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Photoperiodic Mediation of Seasonal Breeding and Immune Function In Rodents: A Multi-Factorial Approach[1]

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SYNOPSIS. **Winter is energetically-demanding; thermoregulatory demands increase when food availability usually decreases. Physiological and behavioral adaptations, including termination of breeding, have evolved among nontropical animals to cope with winter energy shortages. Presumably, selection for mechanisms that permit physiological and behavioral anticipation of seasonal ambient changes have led to current seasonal breeding patterns for many populations. Energetically-challenging winter conditions can directly induce death via hypothermia, starvation, or shock; surviving these demanding conditions likely evokes significant stress responses. The stress of coping with energetically-demanding conditions may increase adrenocortical steroid levels to the extent that immune function is compromised. Individuals would enjoy a survival advantage if seasonally-recurring stressors could be anticipated and countered by shunting energy reserves to bolster immune function. The primary environmental cue that permits physiological anticipation of season is daily photoperiod, a cue that is mediated by melatonin. However, other environmental factors, such as low food availability and ambient temperatures, may interact with photoperiod to affect immune function and disease processes. Laboratory studies of seasonal changes in mammalian immune function consistently report that immune function is enhanced in short day lengths. Prolonged melatonin treatment mimics short days, and also enhances immune function in rodents. In sum, melatonin may be part of an integrative system to coordinate reproductive, immunologic, and other physiological processes to cope successfully with energetic stressors during winter. Social factors influence immune function and changes in social interactions may also contribute to seasonal changes in immune function. The mechanisms by which social factors are transduced into immune responses are largely unspecified. In order to understand the optimization of immune function it is necessary to understand the interaction of factors, on both mechanistic and functional levels, that affect immunity.**

INTRODUCTION

Few animals in nature engage in continuous breeding. Presumably, the costs of winter breeding outweigh the benefits. Although this hypothesis is well-accepted, it has rarely been tested directly (Bronson, 1989; Bronson and Heideman, 1994). Energy availability is generally low during temperate and boreal winters, while thermogenic energy requirements are typically high. Most animals restrict reproductive activities to specific times of the year when food is abundant and survival and reproductive success are most likely. Inhibition of winter breeding is the central component of a suite of energy-savings adaptations (Goldman and Nelson, 1993). In addition to the seasonal onset and offset of reproductive function among nontropical mammals, it is also well-established that endocrine, metabolic, growth, neural, and thermogenic processes undergo seasonal changes to enhance winter energy conservation (Moffatt *et al.*, 1993; Bronson, 1989; Bronson and Heideman, 1994). Mechanisms in virtually every physiological and behavioral system have evolved to cope with this winter energetic "bottleneck"; presumably, animals possessing these adaptations enhance their

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survival, and ultimately increase their reproductive success.

In contrast to other physiological processes, immune function has been assumed to remain relatively constant across the seasons (see Sheldon and Verhulst, 1996). Recent evidence, however, suggests that immune function also varies on a seasonal basis (Nelson and Demas, 1996; Lochmiller *et al.,* 1994). Maintaining maximal immune function is energetically expensive, the cascades of dividing immune cells, the onset and maintenance of fever, and the production of humoral immune factors all require substantial energy (Maier *et al.,* 1994). Mounting an immune response likely requires resources that could otherwise be allocated to other functions (Sheldon and Verhulst, 1996). Thus, it is reasonable to consider immune function from a perspective of energetic trade-offs. Recent studies suggest that individuals "optimize" immune function, and allocate energetic resources between the costs of immune function and other maintenance or reproductive functions (*e.g.,* Festa-Bianchet, 1989). Seasonal changes in immune function are consistent with this energetic perspective and are likely driven by seasonal changes in energy availability and requirements (Nelson and Demas, 1996). According to this hypothesis, animals maintain the highest level of immune function that is energetically possible. The observation that immune function is generally compromised during specific energetically-demanding times such as winter, breeding, migration, or molting, is consistent with the hypothesis that immune function is optimized (John, 1994; Zuk, 1990). Pregnant mammals also display compromised immune function; high levels of progesterone suppress immune function among females (reviewed in McCruden and Stimson, 1991). Traditionally, the functional explanation for immunosuppression during pregnancy has been to protect the fetus from being attacked as foreign tissue by the maternal immune system (McCruden and Stimson, 1991). However, reduced immune function during pregnancy might [also] represent an energy-savings adaptation; this perspective may have been masked by laboratory studies where *ad lib* food is available.

The mechanisms that mediate seasonal changes in other physiological, morphological, and behavioral processes also appear to mediate the seasonal changes in immunity (see below). These proximate mechanisms reflect a complex web of interactive, adaptive processes. Individuals use photoperiodic information to initiate or terminate specific seasonal adaptations, including reproduction, in order to maintain a positive energy balance (reviewed in Bartness and Goldman, 1989; Heldmaier *et al.,* 1989; Saafela and Reiter, 1994). The annual cycle of changing photoperiod can be used by nontropical animals as a very precise temporal cue for the time of year. Ambient photoperiodic information is transduced by the pineal gland into a melatonin signal. Melatonin is secreted from the pineal gland during the night so a relatively long duration of melatonin secretion encodes a long night (*i.e.,* short day) and a relatively short duration of melatonin secretion encodes a short night (*i.e.,* long day) (Bartness *et al.,* 1993). The secretory pattern of melatonin allows individuals to ascertain the time of year and develop seasonally-appropriate adjustments to energy use (reviewed in Bartness and Goldman, 1989; Reiter, 1991).

Environmental and biotic influences on immune function are complex, but not empirically intractable. Low ambient temperatures, reduced food availability, and other stressful winter conditions compromise immune function. Because these stressors are seasonally recurrent, individuals of some species may have evolved mechanisms to anticipate and to counteract these recurring threats to maximal immunity. Short photoperiods have been hypothesized to serve as a cue used by animals to enhance immune function in advance of energy compromising conditions (Nelson and Demas, 1996). Increased duration of melatonin treatment (mimicking long nights) enhances immune function, either directly or indirectly by affecting the secretion of steroid hormones and prolactin (Goldman and Nelson, 1993).

Importantly, social conditions also dramatically influence immune function (Barnard *et al.,* 1994; Karp *et al.,* 1993; Klein

et al., 1997; Rabin and Salvin, 1987; Rabin *et al.,* 1987). The social organization of rodents often changes from highly territorial during the breeding season to the formation of communal groups during the winter (McShea, 1990). The contribution of changes in social organization in the mediation of seasonal fluctuations of immune function remains unspecified. Furthermore, the role of social system (*i.e.,* socially monogamous *versus* polygynous) in seasonal fluctuations in immune function is only now being explored. The physiological processes by which social factors are transduced to enhance or compromise immune function are also unknown. The goal of this paper is to review the literature showing that immune function is not a static process, but an optimized energetically expensive physiological process that is affected by numerous environmental and biotic forces.

STRESS AND IMMUNE FUNCTION

The risk of infection and death is highest when insufficient energy reserves are available to sustain immunity. Stress can compromise immune function (see Ader and Cohen, 1993; Dunn, 1989; O'Leary, 1990 for reviews). Prolonged or severe food shortages may evoke secretion of glucocorticoid hormones (Nakano *et al.,* 1987; Jose and Good, 1973); glucocorticosteroids actively compromise several aspects of immune function (Kelley, 1985; Munck and Guyre, 1991; Maier *et al.,* 1994; and see below). Environmental or biotic conditions perceived as stressful, such as reduced food availability, low ambient temperatures, overcrowding, lack of shelter, or increased predator pressure, can recur seasonally leading to seasonal fluctuations in immune function among individuals, and seasonal changes in population-wide disease and death rates (Lochmiller *et al.,* 1994). A dynamic relationship exists between longevity and reproductive output (Stearns, 1976); all other factors being equal, longer lived individuals produce more offspring and are more fit than individuals that die prematurely.

Laboratory studies have established that stress inhibits immune function (Keller *et al.,* 1983; Laudenslager *et al.,* 1983; reviewed in Ader and Cohen, 1993). "Stress" has been a notoriously ethereal concept in biology. The term, "stress," has been conflated to include both the stressor and the physiological stress response (Sapolsky, 1992). In many cases, the implicit definition of a stressor is anything that increases glucocorticoid secretion; glucocorticoids suppress certain aspects of immune function (Kelley, 1985; Maier *et al.,* 1994; Munck and Guyer, 1991). Traditionally, there has been little concern about the functional explanation of why individuals exposed to stressors evolved mechanisms to compromise immune function (but see Sapolsky, 1992). In other words, what is the adaptive advantage to compromised immune function? To answer that question, it is important to propose an operational definition of "stressor," and to change levels of analysis. Stressors disturb homeostasis. Because restoring homeostasis requires more energy than maintaining homeostasis (Sapolsky, 1992), exposure to a stressor increases energy demands on individuals. During slight homeostatic perturbations, adrenalin mobilizes energy and prepares the individual for "fight or flight" activities. Animals have evolved to function within an optimal range of conditions. During long-term perturbations of homeostasis (*e.g.,* when temperatures are low or food availability becomes scarce) glucocorticoids are released and energy is mobilized to restore homeostatic equilibrium. Immune function may be reduced during reinstatement of homeostasis because both processes are energetically costly. There is a trade-off between "maximal" immune function and "perfect" homeostatic balance. Steroid hormones appear to be one of the physiological mediators of this trade-off.

Immune function is often compromised during the breeding season (John, 1994). The odds of close social interactions and the risk of infection both increase at this time. Presumably, socially polygynous animals are at higher risk for communicable diseases and infections than monogamous animals. As noted above, there is a trade-off between maximal immune and reproductive function. The physiological mechanisms compromising immune function

during breeding are likely mediated by blood androgen concentrations (Alexander and Stimson, 1988; Olsen and Kovacs, 1996). Individuals of monogamous species generally have lower androgen levels than individuals of polygynous species (Wingfield, 1994).

The hormone, melatonin, may also affect the stress response by interacting directly with glucocorticoid secretion. Generally, melatonin enhances immune function, whereas glucocorticoids compromise immune function (Gupta, 1990; Maestroni, 1993; Maier *et al.,* 1994). Melatonin treatment, however, can ameliorate the immunocompromising effects of glucocorticosteroids (Aoyama *et al.,* 1986, 1987; Bolinger *et al.,* 1996). Conversely, glucocorticosteroids can reduce the immunoenhancing properties of melatonin. For example, cortisol treatment of ducklings reduced the number of thymic melatonin receptors (Poon *et al.,* 1994*a, b*).

Previous studies have demonstrated that environmental stressors elevate blood glucocorticoid levels and that high glucocorticoid levels suppress immune function (Ader and Cohen, 1993; Besedovsky and Del Rey, 1991; Black, 1994; Claman, 1972; Hauger *et al.,* 1988). For example, low ambient temperatures are often perceived as stressful, and can potentially compromise immune function (*e.g.,* Claman, 1972; MacMurray *et al.,* 1983; Monjan, 1981). Winter survival in small animals is hypothesized to require a positive balance between short-day enhanced immune status and glucocorticoid-induced immunosuppression (Demas and Nelson, 1996). This immunosuppression may be due to many factors, including overcrowding, increased competition for scarce resources, low ambient temperatures, reduced food availability, increased predator pressure, or lack of shelter. Each of these potential stressors may cause high blood concentrations of glucocorticoids. Winter breeding with its concomitant elevation in sex steroid hormones may also cause immunocompromise (*e.g.,* Lochmiller *et al.,* 1994; Tang *et al.,* 1984). Presumably, winter breeding occurs when other environmental stressors such as temperature and food availability are not severe. The balance of enhanced immune function (*i.e.,* to the point where autoimmune disease becomes a danger) against stressor-induced immunosuppression (*i.e.,* to the point where opportunistic pathogens and parasites overwhelm the host) must be met for animals to survive and become reproductively successful.

Recently, the interaction between photoperiod and temperature was examined on immunoglobulin (IgG) levels and splenic mass in male deer mice (*Peromyscus maniculatus*) (Demas and Nelson, 1996). Animals were maintained in LD 16:8 or LD 8:16 photoperiods and either in 20° or 8°C temperatures. Serum IgG levels were elevated in short day mice maintained at normal room temperature (*i.e.,* 20°) as compared to long-day animals housed at either 20° or 8°C. Long-day deer mice kept at 8°C temperatures had reduced IgG levels as compared to long-day mice maintained at 20°, whereas mice exposed to short days and low temperatures had IgG levels comparable to long-day mice maintained at 20°C. In other words, short days elevated IgG levels over long days. Low temperatures caused a significant reduction in IgG levels. The net effect of short-day enhancement and low temperature reduction of IgG levels is no appreciable difference from baseline (*i.e.,* long-day mice kept at 20°C) (Demas and Nelson, 1996). This adaptive system may help animals cope with seasonal stressors and ultimately increase reproductive fitness. In order to enhance immune function in anticipation of demanding winter conditions animals must initiate these adaptations well in advance of the demanding conditions. The most reliable environmental cue for time of year is the annual pattern of changing photoperiod.

PHOTOPERIODIC CHANGES IN IMMUNE FUNCTION

Short days enhance immune function in individuals with robust reproductive responses to photoperiod. Although, splenic weights of deer mice (*Peromyscus maniculatus*) (Vriend and Lauber, 1973), and Syrian hamsters (*Mesocricetus auratus*) (Brainard *et al.,* 1986, 1987, 1988) were reduced in short days, total splenic lymphocyte numbers, and macrophage counts were significantly higher in hamsters exposed to short days, as compared to animals exposed

to long photoperiods (Brainard *et al.,* 1986, 1988). Thymic mass was unaffected by photoperiod in hamsters (Brainard *et al.,* 1986). Photoperiodic influences on lymphocyte number and total white blood cell count have been reported for deer mice (Blom *et al.,* 1994); short day (LD 8:16) mice possessed more white blood cells than animals maintained in long day lengths (LD 16:8). More recently, short-day deer mice displayed faster healing rates, and higher splenic T-lymphocyte proliferation than long day mice (Demas and Nelson, 1996; Demas *et al.,* 1997*a*; Nelson *et al.,* 1995).

Importantly, all laboratory studies of photoperiodic effects on immune function have reported enhanced immune function in short day lengths (reviewed in Nelson *et al.,* 1995; Nelson and Demas, 1996). Although many field studies support this hypothesis, with data suggesting enhanced immune function and decreased disease prevalence during the winter as compared to the summer, a substantial number of studies have reported the opposite pattern of results (Nelson *et al.,* 1995; Nelson and Demas, 1996); *i.e.,* reduced immune function is coincident with the short days of winter. These conflicting results can be resolved by considering additional environmental factors, not usually manipulated in laboratory studies. For example, winter-associated stressors (*e.g.,* restricted food and low ambient temperatures) appear to counteract short day enhancement of immune function in the lab (Demas and Nelson, 1996). Thus, we predict enhanced immune function should be observed during mild winters when thermoregulatory costs are reduced, whereas compromised immune function should be expected during energetically-challenging winters when thermoregulatory costs can be severe. Long-term field studies are required to test this hypothesis. Although the effects of melatonin on immunity are well-established (see Caroleo *et al.,* 1992*a, b*; Giordano *et al.,* 1993; Guerrero and Reiter, 1992; Pioli *et al.,* 1993; Poon *et al.,* 1994*a, b* for recent reviews), an ecological context is needed to understand the role of melatonin in photoperiodic effects upon immune function, and to suggest why this phenomenon might be adaptive and functional, rather than merely a physiological oddity. Knowledge of the adaptive and functional significance of seasonal fluctuations in immune function may help to provide an improved understanding of the possibilities, as well as the constraints, of melatonin immunotherapy.

Social Factors Influence Immune Function

Social factors, such as male-male interactions or exposure to receptive mates can influence the timing and extent of seasonal reproductive quiescence. Social factors also influence immune function. For example, as compared to house mice (*Mus musculus*) housed five per cage, individually housed mice show higher proliferative responses to the T-cell mitogen, Concanavalin A (Con A) (Rabin *et al.,* 1987), increased primary and secondary antibody responses (Karp *et al.,* 1993; Rabin and Salvin, 1987), more cytokine production (Rabin *et al.,* 1987), and greater resistance to infection (Plaut *et al.,* 1969; Rabin and Salvin, 1987). Although the impact of social factors on immune function has not been fully examined in an ecological context, environmental factors including photoperiod, food intake, and ambient temperature, as well as biotic factors including social organization densities influence immune function. Recently, the effect of social interactions on immune function was examined in polygynous meadow voles (*Microtus pennsylvanicus*) and monogamous prairie voles (*M. ochrogaster*).

Among individually housed animals there were no apparent species or sex differences in proliferative responses to Con A (Klein *et al.,* 1997). Pairing animals with either a same sex or opposite sex conspecific for 28 days unmasked both sex and species differences in immunocompetence (Klein *et al.,* 1997; Fig. 1). Among mixed sex pairs, sex differences were only apparent among the polygynous meadow voles, in which males had higher proliferative responses than conspecific females. Among same sex pairs, the direction of the sex difference depended on the species. Among polygynous meadow voles, males exhibited higher immune responses than females,

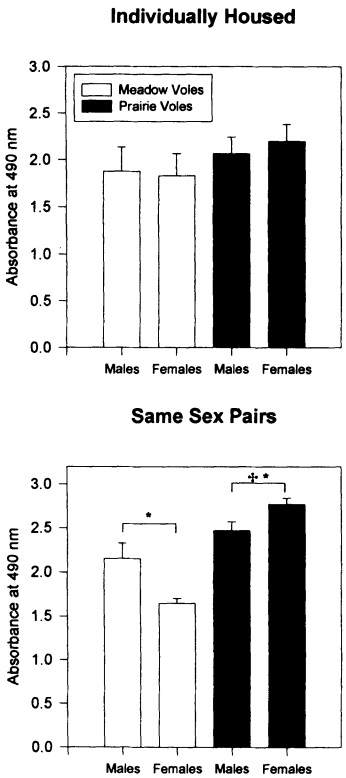

FIG. 1. Upper Panel: Mean (± Standard Error of the Mean [SEM]) splenocyte proliferation values (absorbance units) from individually-housed male and female meadow voles (*Microtus pennsylvanicus*) and prairie voles (*M. ochrogaster*) in response to 10μg of the mitogen, Concanavalin A (Con A). Data are represented as absorbance units as determined by a colorimetric assay. Absorbance units are positively correlated with the amount of cellular division. Lower Panel: Mean (±SEM) splenocyte proliferation values (absorbance units) from mixed-sex pairs of male and female meadow voles (*M. pennsylvanicus*) and prairie voles (*M. ochrogaster*) in response to 10μg of the mitogen, Con A. Asterisks (*) indicate significant sex differences within a species. The plus signs (+) indicate significant species differences in proliferation values (from Klein and Nelson, submitted).

whereas among monogamous prairie voles, females exhibited higher responses to stimulation with Con A than males. Overall, prairie voles displayed higher proliferative responses to stimulation with Con A than meadow voles in both same sex and mixed sex pairs. These data suggest that sex and species differences become apparent only in the context of the social environment in which breeding occurs.

ENERGETIC COSTS OF IMMUNITY

Animals must maintain a relatively constant flow of energy (*i.e.*, energy intake ≥ energy expended) to the body, despite potentially large fluctuations in energetic availability in their environment (Wade and Schneider, 1992). As noted previously, mounting an immune response likely requires resources that could otherwise be allocated to other biological functions (Sheldon and Verhulst, 1996), and immune function should be "optimized" so that individuals can tolerate small infections if the energetic costs of mounting an immune response outweigh the benefits (Behnke *et al.*, 1992). Recent research on bighorn sheep (*Ovis canadensis*) supports the existence of an energetic trade-off between immune and reproductive function (Festa-Bianchet, 1989). Lactation is energetically costly (Bronson, 1989; Fairbain, 1977). Lactating bighorn ewes have increased parasitic infection in fecal samples compared to nonlactating females (Festa-Bianchet, 1989). Elevated parasitic infection likely reflects compromised immune function in lactating ewes, but this hypothesis remains to be tested directly. In fact, few studies have directly assessed the energetic cost of mounting an antibody response, although the initiation of an immune response (*i.e.*, inflammation, activation of cytokines, induction of fever) presumably requires substantial energy. For example, every 1°C increase in body temperature requires a 7–13% increase in caloric energy production, depending on the species (Maier *et al.*, 1994). Recently, precise quantification of the energetic costs of an immune response has been attained in adult house mice (*Mus musculus*) (Demas and Nelson, 1997). House mice were injected with a specific nonreplicating antigen, keyhole limpet hemocyanin (KLH). This substance induces an antibody response without inducing fever or making the treated animal ill (Dixon *et al.*, 1966; Curtis *et al.*, 1970). Both oxygen consumption (ml/kg) and metabolic heat production (kcal/kg) increased in KLH-injected animals (Fig. 2). Mounting an immune response costs energy; presumably, mounting an immune response to a

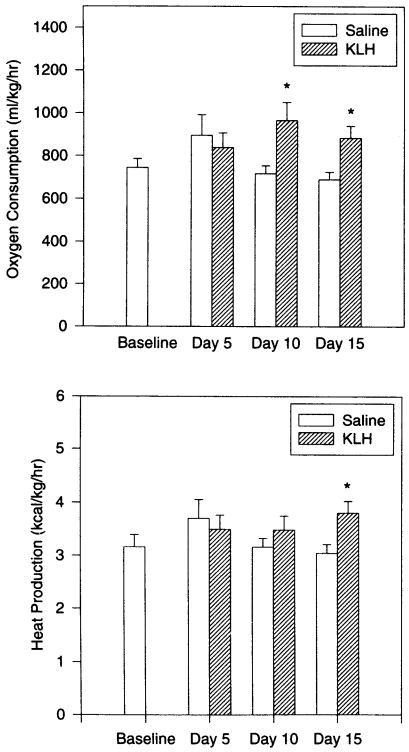

FIG. 2. Upper Panel: Mean (±SEM) oxygen consumption (ml/kg) 5, 10, and 15 days after male house mice were injected sc with 150 μg of KLH suspended in 0.1 cc sterile saline. Lower Panel: Mean (±SEM) metabolic heat production 5, 10, and 15 days after male house mice were injected sc with 150 μg of KLH suspended in 0.1 cc sterile saline. Significant differences ($P < 0.05$) are indicated by the asterisk (from Demas and Nelson, 1997).

replicating antigen such as a virus requires additional energy. Thus, a general energy deficit can increase the risk of infection and death because insufficient energy reserves may be available to sustain immunity.

In another attempt to examine the role of energetics in seasonal changes in immune function in deer mice, the chemical compound 2-deoxy-D-glucose (2-DG) was used to manipulate energy availability at the input end of the energetic equation (Demas *et al.,* 1997*b*). 2-DG is a glucose analog that inhibits cellular utilization of glucose, thus inducing a state of glucoprivation (Smith and Epstein, 1969). 2-DG acts as a metabolic stressor by increasing serum corticosterone levels (Lysle *et al.,* 1988), and 2-DG glucoprivation induces an anestrous state in female Syrian hamsters (*Mesocricetus auratus*) (Schneider *et al.,* 1993), and torpor in female Siberian hamsters (*Phodopus sungorus sungorus*) (Dark *et al.,* 1994). 2-DG induced metabolic stress also affects immune function; 2-DG administration inhibits murine splenic T lymphocyte proliferation in a dose-dependent manner in laboratory strains of rats (*Rattus norvegicus*) (Lysle *et al.,* 1988) and mice (*M. musculus*) (Miller *et al.,* 1994).

Short days buffered deer mice against glucoprivation stress. Long-day deer mice injected with 2-DG had elevated corticosterone levels, as compared to long-day mice injected with saline (Fig. 3); corticosterone levels were not significantly elevated in short-day mice injected with 2-DG. 2-DG-treated long-day mice displayed reduced splenocyte proliferation to Con A as compared to saline-injected mice. Splenocyte proliferation did not differ among short-day deer mice regardless of experimental treatment; short-day animals exhibited enhanced immune function. Overall, short-day mice treated with 2-DG displayed higher splenocyte proliferation than long-day mice treated with 2-DG (Fig. 3) (Demas *et al.,* 1997*b*).

These data are consistent with the hypothesis that short days buffer against metabolic stress. Reduced corticosterone levels in animals maintained on short days or treated with melatonin are likely due to improved metabolic function (Saafela and Reiter, 1994). Accordingly, improved immune function in short days represents one component of numerous winter-coping adaptations that may be mediated by melatonin.

WINTER BREEDING

So-called "out-of-season" breeding occurs in virtually every rodent population examined, suggesting that the energetic bottleneck during winter can be resolved (Nelson, 1987; Kerbeshian *et al.,* 1994). A substantial proportion of individuals not responsive to photoperiod has been reported within every population of photoperiodic animals studied in the lab (reviewed in Nelson, 1987; Kerbeshian *et al.,* 1994). There must be a genetic basis for this variation

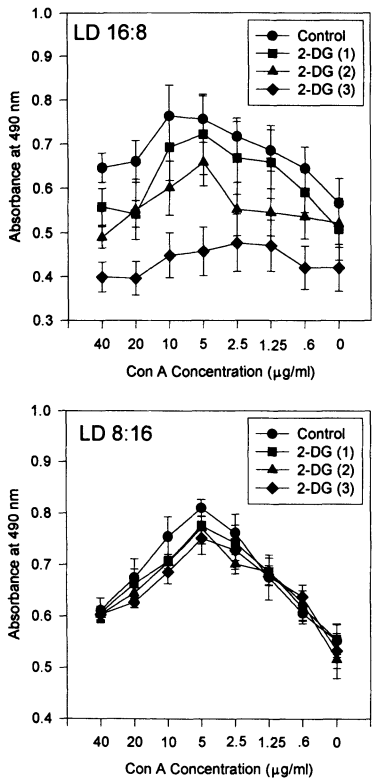

FIG. 3. Mean (±SEM) splenocyte proliferation to Con A (represented as absorbance units) of deer mice housed in long (LD 16:8) (upper panel) or short (LD 8:16) (lower panel) days. Experimental mice received daily injections of 2-DG across 1, 2, or 3 consecutive days. Control mice in each photoperiod received daily ip injections of sterile 0.9% saline across 3 consecutive days. Mice were lightly anesthetized with methoxyflurane vapors (Metofane, Pitman-Moore, Mudelein, IL), weighed, and blood samples obtained from the retro-orbital sinus. Handling time was kept constant and to a minimum; the time from initial removal from the cage to the end of bleeding was <3 min. Blood serum corticosterone concentrations were determined by radioimmunoassay using the ICN Biomedicals, Inc. ^{125}I kit. This assay is highly specific, cross-reacting at less than 0.3 with other steroid hormones. Intra-assay variation was <4.5% Statistically significant differences between means are indicated by an asterisk (from Demas *et al.*, 1997*b*).

because nonresponsiveness to photoperiod is a trait that can be artificially selected (*e.g.*, Kerbeshian *et al.*, 1994; Lynch *et al.*, 1989). Despite growing recognition that variation in responsiveness to photoperiod exists, there is currently no explanation for this variation on a physiological level. Nonresponsive morphs do not vary in their pineal melatonin content, melatonin secretion patterns, brain melatonin receptor numbers, or melatonin receptor binding affinities (Blank *et al.*, 1988; Carlson *et al.*, 1989; Heideman and Bronson, 1991). Exogenous melatonin treatment can induce reproductive regression in some nonresponsive individuals, but is ineffective for other species (see Arendt, 1995 for review). Nonresponsive phenotypes of Siberian hamsters appear to have a defect in the circadian organization (Puchalski and Lynch, 1988, 1994). Responsive hamsters show a free-running activity pattern with a period of 23.86 ± 0.04 hr and respond to brief light pulses with the expected phase delays and phase advances; however, nonresponsive hamsters exhibited a period of 24.04 ± 0.05 hr and responded to light pulses with only phase advances (Puchalski and Lynch, 1988). Thus, as a result of these differences in circadian function, nonresponsive Siberian hamsters appear incapable of proper photoperiodic time measurement and photoperiod-induced seasonality. Individuals of other nonresponsive species display no obvious changes in circadian organization (*e.g.*, Carlson *et al.*, 1989). Many tropical species do not display reproductive responsiveness to short photoperiod. For example, tropical cane mice (*Zygodontomys brevicauda*) do not inhibit reproductive function in response to any number of extrinsic manipulations or even in response to pharmacological treatment with melatonin (Heideman and Bronson, 1991). Reproductive responsiveness to short days varies within populations from 0% (tropical species) to nearly 100% (boreal species) (Dark *et al.*, 1983; Lynch *et al.*, 1981).

The circumstances that shift the cost-benefit ratio to favor winter breeding among individual small mammals remain unidentified (Fairbain, 1977; Moffatt *et al.*, 1993). The existence of winter breeding is evidence that these circumstances exist. Animals that manage to breed successfully should increase their reproductive output over that of nonbreeding conspecifics. However, this hypothetical increase in reproductive fitness will only accrue if the costs of winter-born offspring do not outweigh the likely compromise to survival of

the parents (Stearns, 1976). Because not all animals breed during the winter, it is reasonable to surmise that there must be significant costs associated with winter breeding. Otherwise, the genes allowing winter breeding would spread and dominate in the population (Haldane, 1932). Some of these costs may reflect the survival costs associated with compromised thermogenic ability when breeding (Trayhurn *et al.,* 1982), whereas other costs may reflect energy demands that may not be met if energy resources are scarce.

To what extent does immune function correlate with reproductive responsiveness to photoperiod? Among individual short-day deer mice that showed elevated splenic lymphocyte proliferation, there was no relationship between the degree of testicular regression and the amount of splenocyte proliferation (Demas *et al.,* 1997). These results suggest that photoperiodic responsiveness of immune function is not linked to reproductive responsiveness to day length. However, comparisons between subspecies of *Peromyscus* suggest otherwise. The effects of photoperiod and melatonin treatment on reproductive and immune function were assessed in two subspecies of deer mice (*Peromyscus maniculatus*) from different latitudes of origin (Demas *et al.,* 1997*a*). Short-day *P.m. bairdii* (latitude = 42°51′N) displayed reproductive regression and elevated splenocyte proliferation in response to the T-cell mitogen, Con A, as compared to long-day mice. In contrast, *P.m. luteus* (latitude = 30°37′N) did not undergo reproductive regression in short-days; individuals of this subspecies also failed to exhibit any increase in lymphocyte proliferation to Con A in short days. Other individuals of both subspecies were implanted with empty capsules or capsules that contained melatonin. Individual *P.m. bairdii* implanted with melatonin underwent reproductive regression after 8 weeks of treatment. Individuals of this subspecies also displayed elevated lymphocyte proliferation to Con A compared to mice implanted with empty capsules (Demas *et al.,* 1997*a*). Conversely, *P.m. luteus* implanted with melatonin did not undergo reproductive regression and displayed no significant changes in lymphocyte proliferation. These data suggest that reproductive photoperiodic responsiveness, and more specifically, reproductive responsiveness to melatonin, mediates short-day enhancement of immune function in deer mice. These data also imply that melatonin may not possess universal immunoenhancing properties, and suggest that reproductive and immune responsiveness to day length are linked in these species. The effectiveness of melatonin to influence immune responses may be constrained by reproductive responsiveness to this indole-amine.

CONCLUSION

Few studies have reported the effects of photoperiod on immune function of short-day breeders (*e.g.,* sheep, red deer). Humans may retain minimal reproductive responsiveness to day length (Bronson, 1995). The extent to which humans retain immunologic responsiveness to day length or melatonin remains unspecified. Melatonin appears to be part of an integrated system involved in coordinating reproductive, immunologic and thermoregulatory processes. Energetic trade-offs exist that compromise or enhance immune function in concert with other energetically-demanding requirements (Sheldon and Verhulst, 1996). Environmental factors that compromise immune function, acting via glucocorticoids, include reduced food consumption and low ambient temperatures. Short days enhance immune function, via melatonin. Social factors must also be considered to gain a full understanding of environmentally-induced variation in immune function. For example, it is well established that short day-induced testicular inhibition can be blocked by the presence of a fertile female (Whitsett and Lawton, 1982). Furthermore, social conditions (or social cues) can affect glucocorticoid levels that might affect immune function (DeVries *et al.,* 1995, 1996). Additional studies, on both physiological and adaptive functional levels, are required to understand the interactions among environmental and biotic factors that affect immune function.

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