.jhu.edu,
Tel.: +1 410-516-7396, Fax: +1 410-516-6205

Introduction

Studies of seasonal changes in mammalian physiology have emphasized seasonal fluctuations in reproductive function (reviewed in Bronson and Heideman 1994; Turek and Van Cauter 1994). However, reproduction is only one of many physiological processes that display seasonal fluctuations. For example, many rodent species exhibit seasonal fluctuations in thermoregulation, metabolism, pelage, and body mass (reviewed in Lynch 1973; Moffatt et al. 1993; Ruby and Zucker 1992). Although less commonly studied, seasonal changes in disease and mortality also exist (John 1994; Lochmiller et al. 1994; Nelson and Demas 1996). In many species, disease and death rates typically peak in the fall and winter and reach an annual minimum in spring and summer (reviewed in Nelson and Demas 1996). Many animals die from infectious diseases in the winter that appear to overwhelm immunological defenses, presumably at times when these defenses are lowest. Several field studies have demonstrated reduced immune function and increased disease susceptibility during winter relative to summer (Afoke et al. 1993; Boctor et al. 1989; John 1994; Lochmiller et al. 1994). For example, cotton rats (*Sigmodon hispidus*) have lower white blood cell counts in winter than in summer (Lochmiller et al. 1994). Ground squirrels (*Citellus richardsoni*) display lower antibody concentrations in response to sheep red blood cells in winter than in summer (Sidky et al. 1972). Additionally, outbreaks of European brown hare syndrome display a strong seasonal fluctuation in *Lepus europeus* with peak incidence during the winter (Gavier-Widen and Morner 1991).

Suppression of immune function during the winter likely reflects physiological coping with environmental

conditions (e.g., reduced ambient temperatures, reduced food availability) that create energetic shortages (Demas and Nelson 1996; Demas et al. 1997b). One direct result of reduced energetic availability appears to be compromised immune function (Demas and Nelson 1997b; Miller et al. 1994; Sheldon and Verhulst 1996).

To increase the chances of surviving the energetic demands of winter, individuals of some rodent species may have evolved mechanisms to bolster immune function prior to the deterioration of environmental conditions (Demas and Nelson 1996; Nelson and Demas 1996). Consistent with this hypothesis, the results of most laboratory studies examining photoperiodic changes in immune function report enhanced immune function among individuals maintained in short, winter-like days (Brainard et al. 1988; Champney and McMurray 1991; Demas and Nelson 1996; Vaughan et al. 1987). Although the precise mechanisms mediating enhanced immune function in short photoperiods are not known, it has been suggested that increased immune function may be due to increased duration of melatonin secretion in short days (Brainard et al. 1988; Champney and McMurray 1991; Demas et al. 1996). Melatonin can act directly on target tissue within the immune system leading to enhanced immune function (Poon et al. 1994). Consistent with this direct effect, treatment of animals with exogenous melatonin enhances both humoral and cell-mediated immunity in mammalian species that are, in general, reproductively non-responsive to melatonin (i.e., rats, house mice, humans) (Guerrero and Reiter 1992; Maestroni 1993). Additionally, melatonin receptors have been identified on circulating lymphocytes, thymocytes, and splenocytes in these same species (Calvo et al. 1995; Lopez-Gonzales et al. 1992; Rafii-El-Idrissi et al. 1995).

However, melatonin is only one of several hormones affected by photoperiod. Short days change the pattern of melatonin secretion, which inhibits reproductive function in many rodent species, along with concomitant decreases in circulating concentrations of gonadal steroid hormones and prolactin (e.g., Gaston and Menaker 1967; Johnston and Zucker 1979; Seegal and Goldman 1975). Both estrogens and androgens affect immune function. Testosterone, the primary androgenic hormone secreted in males of most species, typically suppresses immune function (reviewed in Grossman 1984, 1985; Konstadoulakis et al. 1995; McCruden and Stimson 1991). For example, castration of adult male rodents increases both humoral and cellular immunity, as well as increasing lymphatic organ mass (e.g., spleen, thymus) (Schuurs and Verheul 1990). Additionally, treatment of castrated males with physiological doses of testosterone returns immune function to pre-castration levels (Grossman 1984; Schuurs and Verheul, 1990). Thus, increased immune function in male rodents housed in short days or treated with melatonin may be due to reduced circulating testosterone concentrations (i.e., a "release" from the immunocompromising effects of testosterone) rather than a direct effect of melatonin on immune function. If true, then castrated male deer mice (with basal levels of testosterone) should not display enhanced immune function in short days.

In contrast to testosterone, estradiol, the primary estrogenic hormone secreted in females, generally enhances immune function (Grossman 1984, 1985). Treatment of intact male or female rats with physiological or supraphysiological doses of estrogen increases antibody response to a variety of antigens (Brick et al. 1985; Inman 1978; Myers and Peterson 1985). Thus, if photoperiod-mediated changes in immune function are due to fluctuations in circulating estrogen concentrations, then female deer mice maintained in short days or administered short-day levels of melatonin should display reduced circulating estrogen concentrations and reduced immune function (i.e., a "release" from the immunoenhancing effects of estrogen). Additionally, photoperiodic changes in immune function should be abolished in ovariectomized mice (with basal circulating levels of estrogen). However, the effects of estrogen on immune function have been examined almost exclusively in long-day-housed animals and in species that are generally reproductively unresponsive to photoperiod. The role of estrogen in mediating photoperiodic changes in immune function has not been directly tested and remains an empirical question.

Alternatively, photoperiodic changes in immune function may be mediated by glucocorticoid hormones (reviewed in Nakono et al. 1987). Glucocorticoids, primarily corticosterone in rodents, are released from the adrenal glands in response to stress and can suppress immune function (Ader and Cohen 1993; Baxter and Forsham 1972; Black 1994; Clamin 1972). Suppressed immune function during winter typically reported in field studies likely reflects increased glucocorticoid secretion in response to environmental stressors (e.g., low ambient temperatures, reduced food availability) (Nelson and Demas 1996). Similarly, experimental manipulation of these factors can suppress immune function in the laboratory (Bhatnagar et al. 1995; Demas and Nelson 1996; Demas and Nelson 1998; Murphy and Wideman 1992). Maintaining animals in short days or administration of either melatonin or a melatonin agonist attenuates the glucocorticoid stress response and enhances immunity (Bolinger et al. 1996; Demas et al. 1997b; Maestroni et al. 1986). Similarly, chronic melatonin treatment decreases the density of glucocorticoid receptors in rats (Persengiev et al. 1991) and may alter the physiological response to glucocorticoids. Thus, short-day enhancement of immune function may represent the indirect effects of melatonin on glucocorticoids. The present studies were conducted to test the hypothesis that short-day enhancement of immune function is mediated directly by melatonin rather than indirectly via reduced gonadal steroid concentrations.

Materials and methods

Experiment 1

Sixty adult male (> 60 days of age) mice (*Peromyscus maniculatus bairdii*) were obtained from our laboratory breeding colony. This colony was originally derived from the *Peromyscus* Genetic Stock Center at the University of South Carolina, Columbia, S.C. These animals are descendants of deer mice originally trapped near East Lansing, Michigan (latitude = 42°51′N). Deer mice were weaned at 21 days of age and housed with same-sex siblings. Temperature was kept constant at 20 °C and relative humidity was maintained at 50 ± 5%. Food (Agway Prolab 1000, Syracuse, N.Y.) and tap water were provided ad libitum throughout the experiment. Two weeks prior to the initiation of the experiment, all animals were individually housed in polypropylene cages (27.8 × 7.5 × 13.0 cm) in colony rooms with a 24 h LD 16:8 light cycle [lights on 0600 hours Eastern Standard Time (EST)].

Forty male deer mice were castrated under sodium pentobarbital anesthesia (50 mg/kg); half of the castrated deer mice were implanted s.c. with a 15-mm-long Silastic capsules (1.47 mm i.d., 1.95 mm o.d., Dow Corning Corporation, Midland, Mich.) filled with testosterone proprionate crystals (10 mm; Sigma, St. Louis, Mo.) to maintain normal physiological blood levels of this hormone (Kriegsfeld et al. 1997). The remaining castrated deer mice were implanted with empty capsules. Twenty intact deer mice underwent sham operations and were subsequently implanted with empty capsules.

Animals were allowed to recover for 1 week and were then randomly selected and assigned to one of two photoperiodic conditions. Half of the animals from each experimental condition (n = 10/group) were housed under a short-day photoperiod (LD 8:16), while the remaining animals (n = 10/group) were housed under long days (LD 16:8). Animals were maintained in their respective conditions for 10 weeks. Subsequently, they were brought into the surgery room, lightly anesthetized with methoxyflurane vapors, and blood samples were taken from the retro-orbital sinus. Handling time was kept consistent and to a minimum; the time from initial removal from the cage to the end of bleeding was less than 3 min. Animals were then killed by cervical dislocation between 0900 and 1000 hours EST and their spleens were removed under aseptic conditions and immediately suspended in culture medium (RPMI-1640/HEPES). Paired testes and epididymides, seminal vesicles, and epididymal fat pads were removed and cleaned of connective tissue. Seminal vesicles were compressed with a glass vial to remove seminal fluid.

Experiment 2

Forty-two female deer mice (> 60 days of age) were obtained from our breeding colony as described above. Twenty-eight mice were ovariectomized under sodium pentobarbital anesthesia (50 mg/kg). Half of the ovariectomized deer mice were implanted s.c. with a 15-mm-long Silastic capsule (1.47 mm i.d., 1.95 mm o.d., Dow Corning Corporation, Midland, Mich.) filled with 17β-estradiol crystals (10 mm; Sigma, St. Louis, Mo.) to maintain normal physiological blood levels of this hormone (Bronson 1976). The other half were implanted with empty capsules. Fourteen intact deer mice underwent sham operations and were implanted with empty capsules only. Animals were allowed to recover for 1 week and then randomly selected and assigned to long (n = 7/group) or short days (n = 7/group) as in experiment 1. Mice were maintained in their respective conditions for 10 weeks and then killed. Spleens were removed under aseptic conditions and immediately suspended in culture medium (RPMI-1640/HEPES). Uterine horns and ovarian fat pads were removed, cleaned of connective tissue, and weighed.

Silastic capsules containing testosterone in experiment 1 or 17β-estradiol in experiment 2 were soaked in physiological saline at 37 °C for 24 h to prevent a bolus of hormone from being released when implanted. All organs from both experiments were weighed by laboratory assistants naive to the experimental hypotheses and treatment assignments.

Immunological assay

Splenocyte proliferation in response to the T-cell mitogen concanavalin A (Con A) was determined using a colormetric assay based on the tetrazolium salt 3-(4,5-demethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS). Splenocytes were separated from tissue by compressing the whole spleen between sterile frosted glass slides; separated cells were suspended in 4 ml of culture medium (RPMI-1640/HEPES) supplemented with 1% penicillin (5000 U ml^{-1})/streptomycin (5000 µl/ml^{-1}), 1% L-glutamine (2 mM/ml), 0.1% 2-mercaptoethanol (5×10^{-2} M/ml), and 10% heat-inactivated fetal bovine serum). Splenocyte counts and viability were determined with a hemacytometer and trypan blue exclusion. Viable cells (which exceed 95%) were adjusted to 2×10^6 cells/ml by dilution with culture medium, and 50 µl aliquots of each cell suspension (i.e., 100 000 cells) were added to the wells of sterile flat-bottom 96-well culture plates. Con A (Sigma Chemical, St. Louis, Mo.) was diluted with culture medium to concentrations of 40, 20, 10, 5, 2.5, 1.25, and 0.6 µg/ml; 50 µl of each mitogen concentration was added to the wells of the plate containing the spleen cell suspensions to yield a final volume of 100 µl/well (each in duplicate). Plates were incubated at 37 °C with 5% CO_2 for 48 h prior to addition of 20 µl of MTS/PMS solution [Promega; 0.92 mg/ml of phenazine methosulphate (PMS) in sterile Dulbecco's phosphate buffered saline] per well. Plates were then incubated at 37 °C with 5% CO_2 for an additional 4 h. The optical density (OD) of each well was determined with a microplate reader (Bio-Rad: model No. 3550) equipped with a 490 nm wavelength filter. Mean OD values for each set of duplicates were used in subsequent statistical analyses. Dose response curves were constructed using group means of the mean OD values at each mitogen concentration and unstimulated cultures.

Steroid hormone assays

Serum testosterone, estradiol, and corticosterone concentrations were determined by radioimmunoassay (RIA) with the use of ^{125}I kits purchased from ICN Biomedicals (Costa Mesa, Calif.). All instructions furnished by ICN were followed. Each sample was assayed in duplicate. All of the steroid assays were highly specific; cross reactions with other steroid hormones were less than 0.3%. The coefficient of variability was less than 15% for all samples. Serum testosterone, estradiol, and corticosterone concentrations were determined in separate RIAs.

Statistical analyses

Reproductive organ mass data were analyzed using a 2 (photoperiod) × 3 (surgical manipulation) analysis of variance (ANOVA). Analyses on ranked sums were conducted in cases where a violation of normality occurred. Splenocyte proliferation data were analyzed using a 2 (photoperiod) × 3 (surgical manipulation) × 8 (Con A concentration) mixed model ANOVA. Post-hoc comparisons between pairwise means were conducted using Tukey HSD tests and differences between group means were considered statistically significant if $P < 0.05$.

Results

Experiment 1

One animal from the long-day, sham-castrated group and one animal from the short-day, castrated,

testosterone-treated group died before the end of the experiment and these animals were not included in subsequent data analyses. The remaining groups consisted of the ten originally assigned mice. Short-day mice displayed significantly higher splenocyte proliferation to Con A than did long-day animals regardless of surgical manipulation ($P < 0.05$ in all cases). However, there were no differences in lymphocyte proliferation among castrated, sham-operated, or castrated testosterone-treated mice within either photoperiod ($P > 0.05$ in both cases). Sham-castrated deer mice housed in short days had significantly smaller paired testes and epididymides, seminal vesicles, and epididymal white adipose tissue (EWAT) than sham-castrated animals housed in long days ($P < 0.05$ in all cases) (Fig. 1; Table 1). Additionally, castrated long-day mice had significantly smaller paired epididymides, seminal vesicles, EWAT, and serum testosterone concentrations compared to either sham-operated or castrated, testosterone-treated long-day animals ($P < 0.05$ in all cases) (Fig. 1; Table 1). Castrated mice treated with testosterone did not display photoperiodic changes in reproductive organ masses or serum testosterone concentrations ($P > 0.05$ in all cases). There was no difference in serum testosterone concentrations between long- and short-day mice ($P > 0.05$ in both cases). Body mass and serum corticosterone concentrations did not differ among any of the experimental groups ($P > 0.05$ in both cases).

Experiment 2

One mouse from the long-day, sham-ovariectomy group died before the end of the experiment and this animal was not used in subsequent data analyses. All the remaining groups consisted of the seven originally assigned mice. Short-day ovariectomized and ovariectomized, estrogen-treated mice displayed significantly higher splenocyte proliferation to Con A than did long-day animals ($P < 0.05$ in both cases). Short-day, sham-ovariectomized mice did not differ from long-day mice in splenocyte proliferation ($P > 0.05$). There were no differences in lymphocyte proliferation between ovariectomized, ovariectomized estradiol-treated, or sham-operated mice within each photoperiodic condition ($P > 0.05$ in all cases) (Fig. 2). Sham-ovariectomized deer mice housed in short days had significantly smaller uterine horns and serum estradiol concentrations than animals housed in long days ($P < 0.05$) (Fig. 2). Both long and short-day ovariectomized animals had significantly smaller uterine horns and serum estradiol concentrations than did either sham-operated or ovariectomized, estradiol-treated animals maintained in long days ($P < 0.05$ in all cases). Body mass and serum corticosterone concentrations did not differ among any of the experimental groups ($P > 0.05$ in all cases; Table 2).

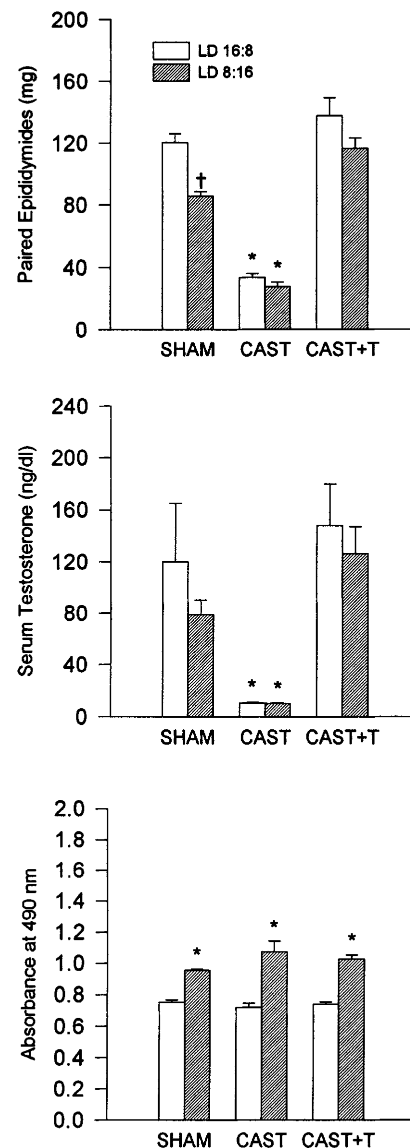

Fig. 1 Paired epididymal masses (*top panel*), serum testosterone concentrations (*middle panel*), and splenocyte proliferation to Concanavalin A (*Con A*) (10 μg/ml, measured in absorbance units) (*bottom panel*) in castrated deer mice (*CAST*), castrated mice given testosterone replacement (*CAST + T*), and sham-operated mice (*SHAM*) maintained in either long- (LD 16:8) (open bars) or short days (LD 8:16) (filled bars). Columns with no symbol or sharing the same symbol are statistically equivalent. Columns with different symbols are significantly different at $P < 0.05$. Splenocyte proliferation is measured in absorbance units at 490 nm. Only the optimal concentration of Con A (i.e., the concentration that stimulated the highest amount of cell proliferation) is graphically represented

Discussion

Both male and female deer mice responded to short days by displaying reproductive regression and elevated splenocyte proliferation in response to Con A. Surgical manipulation (i.e., castration or ovariectomy) reduced circulating gonadal steroid hormones in males and females, respectively, but had no effect on splenocyte

Table 1 Mean (±SEM) body, seminal vesicle, and fat pad masses and serum corticosterone concentrations in male deer mice. Entries without symbols or sharing symbols are statistically equivalent. Entries with different symbols are significantly different at $P < 0.05$. (*SHAM* sham-operated, *CAST* casterated, *T* testosterone replacement, *EWAT* epididymal white adipose tissue, *LD* light:dark regime)

	LD 16:8			LD 8:16		
	SHAM	CAST	CAST+T	SHAM	CAST	CAST+T
Body mass (g)	20.1 ± 0.5	19.4 ± 0.6	19.6 ± 0.5	20.8 ± 0.7	20.6 ± 0.5	19.5 ± 0.4
Seminal vesicles (mg)	140.5 ± 0.7	34.1 ± 8.7†	130.1 ± 9.5	84.3 ± 2.0*	28.9 ± 1.8*	117.8 ± 8.6*
EWAT (mg)	226.7 ± 17.8	68.6 ± 13.5*	209.7 ± 21.5	219.7 ± 13.1	90.1 ± 10.3*	168.7 ± 25.8
Corticosterone (ng/ml)	135.3 ± 21.3	117.3 ± 20.2	112.1 ± 21.3	115.3 ± 26.1	110.6 ± 28.6	175.3 ± 28.6

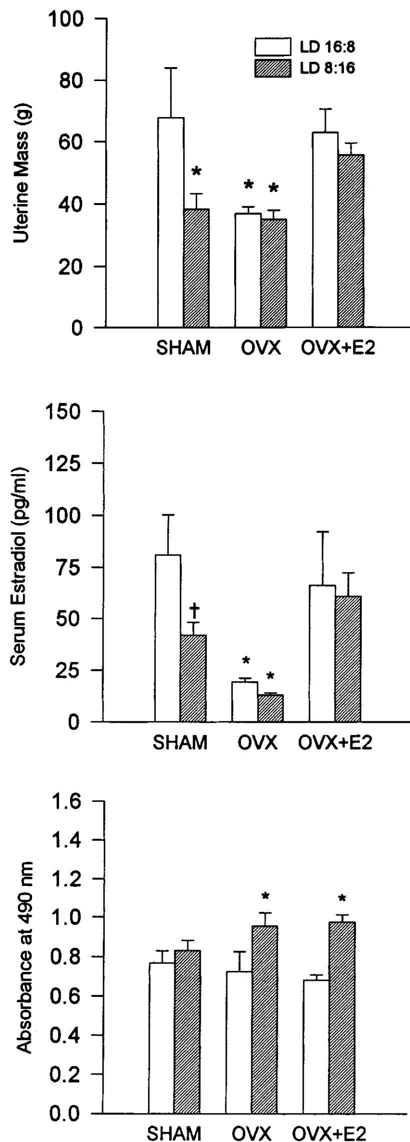

Fig. 2 Uterine horn masses (*top panel*), serum estradiol concentrations (*middle panel*), and splenocyte proliferation to Con A (measured in absorbance units) (*bottom panel*) in ovariectomized deer mice (*OVX*), ovariectomized mice given estradiol replacement (*OVX + E2*), and sham-operated mice (*SHAM*) maintained in either long- (LD 16:8) or short days (LD 8:16). As in Fig. 1, only the optimal concentration of Con A (10 μg/ml) is graphically represented. Other symbols and conventions are as in Fig. 1

proliferation in either sex. Further, hormone replacement returned both serum testosterone and estradiol to pre-surgery concentrations, but did not affect immune function. Corticosterone concentrations did not differ among any of the experimental groups in either experiment. These results suggest that short-day enhancement of immune function is not mediated by changes in steroid hormone concentrations in male or female deer mice. Rather, these results are consistent with a direct effect of melatonin on immune function.

The pineal gland and its primary hormone melatonin can affect lymphatic tissue size as well as immune cell numbers and function (reviewed in Nelson et al. 1995; Demas and Nelson 1998b; Vaughan et al. 1987). In almost all cases examined to date, administration of melatonin enhances both humoral and cell-mediated immunity in rodents (reviewed in Maestroni 1993; Nelson et al. 1995). For example, exogenous melatonin treatment elevates both in vivo and in vitro antibody responses in both normal and immunodeficient mice (Guerrero and Reiter 1992; Maestroni 1993). Melatonin also enhances splenocyte proliferation to the T-cell mitogen Con A (Demas et al. 1996). Similarly, pinealectomy impairs both cellular immune responses and natural killer cell activity, and causes premature involution of immune tissue in rodents (Csaba and Barath 1975; Del Gobbo et al. 1989; Jancovic et al. 1970).

The mechanisms by which melatonin mediates immune function are not known, although melatonin has been suggested to exert a direct effect on lymphoid tissue (Brainard et al. 1988; Champney and McMurray 1991; Poon et al. 1994). Consistent with the hypothesis of a direct effect on immunity, melatonin receptors have been isolated on circulating lymphocytes (Calvo et al. 1995; Liu and Pang 1993; Pang and Pang 1992), as well as on thymoctes and splenocytes (Lopez-Gonzales et al. 1993; Rafii-El-Idrissi et al. 1995). Alternatively, melatonin can act indirectly on immune function by altering circulating non-steroidal hormone concentrations. For example, exposure to short days or infusions of melatonin that mimic short-day concentrations, significantly reduces prolactin concentrations in virtually all rodent species examined (Bartness et al. 1993; Goldman and Nelson 1993). Prolactin can have diverse effects on the immune system. Generally, prolactin enhances or maintains normal immune function (Nelson et al. 1995; Reber 1993). However, prolactin can also inhibit immunity,

Table 2 Mean (±SEM) body and uterine horn masses and serum corticosterone concentrations in female deer mice. Symbols and conventions as in Table 1. (*OVX* ovariectomized mice, E_2 estradiol replacement)

	LD 16:8			LD 8:16		
	SHAM	OVX	OVX+E2	SHAM	OVX	OVX+E2
Body mass (g)	20.3 ± 0.5	20.3 ± 0.4	19.4 ± 0.3	20.2 ± 0.6	19.6 ± 0.5	19.5 ± 0.9
Uterine mass (mg)	67.8 ± 16.2	36.8 ± 2.3*	62.9 ± 7.6	38.3 ± 5.1*	35.0 ± 2.9*	55.6 ± 3.9
Corticosterone (ng/ml)	216.9 ± 78.8	360.3 ± 80.2	309.1 ± 59.0	169.9 ± 59.0	345.9 ± 78.6	198.4 ± 32.6

especially at high or low circulating concentrations (Reber 1993). Thus, prolactin may also play a role in mediating photoperiodic changes in immune function. The degree of reproductive regression observed in both short-day males and females, although statistically significant, was uncharacteristically small for this species. This lack of full gonadal regression likely explains why short-day sham-castrated animals did not have significantly lower serum testosterone concentrations compared to long-day sham-operated mice. Additionally, incomplete reproductive regression may also explain the lack of enhanced immune function in short-day, sham-ovariectomized mice compared to long-day, sham-operated animals. Future studies using animals that demonstrate more robust gonadal regression should address this issue.

An alternative explanation for short-day enhancement of immune function is that photoperiod alters the negative feedback of glucocorticoid hormones. For example, reduced androgen concentrations in short-day rodent species result from increased responsiveness of the hypothalamic-pituitary-gonadal axis to the negative feedback of androgens (Turek and Ellis 1981). A similar mechanism may reduce glucocorticoid secretion in short days. Given that glucocorticoids are generally immunosuppressive, decreased release of these hormones can bolster immune function. Unlike previous studies (e.g., Demas and Nelson 1996), serum corticosterone concentrations were not reduced in short compared to long days in the present study. Additionally, corticosterone concentrations did not differ among any experimental group in either experiment; thus, enhanced immune function in short days is not likely explained by changes in glucocorticoid secretion, at least, not at 10 weeks after onset of short days. However, because corticosterone is secreted in a circadian pattern and serum corticosterone concentrations were sampled at only one time point, the possibility that changes in photoperiod altered the circadian rhythm of corticosterone release cannot be ruled out in the present study.

Taken together, the present findings suggest that immune function is enhanced in short days in both male and female deer mice and that enhanced immune function does not appear to be due to reductions in circulating steroid hormone concentrations. Rather, these data suggest that increased splenocyte proliferation in short days is mediated by a direct action of the pineal indole-amine melatonin, or possibly prolactin, on immune function. However, further studies are required to determine whether enhanced immune function in short days is due to increases in the duration of nightly melatonin secretion or increased target-tissue responsiveness to melatonin in short compared to long days.

Acknowledgements We thank Brooke Buckley, Joyce Hairston, Violette Renard, and Sue Yang for technical support and Lance Kriegsfeld and Sabra Klein for valuable comments on the manuscript. We are also grateful to Ed Silverman and for expert animal care. This study was supported by NICHD grant MH 57535 and NSF grant IBN 97-23420 and in part by NICHD grant P30-HD-06268. This research protocol conforms with federal NIH and USDA regulations, and was conducted with approval of our local Institutional Animal Care and Use Committee.

References

Ader R, Cohen N (1993) Psychoneuroimmunology: conditioning and stress. Annu Rev Psychol 44: 53–85

Afoke AO, Eeg-Olofsson O, Hed J, Kjellman NM, Lindblom B, Ludvigsson J (1993) Seasonal variation and sex differences of circulating macrophages, immunoglobulins, and lymphocytes in healthy school children. Scand J Immunol 37: 209–215

Bartness TJ, Powers JB, Hastings MH, Bittman EL, Goldman BD (1993) The timed infusion paradigm for melatonin delivery: what has it taught us about the melatonin signal, its reception, and the photoperiodic control of seasonal responses? J Pineal Res 15: 161–190

Baxter J, Forsham P (1972) The effects of glucocorticoids. Am J Med 53: 573–589

Bhatnagar S, Mitchell JB, Betito K, Boksa P, Meaney MJ (1995) Effects of chronic intermittant cold stress on pituitary adrenocortical and sympathetic adrenomedullary functioning. Physiol Behav 57: 633–639

Black PH (1994) Central nervous system interactions: psychoneuroendocrinology of stress and its immune consequences. Antimicrob Agents Chemother 38: 1–6

Boctor FN, Charmy RA, Cooper EL (1989) Seasonal differences in the rhythmicity of human male and female lymphocyte blastogenic responses. Immunol Invest 18: 775–784

Bolinger M, Olson SL, Delagrange P, Turek F (1996) Melatonin agonist attenuates a stress response and permits growth hormone release in male golden hamsters. Amelia Islands, Florida

Brainard GC, Watson-Whitmyer M, Knobler RL, Lubin FD (1988) Neuroendocrine regulation of immune parameters: photoperiod control of the spleen in Syrian hamsters. Ann NY Acad Sci 540: 704–706

Brick JE, Wilson DA, Walker SE (1985) Hormonal modulation to thymus-independent and thymus-dependent antigens in autoimmune NZB/W mice. J Immunol 134: 3693–3698

Bronson FH, Heideman PD (1994) Seasonal regulation of reproduction in mammals. In: Knobil E, Neill JD (eds) The physiology of reproduction, vol 2, 2nd edn. Raven Press, New York, pp 541–584

Bronson HH (1976) Serum FSH, LH, and prolactin in adult ovariectomized mice bearing Silastic implants of estradiol: responses to social cues. Biol Reprod 15: 147–152

Calvo JR, Rafii-El-Idrissi M, Pozo D, Guerrero JM (1995) Immunomodulatory role of melatonin: specific binding sites in human and rodent lymphoid cells. J Pineal Res 18: 119–126

Champney TH, McMurray DN (1991) Spleen morphology and lymphoproliferative activity in short photoperiod exposed hamsters. In: Franschini F, Reiter RJ (eds) Role of melatonin and pineal peptides in neuroimmunomodulation. Plenum Press, New York, pp 219–223

Clamin HN (1972) Corticosteroids and lymphoid cells. N Engl J Med 287: 388–397

Csaba G, Barath P (1975) Morphological changes in the lung, thymus and the thyroid gland after postnatal extirpation of pineal body. Endocrinol Exp 9: 59–67

Del Gobbo V, Libri V, Villani N, Calio R, Nistico G (1989) Pinealectomy inhibits interleukin-2 production and natural killer cell activity in mice. Int J Immunopharm 11: 567–573

Demas GE, Nelson RJ (1996) Photoperiod and temperature interact to affect immune parameters in adult deer mice (*Peromyscus maniculatus*). J Biol Rhythms 11: 94–102

Demas GE, Nelson RJ (1998) Photoperiod, temperature, and food availability interact to affect reproductive and immune function in adult male deer mice (*Peromyscus maniculatus*). J Biol Rhythms 13: 253–262

Demas GE, Klein SL, Nelson RJ (1996) Reproductive and immune response to photoperiod and melatonin are linked in *Peromyscus* subspecies. J Comp Physiol A 179: 819–825

Demas GE, Nelson RJ (1998b) Exogenous melatonin enhances cell-mediated, but not humoral, immune function in adult male deer mice (*Peromyscus maniculatus*). J Biol Rhythms 13: 245–252

Demas GE, Chefer V, Talan MI, Nelson RJ (1997a) Metabolic costs of mounting an antigen-stimulated immune response in adult and aged C57BL/6J mice. Am J Physiol 273: R1631–R1637

Demas GE, DeVries AC, Nelson RJ (1997b) Effects of photoperiod and 2-deoxy-D-glucose-induced metabolic stress on immune function in female deer mice. Am J Physiol 272: R1762–R1767

Gaston S, Menaker M (1967) Photoperiodic control of hamster testis. Science 167: 925–928

Gavier-Widen D, Morner T (1991) Epidemiology and diagnosis of the European brown hare syndrome in Scandinavian countries: a review. Rev Sci Tech 10: 453–458

Goldman BD, Nelson RJ (1993) Melatonin and seasonality in mammals. In: Yu HS, Reiter RJ (eds) Melatonin: biosynthesis, physiological effects and clinical applications. Raven Press, New York, pp 225–252

Grossman CJ (1984) Regulation of the immune system by sex steroids. Endocr Rev 5: 435–455

Grossman CJ (1985) Interactions between the gonadal steroids and the immune system. Science 227: 257–261

Guerrero JM, Reiter RJ (1992) A brief survey of pineal gland-immune system interrelationships. Endocr Res 18: 91–113

Inman RD (1978) Immunologic sex differences and the female preponderance in systemic lupus erythematosus Arthritis Rheum 21: 849–852

Jankovic BD, Isavic K, Petrovic S (1970) Effect of pinealectomy on immune reactions in the rat. Immunology 18: 1–16

John JL (1994) The avian spleen: a neglected organ. Q Rev Biol 69: 327–351

Johnston PG, Zucker I (1979) Photoperiodic influences on gonadal development and maintenance in the cotton rat, *Sigmodon hispidus*. Biol Reprod 21: 1–8

Konstadoulakis MM, Syrigos KN, Baxevanis CN, Syrigou EI, Papamichail M, Peveretos P, Anapliotou M, Golematis BC (1995) Effect of testosterone administration, pre- and postnatally, on the immune system of rats. Horm Metab Res 27: 275–278

Kriegsfeld LJ, Dawson TM, Dawson VL, Nlson RJ, Snyder SH (1997) Aggressive behavior in male mice lacking the gene for neuronal nitric oxide synthase requires testosterone. Brain Res 769: 66–70

Liu ZM, Pang SF (1993) [^{125}I]iodomelatonin binding sites in the bursa of Fabricus of birds: binding characteristics, subcellular distribution, diurnal variations and age studies. J Endocrinol 138: 51–57

Lochmiller RL, Vestey MR, McMurray ST (1994) Temporal variation in humoral and cell-mediated immune response in a *Sigmodon hispidus* population. Ecology 75: 236–245

Lopez-Gonzales MA, Calvo JR, Osuna C, Guerrero JM (1992) Interaction of melatonin with human lymphocytes: evidence for binding sites coupled to potentiation of cyclic AMP stimulated vasoactive intestinal peptide and activation of cyclic GMP. J Pineal Res 12: 97–104

Lynch GR (1973) Seasonal changes in thermogenesis, organ weights and body composition in the white-footed mouse, *Peromyscus leucopus*. Oecologia 13: 363–376

Maestroni GJ (1993) The immunoendocrine role of melatonin. J Pineal Res 14: 1–10

Maestroni GJ, Conti A, Pierpaoli W (1986) Role of the pineal gland in immunity. Circadian synthesis and release of melatonin modulates the antibody response and antagonizes the immunosuppressive effect of corticosterone. J Neuroimmunol 13: 19–30

McCruden AB, Stimson WH (1991) Sex hormones and immune function. In: R Ader, J Cohen (eds) Psychoneuroimmunology. Academic Press, New York, pp 475–493

Miller ES, Klinger JC, Akin C, Koebel DA, Sonnenfield G (1994) Inhibition of murine splenic T lymphocyte proliferation by 2-deoxy-D-glucose-induced metabolic stress. J Neuroimmunol 52: 165–173

Moffatt CA, DeVries AC, Nelson RJ (1993) Winter adaptations of male deer mice (*Peromyscus maniculatus*) and prairie voles (*Microtus ochrogaster*) that vary in reproductive responsiveness to photoperiod. J Biol Rhythms 8: 221–232

Murphy HM, Wideman CH (1992) Vasopressin, corticosterone levels, and gastric ulcers during food-restriction stress. Peptides 13: 373–376

Myers MJ, Peterson BH (1985) Estradiol induced alterations of the immune system I. Enhancement of IgM production. Int J Immunopharmacol 7: 207–213

Nelson RJ, Demas GE (1996) Seasonal changes in immune function. Q Rev Biol 71: 512–548

Nelson RJ, Demas GE, Klein SL, Kriegsfeld LJ (1995) The influence of season, photoperiod, and pineal melatonin on immune function. J Pineal Res 19: 149–165

Pang CS, Pang SF (1992) High affinity specific binding of 2-[^{125}I]iodomelatonin by spleen membrane preparations of chicken. J Pineal Res 12: 167–173

Persengiev S, Patchev V, Velev B (1991) Melaton in effects on thymus steroid receptors in the course of primary antibody responses: significance of circulating glucocorticoid levels. Int J Biochem 23: 1487–1489

Poon AM, Liu ZM, Pang CS, Pang SF (1994) Evidence for a direct action of melatonin on the immune system. Biol Signals 3: 107–117

Rafii-El-Idrissi M, Calvo JR, Pozo D, Harmouch A, Guerrero JM (1995) Specific binding of 2-[^{125}I]iodomelatonin by rat splenocytes: characterization and its role on regulation of cyclic AMP production. J Neuroimmunol 57: 171–178

Reber PM (1993) Prolactin and immunomodulation. Am J Med 95: 637–644

Ruby NR, Zucker I (1992) Daily torpor in the absence of the suprachiasmatic nucleus in Siberian hamsters. Am J Physiol 263: R353–R362

Schuurs AHWM, Verheul HAM (1990) Effects of gender and sex steroids on the immune response. J Steroid Biochem 35: 157–172

Seegal RF, Goldman BD (1975) Effects of photoperiod of cyclicity and serum gonadotropins in the Syrian hamster. Biol Reprod 12: 223–231

Sheldon BC, Verhulst S (1996) Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. Trends Evol Ecol 11: 317–321

Sidky YA, Hayward JS, Ruth RF (1972) Seasonal variations of the immune response of ground squirrels kept at 22–24°. Can J Physiol Pharmacol 50: 203–206

Turek FW, Ellis GB (1981) Steroid-dependent and steroid-independent aspects of the photoperiodic control of seasonal reproductive cycles in male hamsters. In: Follet BK, Follet DE (eds) Biological clocks in seasonal reproductive cycles. Halstead, New York, pp 251–260

Turek FW, Van Cauter E (1994) Rhythms in reproduction. In: Knobil E, Neill JD (eds) The physiology of reproduction, vol 2, 2nd edn. Raven Press, New York, pp 487–540

Vaughan MK, Hubbard GB, Champney TH, Vaughan GM, Little JC (1987) Splenic hypertrophy and extramedullary hematopoiesis induced in male Syrian hamsters by short photoperiod or melatonin injections and reversed by melatonin pellets or pinealectomy. Am J Anat 179: 131–136

Communicated by L.C.-H. Wang