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## Photoperiodism in Mammals: Regulation of Nonreproductive Traits

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As noted in previous chapters, many plants and animals are exposed to annual fluctuations in the deterioration and renewal of their environments. Organisms tend to restrict energetically expensive processes and activities to a specific time of the year. Animals migrate or limit activity when food availability is low; for example, reproduction, preparation for migration, and other energetically demanding activities have evolved to coincide with abundant local food resources or other environmental conditions that promote survival. Thus, precise timing of physiology and behavior is critical for individual reproductive success and subsequent fitness. Importantly, vertebrate animals have evolved to fill both temporal and spatial niches.

Some physiological and behavioral adjustments occur in direct response to environmental fluctuations that have an obvious and immediate adaptive function. For example, reduced food or water availability can inhibit breeding (Nelson, 1987; Bronson, 1988). Such environmental factors have been termed the “ultimate factors” underlying seasonality (Baker, 1938). Many animals need to forecast the optimal time to breed so that spermatogenesis, territorial defense, or any other time-consuming adaptations can be developed prior to the onset of the breeding season. Thus, seasonally breeding vertebrate animals often must detect and respond to environmental cues that accurately signal, well in advance, the arrival or departure of seasons favoring reproductive success. The environmental cues used to anticipate environmental change may or may not have direct survival value. Such cues are called “proximate factors” (Baker, 1938). Photoperiod (day length) is the most notable example of a proximate factor. The annual changes in day length serve as a precise reference for the time of year. Under some circumstances, proximate and ultimate factors are identical (Nelson, 1987). For example, some individuals may not begin breeding until food cues are detected (Bronson, 1988).

In many cases, there are trade-offs (i.e., direct or indirect antagonistic interactions between two physiological processes that may have fitness consequences for organisms) among expensive physiological activities. Often individuals trade off energy between reproductive functions during mild seasons and survival functions

during winter. For example, investment in reproduction and growth is curtailed, whereas investment in immune function is bolstered during winter.

This chapter addresses the physiological and cellular mechanisms underlying the detection of and response to environmental factors in regulating nonreproductive seasonal adaptations. Although the majority of the research within the area of mammalian seasonality has focused on seasonal changes in reproduction (see chapter 20), pronounced fluctuations in other nonreproductive responses, including changes in energy balance, immune function, and behavior, occur as well. Most research has focused on the role of photoperiod; presumably, with only two bits of data, length of day and direction of change in the photoperiod, individuals can precisely determine time of year and might then use this information to anticipate subsequent seasonal environmental changes.

## PHOTOPERIODIC REGULATION OF ENERGY BALANCE

### Introduction to Seasonal Variation in Energy Requirements

In many photoperiodic species, substantial changes in both metabolism and food intake occur when animals are transferred from long “summerlike” to short “winterlike” days, leading to appreciable changes in body weight and total body fat. Although a wide variety of mammalian species undergo seasonal cycles of body fat, the vast majority of research on these seasonal responses has focused on Siberian hamsters (*Phodopus sungorus*) and Syrian hamsters (*Mesocricetus auratus*) (Wade and Bartness, 1984b; Bartness et al., 2002; Morgan et al., 2003). For example, adult male Siberian hamsters housed in long days (16/8 h light–dark cycle [LD 16:8]) display relatively constant body masses; transfer to short days (LD 8:16), however, results in gradual and progressive loss in body weight (Wade and Bartness, 1984b; Bartness et al., 2002; Morgan et al., 2003). Although some of this weight loss is driven by decreased testis and muscle mass, the majority of the weight loss occurs in the form of decreased adiposity (Mercer et al., 2001).

Approximately 30–40% of the initial long-day body fat is lost by approximately 12–16 weeks in short days. Indeed, if long-day Siberian hamsters are transferred to short days and subsequently food restricted during their progressive decline in body mass, then these animals lose a greater amount of body mass than do short-day animals fed ad libitum. When short-day food-restricted hamsters are allowed to refeed, they substantially increase their food intake to compensate for food restriction. Interestingly, however, these animals do not return to their pre-food restriction levels. Rather, they regain a stable body mass at the reduced level that is consistent with the progressive short-day–induced decrease in body mass (Steinlechner et al., 1983). This and subsequent studies have confirmed the notion that body mass (and body fat) is a highly regulated, photoperiod-dependent, physiological response.

## Neuroendocrine Mechanisms

### *Steroid Hormones*

In a large number of rodent species, including hamsters (Hoffman et al., 1965), voles (Dark et al., 1983), deer mice (*Peromyscus maniculatus*) (Whitsett et al., 1983), and lemmings (Hasler et al., 1976), the gonads regress in response to exposure to short days. Rodents also display marked differences in body mass adjustments in response to photoperiod. For example, prairie voles (*Microtus ochrogaster*) (Kriegsfeld and Nelson, 1996), Syrian hamsters (Bartness and Wade, 1984; Campbell et al., 1983), and collared lemmings (*Dicrostonyx groenlandicus*) (Gower et al., 1994) increase body mass in short days, whereas Siberian hamsters (Hoffmann, 1973; Steinlechner and Heldmaier, 1982; Wade and Bartness, 1984a), meadow voles (*Microtus pennsylvanicus*) (Dark et al., 1983; Dark and Zucker, 1984), and deer mice (Blank and Freeman, 1991; Nelson et al., 1992) display short-day decreases in body mass. Furthermore, in many cases, the direction of the short-day change in body mass can be mimicked by gonadectomies, suggesting that photoperiodic changes in body mass are driven by changes in gonadal steroids. For example, gonadectomized long-day Syrian hamsters, prairie voles, and collared lemmings display increased body (and fat) masses comparable to those seen when intact animals are housed in short days (Morin and Fleming, 1978; Gower et al., 1994; Bartness, 1996). In contrast, gonadectomized Siberian hamsters, deer mice and meadow voles display short-day-like decreases in body mass and adiposity (Dark and Zucker, 1984, 1986; Wade and Bartness, 1984b; Blank and Freeman, 1991; Bartness, 1996). Short-day changes in gonadal steroid concentrations, however, cannot account for the total change in total body fat following short-day exposure. For example, exposure of gonadectomized Siberian hamsters to short days results in a further reduction in body mass (Wade and Bartness, 1984b). Furthermore, animals that experience prolonged exposure to short days experience "spontaneous recrudescence" during which they become refractory to the short-day signal and return to long-day gonadal and body masses (Dark and Zucker, 1984; Wade and Bartness, 1984b). Body masses return to long-day levels even in animals that were gonadectomized before short-day exposure (Hoffman et al., 1982). These findings suggest that, although gonadal steroids play an important role in regulating photoperiod changes in energy balance, other factors contribute to these changes.

### *Pancreatic Peptides*

In most vertebrate species, peptides produced by the alpha and beta cells of endocrine pancreas act in a yin-yang fashion to coordinate storage (insulin) or liberation (glucagon) of glucose to be used as energy. Despite extensive research on these hormones in nonphotoperiodic "model systems" (e.g., rats, mice), much less is known about the role of these peptide in the photoperiodic regulation of body fat. In fact, among photoperiodic animals, seasonal fluctuations have only been investigated in two species of hamsters, Syrian and Siberian hamsters. As expected due to their differential responses to short days, Siberian hamsters display decreased serum

insulin concentrations (Bartness et al., 1991), whereas Syrian hamsters increase serum insulin in response to short day lengths (Cincotta et al., 1991, 1993; Cincotta and Meier, 1995). In both species, insulin levels correlate with the level of adiposity. Siberian hamsters experiencing experimentally induced diabetes mellitus (via streptozotocin) and transferred to short days display normal decreases in body mass, body fat and food intake, suggesting that insulin does not play a critical role in photoperiod changes in energy, at least in this species. Insulin levels have been directly manipulated in Syrian hamsters via subdiaphragmatic vagotomies, which block insulin secretion indirectly via disruption of parasympathetic control of insulin release. Short-day hamsters that received subdiaphragmatic vagotomies continued to display gonadal regression, but the typical short-day decrease in body fat was blocked in these animals (Miceli et al., 1989). Although these data suggest that insulin may play a role in short-day increases in body fat, they are difficult to interpret because vagotomies generate many nonspecific physiological effects. Thus, the failure of short-day vagotomized animals to display photoperiodic changes in total body fat does not necessarily support the idea that insulin contributes to short-day-induced changes in body fat in this or other species.

In contrast to insulin, the pancreatic peptide glucagon plays an important role in stimulating lipolysis of white adipose tissue and thermogenesis in brown adipose tissue. This latter effect plays an important role in the thermogenic response to exposure to low ambient temperatures; thus, it seems plausible that increases in glucagon may play a role in regulating photoperiodic changes in energy balance, at least in species that undergo short-day decreases in body fat. Despite this intriguing possibility, a role for glucagon in regulating seasonal adiposity has not been examined in any photoperiodic species studied to date. Future studies will be needed to answer this question.

#### *Leptin (OB Protein)*

All mammals that undergo seasonal changes in reproduction and body mass display seasonal/photoperiodic changes in leptin, a peptide hormone produced almost exclusively by adipose tissue (Woods and Seeley, 2000; Drazen et al., 2001). Importantly, in both photoperiodic and nonphotoperiodic species, circulating levels of leptin are highly correlated with total body fat, suggesting that leptin serves an important role as a peripheral signal of adiposity. For example, serum leptin concentration correlates positively with body fat in Siberian hamsters over their yearly cycle (Drazen et al., 2000b; Horton et al., 2000). In addition, white adipose tissue leptin gene expression, circulating leptin concentrations, and leptin receptor gene expression are all reduced in short days compared with long days, consistent with short-day-induced decreases in body fat (Klingenspor et al., 1996a; Drazen et al., 2000b; Mercer et al., 2000b; Demas et al., 2002). Given that reduced leptin receptor gene expression contributes to a decrease in sensitivity to leptin, reduced gene expression in short days may reduce leptin sensitivity in short-day hamsters, and this indeed seems to be the case in Siberian hamsters (Mercer et al., 2000b). Note, however, that the dogma associated with the regulation of body fat by leptin

states that when body fat levels decrease, the decrease in leptin triggers increases in food intake. The data above support the first portion of this dogma (i.e., short-day-induced decreases in body fat are associated with decreases in leptin gene expression by white fat; Klingenspor et al., 1996b) and circulating leptin concentrations (Klingenspor et al., 1996b; Drazen et al., 2000b; Horton et al., 2000); however, food intake is *decreased* in short photoperiods *not* increased, especially when body fat is at its seasonal nadir (Wade and Bartness, 1984b).

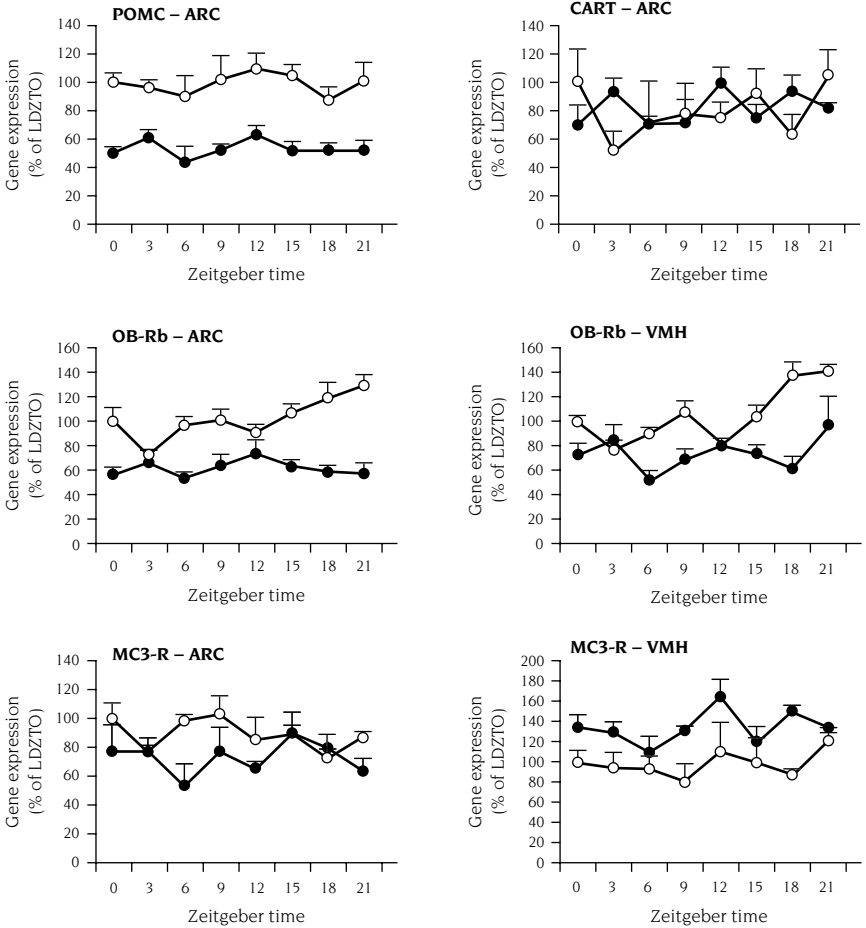
At least one of the energy-related short-day-induced changes by Siberian hamsters is reversed by peripheral chronic administration of leptin; the increase in food intake occurring when these animals are switched from short to long days is blocked by exogenous leptin (Drazen et al., 2001). Unlike other species, however, such as standard strains of laboratory rats and mice, leptin administration did not affect food intake when Siberian hamsters were at the body and lipid mass peaks in long days (Drazen et al., 2001). This result contrasts with the findings of two earlier studies of Siberian hamsters where peripheral leptin injections decreased food intake to the same extent in both long and short days, but reduced body and fat pad mass to a greater extent in short days (Atcha et al., 2000; Klingenspor et al., 2000). The precise reasons for these discrepancies are unknown, but in part may be due to differences in leptin administration, as well as other methodological considerations (for discussion, see Drazen et al., 2001). The most robust effect of leptin on food intake in rats and mice is when it is given intracerebroventricularly, and to our knowledge, this has not been done in Siberian hamsters. Although Siberian hamsters do not increase food intake after a fast, release from a less than complete food restriction can stimulate food intake (Fine and Bartness, 1996; Rousseau et al., 2003), and chronic peripheral leptin administration does not block this increase, nor does it have any effect on body or lipid mass in these animals (Rousseau et al., 2002). Despite the varied leptin-induced responses across these experiments, there is the tendency for leptin to act differentially between the photoperiods to affect energy balance and food intake. Therefore, seasonal changes in circulating leptin concentrations, coupled with changes in leptin sensitivity, may serve as part of an adaptive mechanism for increasing the odds of winter survival when food availability is decreased and adipose tissue stores are at their nadir (for review, see Rousseau et al., 2003).

Lastly, receptors for leptin (Ob-Rb) are located in several regions of the hypothalamus, and leptin binding to these receptors can contribute to photoperiodic changes in body mass and adiposity. As demonstrated for nonphotoperiodic rodents, photoperiodic rodents such as the Siberian hamster displays Ob-Rb in the hypothalamus, particularly in the areas of the arcuate nucleus (Arc), the ventromedial nucleus (VMN), the dorsomedial nucleus (DMN), the paraventricular nucleus (PVN), and the lateral hypothalamic area (LH) (Mercer et al., 2000a). In addition, Ob-Rb also respond to photoperiod; short-day hamsters display decreases in hypothalamic Ob-Rb compared with long-day animals (Mercer et al., 2000a, 2001). Importantly for the regulation of body mass, the neurons containing these receptors colocalize with other hypothalamic peptides that are involved in the regulation of energy balance; these peptides are described in the following section.

### *Hypothalamic Neuropeptides*

A large number of neuropeptides are localized within specific regions of the hypothalamus in mammals which appear to play a critical role in the regulation of food intake (reviewed in Adam and Mercer, 2004). Among these peptides, a core group of peptides have been identified and these peptides have been categorized into those that stimulate food intake (orexigenic) and those that inhibit food intake (anorexigenic). Those peptides receiving most experimental attention are the orexigenic peptides neuropeptide Y (NPY) and agouti gene-related peptide (AGRP), and the anorexigenic peptides pro-opiomelanocortin (POMC) and cocaine- and amphetamine-related transcript (CART). Considerable evidence exists in support of the idea that changes in one or more of these peptides are critical to the regulation of food intake and thus energy balance. Although considerably less is known about the potential role of these peptides in the seasonal regulation of body mass and energy balance, recent evidence suggests that these peptides play an important role in photoperiodic species (figure 19.1). Recently, hypothalamic peptide gene expression has been examined in long- and short-day-housed Siberian hamsters (Mercer et al., 2000a, 2001). In general, these studies consistently report decreases in POMC and increased expression in CART in short days, whereas both AGRP and NPY are unaffected by photoperiod.

As with other rodent species, the hypothalamic peptide NPY acts as an orexigenic molecule by triggering robust increases in food intake (Boss-Williams and Bartness, 1996). In addition, fasting-induced decreases in body fat in nonphotoperiodic species suggest increases in NPY gene expression (Adam and Mercer, 2004). In contrast, photoperiod has no effect on NPY gene expression (Adam and Mercer, 2004). Additionally, based on food restriction studies, one would expect increased CART expression in short days, consistent with that seen in fasted animals. As with NPY, however, CART expression does not fit this simple prediction. In fact, CART gene expression actually changes in the opposite direction as predicted, with decreased expression seen in short-day-housed animals. Similar findings have been reported in the seasonally breeding sheep. Unlike rodents, sheep are short-day breeders, breeding during autumn and winter and inhibiting breeding during long days. As with rodents, however, there are pronounced decreases in leptin in short days. Food restriction or food deprivation in sheep results in increases in NPY and AGRP gene expression, and decreases in CART and POMC gene expression, a finding consistent with rodents and with the decrease in leptin levels. However, when leptin is decreased via exposure to short days, there is no change in NPY or Ob-Rb, and up-regulated CART and POMC expression (Marie et al., 2001). A subsequent study in sheep confirmed the up-regulation in POMC gene expression, but reported increases in Ob-Rb and NPY expression in short-day-housed sheep compared with long-day animals (Clarke et al., 2003). Thus, it is clear that some discrepancies exist in the literature. Despite these discrepancies, however, taken together, the data from food restriction studies combined with data from photoperiodic analyses, in both

**FIGURE 19.1.**

Twenty-four-hour profiles of hypothalamic gene expression from adult male Siberian hamsters housed in long days (open circles) or short days (solid circles) for 12 weeks and killed at 3-h intervals throughout the respective LD cycles. Modified from Ellis et al. (2008).

sheep and hamsters, suggest that short-day animals, unlike food-restricted animals, maintain a state of energy balance despite decrease food intake and loss of body fat (Adam and Mercer, 2004).

## Central Nervous System Regulation

Both white adipose tissue (WAT) and brown adipose tissue (BAT) receive innervation from the autonomic nervous system (ANS). Although sympathetic nervous

system (SNS) innervation of BAT has been well established, SNS control of WAT has been more recently elucidated. Unlike SNS, no convincing evidence in support of parasympathetic innervation of WAT has been documented to date (reviewed in Bartness et al., 2002). Studies employing a variety of monosynaptic and transsynaptic tract tracers, as well as functional studies involved physical or pharmacological denervations of SNS nerves innervating WAT, have demonstrated that SNS input plays an important role in the regulation of lipid mobilization. More important for this chapter, seasonal or photoperiodic changes in SNS outflow may play a critical role in photoperiodic regulation of body fat. For example, exposure to short days increases adipocyte sensitivity to noradrenergic stimulation in Siberian hamsters (Bowers et al., 2005). Furthermore, SNS denervations of WAT, coupled with adrenal demedullations (which eliminate noradrenergic outflow), can block short-day decreases in body fat in Siberian hamsters (Demas and Bartness, 2001). These short-day-induced changes in lipid mobilization, like changes in reproduction, are likely driven by pineal melatonin; central nervous system (CNS) neurons that are part of the sympathetic outflow to WAT express melatonin (MEL1a) receptors. Thus, seasonally coded melatonin signals can act directly on SNS targets to regulate photoperiodic changes in today body fat, at least in hamsters, and likely other seasonally breeding rodents.

## PHOTOPERIODIC REGULATION OF IMMUNE FUNCTION

Seasonal variation is observed in most major classes of diseases among vertebrates (Nelson et al., 2002; Nelson, 2004). Although much of the seasonality, for instance, in infectious diseases is related to specific environmental conditions and life-history traits of pathogens and vectors, there are also prominent fluctuations in host immune function that can affect disease parameters. In common with other nonreproductive adaptations to day length, changes in the immune system appear to be the result of “eavesdropping” on the neuroendocrine signals that tie photoperiod information to the reproductive system. Over evolutionary time adjusting immunological function in concert with changes in the reproductive system must have provided a competitive advantage. As the two systems are largely under the control of the pineal melatonin rhythm (Nelson and Demas, 1997), marked adjustments in immunological function can be induced in the laboratory by altering day length.

Why should immune function vary seasonally? Logically, it would seem that organisms would favor strategies in which they maintained the maximal immune defenses possible without inducing autoimmunity (Nelson et al., 2002). However, immune defenses are energetically costly to maintain and utilize (see below; see also Demas et al., 1997a; Martin et al., 2003), and so competing physiological processes reduce the energy available for immune defense. In a seasonal context, this is usually conceptualized via energetic trade-offs between the reproductive and immune systems (Deerenberg et al., 1997; Prendergast et al., 2004a; Martin et al.,



2008). Said another way, maximal reproductive output is not fully compatible with high levels of immune defenses. Indeed, there are many examples of short winterlike photoperiods that suppress reproduction also enhancing various immunological processes across vertebrate taxa (reviewed in Nelson and Demas, 1996; Nelson et al., 2002). From a life history perspective, individuals invest in survival mechanisms prior to puberty, whereas they invest in reproduction after puberty. Seasonally breeding animals fluctuate between reproductive condition and retrogression to a prepubertal state. We have conceptualized enhanced immune function outside of the breeding season in short days among small mammals and birds as representing individuals investment in overwinter survival (Nelson and Demas, 1996).

Previously, we outlined a scheme in which harsh winter conditions could both directly kill small vertebrates via starvation or hypothermia and indirectly kill by rendering them more susceptible to infectious diseases (Nelson and Demas, 1996), particularly via chronic-stress-induced glucocorticoid secretion (McEwen et al., 1997) and other immunosuppressive mediators. The primary hypothesis that has driven research in our laboratories has centered on the assumption that animals boost immune function during the winter in order to maintain immune function at viable levels despite the effects of harsh winter conditions and the associated stress responses (Nelson and Demas, 1996; Sinclair and Lochmiller, 2000; Nelson et al., 2002). As changes in day length reliably predict a set of environmental conditions, including low temperatures and reduced food availability, a boosting of immune responses can prophylactically block compromised immune function. This hypothesis suggests that the short-day enhancement of immune function seen in the laboratory would be limited in the wild during particularly harsh winters during conditions of reduced food availability, pronounced stressors, and higher thermoregulatory demands. Our research has largely borne out these predictions as low temperatures or food restriction can reduce short-day enhancement of immune activity among rodents (Demas and Nelson, 1996; Bilbo and Nelson, 2002). An alternative explanation is that maintenance and use of the reproductive system shunts energy away from the immune system and short-day enhancement of immune function actually represents a disinhibition mediated by regression of the reproductive tract. A thorough review of these two competing hypotheses is beyond the scope of this chapter (but see section below on sex steroids; for a review, see Martin et al., 2008), but most data in mammals support the former rather than the latter hypothesis.

The argument requires significantly more nuance as the vertebrate immune system is complex and consists of multiple partially redundant subsystems that do not necessarily covary (Demas and Nelson, 1998a; Martin et al., 2007). Thus, it would be an oversimplification and inaccurate to state that all aspects of immune defenses are enhanced in the nonbreeding season. Some (perhaps most) immunological processes are enhanced by short day lengths, but others are inhibited (Nelson and Demas, 1996; Yellon et al., 1999a; Drazen et al., 2001; Bilbo et al., 2002a).

## The Vertebrate Immune System

The vertebrate immune system is a complex set of interacting tissues, cells, and soluble proteins diffusely distributed throughout the body. Collectively, these systems serve to prevent infection and also control and expel pathogens if infections do take place (Janeway et al., 1999). Broadly, the immune system can be broken down into two basic categories that differ in their evolutionary and developmental origins. The innate immune system consists of a variety of cells (e.g., macrophages, granulocytes, and natural killer cells) that are principally responsible for rapid and nonspecific antimicrobial actions and for the removal of extracellular pathogens. Innate immune cells contain pattern recognition receptors for common microbial components and are capable of initiating an immune response rapidly and also can directly destroy many types of invaders. However, activation of the innate immune system can be particularly damaging to host tissue as the responses tend to be extremely nonspecific. Importantly, there are strong interconnections between the innate immune system and the nervous and neuroendocrine systems, as we discuss below.

In contrast, the acquired or adaptive immune system consists of cell-mediated (T-cell) and humoral (antibody-mediated; B-cell) arms. Cell-mediated immune function is primarily responsible for controlling intracellular infection (cytotoxic T-cells) and for coordinating immune responses in B-cells and other immunological cells (T-helper cells). B-cells produce antibodies to foreign cells or proteins and thus target invaders for destruction by other arms of the immune system. The adaptive immune system is relatively recent evolutionarily and is characterized by membrane bound receptors on B- and T-cells, which can detect foreign antigens. These receptors are generated in development by a process of gene recombination. Adaptive immunity is distinct from innate mechanisms because (1) they are slower acting than innate immunity and (2) adaptive immune cells retain immunological “memory” and thus can respond vigorously to a foreign antigen that it has previously encountered. Immunologists traditionally assail the distinctions between the two systems and new interconnections are continually discovered, but for our purposes it is advantageous to discuss the systems separately (e.g., Chan et al., 2006). Various measures of cell-mediated immune function are nearly always enhanced by short day lengths in a variety of reproductively photoperiodic rodent species (Demas and Nelson, 1998a; Bilbo et al., 2002a; Weil et al., 2006b).

## Photoperiodic Modulation of Immune Function

Historically, many ecological immunology investigators have measured the size of various immunological tissues in early studies of the seasonality of immune function. This approach, though relatively uninformative about specific immune defenses, supports the notion that there is reception of photoperiod information by immunological tissues. Major organs of the immune system include the spleen,

thymus, Bursa of Fabricius (in birds), bone marrow, and lymph nodes. These tissues perform various immunological processes including supporting leukocyte (white blood cell) development, filtering blood-borne antigens, and providing anatomical loci for interaction between innate and adaptive immune cells. Implicit in the measurement of these tissues is the argument that increased size of these tissues represents greater immune activity. This perspective is taken from seasonal reproductive function in which larger gonads are consistent with increased function. Short days increase the mass of these tissues in a variety of free-living species. For instance, splenic masses are larger in short days in Norway rats (*Rattus norvegicus*) and Syrian hamsters (Wurtman and Weisel, 1969; Vriend and Lauber, 1973; Vaughan et al., 1985). Similar patterns are evident in birds as spleen and thymus mass are at their nadir during vernal recrudescence (Fänge and Silverin, 1985; John, 1994; Silverin et al., 1999). In other rodents, however, such as the short-tailed vole (*Microtus agrestis*) and European ground squirrel (*Spermophilus citellus*), splenic mass is reduced during winter. These data indicate the importance of comparing laboratory and fieldwork and also emphasize the need for more sensitive immunological measures. However, it is highly suggestive that immunological tissues (and thus putatively some aspects of immune function) are responsive to the changing seasons.

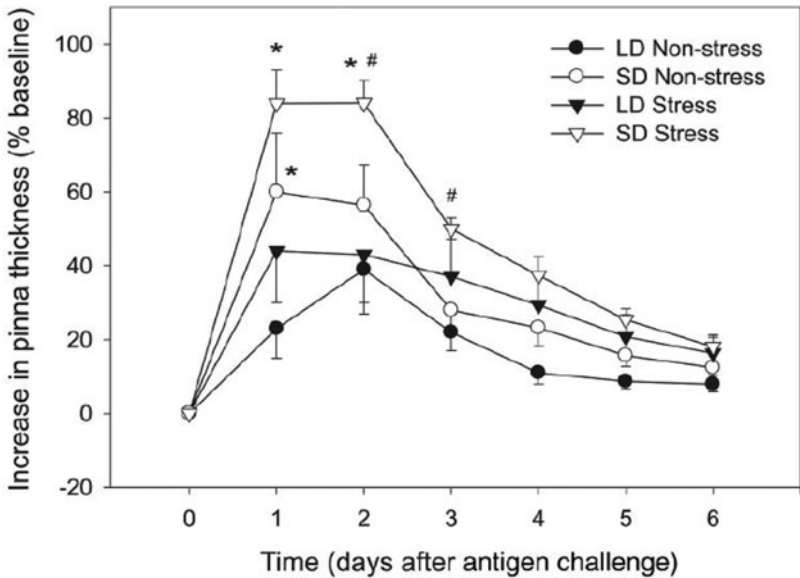
In addition to increasing immune activity, short day lengths also appear to buffer the immune system from suppression by metabolic stressors. 2-Deoxy-D-glucose (2-DG) interferes with the cellular uptake and utilization of glucose a key energy source for the immune system. Leukocyte proliferation is inhibited by 2-DG in female deer mice housed in long but not short days. 2-DG also elevated corticosterone concentrations in long-day but not short-day mice (Demas et al., 1997b). In contrast, Siberian hamsters enhanced delayed-type hypersensitivity (DTH) responses in short days; this effect is potentiated by access to a running wheel. However, the enhancing effects of both short days and exercise availability are blocked by food restriction. Together, these data indicate that short-day animals increase and defend enhanced immune responses against suppression by metabolic stressors (Bilbo and Nelson, 2004).

#### *Adaptive Immune Function*

The adaptive immune system, especially the cell-mediated arm of the system, is typically responsive to changes in day length. Cell-mediated immune function is generally measured by assessing leukocyte counts, lymphatic tissue masses, leukocyte proliferation to a mitogen in vitro (a putative index of the responsiveness of the cells to stimulation in vivo), and also DTH responses (in vivo assay of cell-mediated immune function that integrates multiple immune parameters including antigen processing and presentation, leukocyte targeting, and extravasation, as well as T-cell-mediated inflammation). DTH represents an ecologically valid response to antigenic challenge. As a general rule, functional assays of immune activity (e.g., DTH or proliferative responses) are more meaningful than morphological measures such as spleen size (Martin et al., 2006).

Cotton rats (*Sigmodon hispidus*) enhance splenocyte (spleen lymphocytes) proliferation in response to the mitogen, concanavalin A during February relative to other months of year. In the laboratory, enhanced cell-mediated immune function in short day lengths has been reported in a variety of rodent species including several *Peromyscus* species, Siberian hamsters, meadow voles, and collared lemmings (Demas and Nelson, 1998b; Bilbo et al., 2002a; Pyter et al., 2005c; Weil et al., 2006b). DTH responses in Siberian hamsters are particularly responsive to changes in day length (figure 19.2). Short days increase total circulating leukocytes, lymphocytes, and T-cells and also increase cutaneous immune function, and these effects are boosted by acute stress. Similarly, short days enhance wound healing in deer mice (Nelson and Blom, 1994); stress enhances cutaneous wound healing in short- but not long-day hamsters (Kinsey et al., 2003), suggesting that neuroendocrine modulation of cell-mediated immunity is photoperiod responsive.

Humoral immune function tends to be more variable than cell-mediated responses. Photoperiod differences depend on the species and specific end points measured, but antigen-specific immune antibody responses are blunted in Siberian hamsters (e.g., Yellon et al., 1999b; Drazen et al., 2001; Yellon, 2007). Further, exogenous melatonin does not enhance antibody production in deer mice or Syrian



**FIGURE 19.2.**

Short-day (SD) hamsters exhibited an enhanced DTH response percentage during both stress and nonstress conditions compared to long-day (LD) hamsters. Restraint stress significantly increased the inflammatory response during the two to three days after challenge with the allergen dinitrofluorobenzene in short-day hamsters only. Reprinted with permission from Bilbo et al. (2002a).

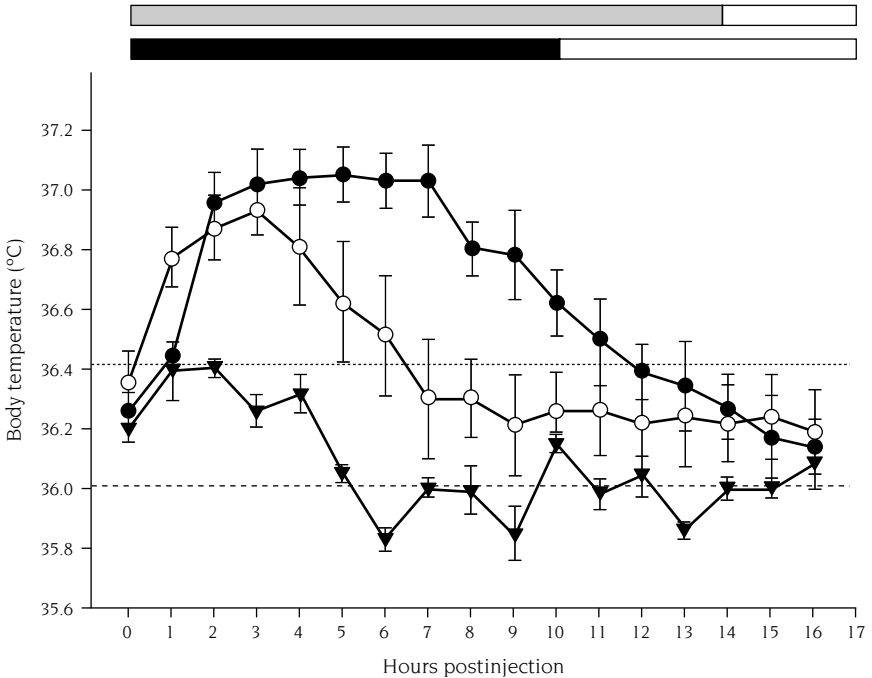
hamsters (Demas and Nelson, 1998a; Drazen et al., 2002). Antigen-specific antibody production, however, was enhanced in Syrian hamsters by exposure to short days; total circulating antibodies (natural antibodies) showed a similar response in deer mice (Demas and Nelson, 1996; Drazen et al., 2002), although antigen-specific antibody production did not vary by photoperiod (Nelson and Blom, 1994). In mice, melatonin can enhance antibody responses *in vivo* and this effect is mediated by the MT2 (Drazen and Nelson, 2001), the melatonin receptor missing in Siberian hamsters (Weaver et al., 1996). Photoperiod adjustments in adaptive immune responses are more common and distinct in the cell-mediated rather than humoral arm of the immune system. Presumably, this reflects investments in the immune defenses best suited for host defense at different times of the year (Martin et al., 2008).

In addition to regulating immune function acutely, several studies have reported that past photoperiod exposure can modulate or organize immune responses later. Prenatal photoperiod information is transferred to developing fetuses *in utero*, and this information is processed by the immune system; immune cell counts were altered even when mice were cross-fostered to dams in the opposite photoperiod (Horton, 1984, 1985; Blom et al., 1994). Siberian hamsters housed in short days both perinatally and postweaning exhibit strong DTH responses. However, exposure to long days during either of those developmental periods prevented the enhanced DTH responses (Weil et al., 2006c), indicating that early-life photoperiod organizes and directs how photoperiod exposure later in life alters the immune system. Additionally, adaptive immune responses produce immunological memory that persists far beyond the initial response. To determine whether photoperiod altered the induction, retention, or expression of immunological memories, Siberian hamsters were acclimated to either long or short days and then exposed to the T-cell antigen dinitrofluorobenzene. Some hamsters were maintained in their current photoperiod or transferred to the opposite one. Day length modulated both the acquisition and retrieval of immunological memory. Short day lengths during both the initial exposure to an antigen or during a subsequent challenge boosted cell-mediated immune responses. This effect was mitigated by exposure to long days after the initial exposure. Similarly, short days during the secondary exposure enhanced responses, but to a lesser degree if the hamster had been in long days during the initial exposure (Prendergast et al., 2004b).

#### *Innate Immune Function*

The innate immune system is a broadly effective but nonspecific defense system that can readily protect hosts against invading microbes. The innate arm of the immune system is relatively understudied compared to the adaptive side. Our research groups have generally observed a suppression of most aspects of innate immune function in short day lengths. It seems possible that the full investment in energetically expensive innate immune activity such as fever is prohibited by the tight energy budget associated with winter (Lochmiller and Deerenberg, 2000). In Siberian hamsters, short days appear to suppress most aspects of the innate immune system. For instance, phagocytosis (engulfing bacteria) and oxidative burst activity

(index of cytotoxic potential) by granulocytes and monocytes were inhibited by short day lengths (Yellon et al., 1999a). We sought to expand on these findings using an integrative measure of innate immune activity. We challenged Siberian hamsters with lipopolysaccharide (LPS), a component of gram-negative bacterial cell walls that activates the immune system and recorded body temperatures and food intake over the subsequent days (Bilbo et al., 2002b). These measures provide an index of a large number of immunological processes, including LPS recognition, cytokine production, and neuroimmune communication. Additionally, peripheral inflammation also induces a suite of behavioral responses, including anorexia, lethargy, and reduced social interactions, that have been termed “sickness behaviors” and are mediated by cytokine signaling in the brain (Hart, 1988; Dantzer, 2001). Short day lengths suppressed both the amplitude and duration of febrile responses, weight loss, and reduction in food and sweetened milk intake associated with simulated infection (figure 19.3). This effect was associated with attenuation of both



**FIGURE 19.3.**

Body temperature from 0 to 16 h post-LPS injections in long-day (black circles) and short-day (open circles) hamsters, and after saline injections in control hamsters (triangles). Black and gray bars above the graph indicate the active (dark) phase of the light–dark cycle in long- versus short-day hamsters, respectively. Horizontal dashed and dotted lines represent mean baseline body temperatures during the inactive (36°C) versus active (36.4°C) phases, respectively. Reprinted with permission from Bilbo et al. (2002b).

in vivo and in vitro proinflammatory cytokine production at both the protein and mRNA levels (Bilbo et al., 2002b; Pyter et al., 2005b). Additionally, expression of cyclooxygenase-2, the rate-limiting enzyme in the production of the inflammatory mediator prostaglandin E<sub>2</sub>, was also reduced (Bilbo et al., 2003). High doses of LPS produce a condition termed endotoxemia that is similar to the clinical condition of sepsis. Short day lengths protected hamsters from lethal endotoxemia and reduced the dose of LPS that was lethal in 50% of animals by more than 90%. This was associated with reduced expression of the proinflammatory cytokine tumor necrosis factor  $\alpha$  (Prendergast et al., 2003a).

Several possible explanations exist for these data. First, reduced cytokine production in short day lengths could be the key to a reduction in all downstream events. Treatment with interleukin-1 $\beta$  produces a sickness response similar to that created with LPS (Wen and Prendergast, 2007). However, short days attenuated the febrile response to an equivalent amount of interleukin-1 $\beta$ , indicating that differential cytokine production cannot completely explain photoperiod differences in inflammatory responses. Additionally, there are no photoperiod differences in gene expression of the principal LPS detector, toll-like receptor 4 (TLR4), in the spleen or peritoneal macrophages of Siberian hamsters (Navara et al., 2007). Taken together, these data suggest that the intracellular signaling pathways connecting TLR and cytokine receptor to gene expression changes are blunted by exposure to short days. Future studies should examine the nuclear factor $\kappa$ B signaling pathway as it is involved in both TLR and cytokine receptor signaling (Carmody and Chen, 2007). Finally, the possibility has to be considered that melatonin acting in the CNS mediates photoperiod modulation of sickness responses. Melatonin administration in the suprachiasmatic nucleus reduced the behavioral, but not febrile or cytokine, responses to acute LPS (Freeman et al., 2007).

## **Proximate Mediators of Photoperiod Changes in the Immune System**

Researchers have consistently reported large effects of day length on various aspects of immune activity. In trying to identify proximate mediators of photoperiod changes in the immune system, our research quickly centered on the pineal indole melatonin. Photoperiod changes in immune function, as with most day-length-dependent traits, can be reproduced with the administration of appropriately timed exogenous melatonin (Bilbo and Nelson, 2002; Hotchkiss and Nelson, 2002; Prendergast et al., 2003b); similarly, immune adjustments to chronic short-day exposure are not evident in pinealectomized animals (Yellon, 2007). However, it was not immediately evident in studies of photoperiodic modulation of immune function whether melatonin was acting directly on the immune system or alternatively whether melatonin-induced changes in other neuroendocrine systems (e.g., neuropeptides, sex steroid hormones, or glucocorticoids) could be the key mediator of photoperiod adjustments in the immune system.

### Melatonin

The principal physiological mediator of day length is the neurohormone melatonin. Pineal melatonin is secreted at night, and its production is under the control of a multisynaptic pathway originating in photosensitive cells in the retina. Melatonin interacts with high-affinity G-protein-coupled membrane receptors on a variety of tissues. Most mammals have two melatonin receptor subtypes: melatonin receptor 1a (Mel 1a or mt1) and melatonin receptor 1b (Mel 1b or mt2) that are widely distributed on a large variety of cell types in the CNS and in the periphery. In birds, a third melatonin receptor subtype (1c) has also been identified (Reppert et al., 1995). Importantly, melatonin receptors are present on a variety of immune cell types and tissues (Garcia-Perganeda et al., 1997; Pozo et al., 1997; Barjavel et al., 1998; Konakchieva et al., 1999). Melatonin is a potent immune modulator in a variety of species across taxa, including laboratory rodents, ungulates, and passerines (Bentley et al., 1998; Bilbo and Nelson, 2002; Dahl et al., 2002; Guerrero and Reiter, 2002).

In domesticated laboratory animals, melatonin consistently enhances nearly all aspects of immune function (Guerrero and Reiter, 2002). It should be noted that many laboratory mouse strains have a defect in the *N*-acetyltransferase gene (the rate-limiting enzyme in melatonin synthesis) (Goto et al., 1989; Stehle et al., 2002), and as such, nighttime melatonin rhythms are blunted or absent, suggesting that exogenous melatonin effects on the immune system must be interpreted carefully. However, in general, surgical or functional (i.e., housing in constant light which suppresses pineal melatonin production) pinealectomy reduces thymic and splenic masses in laboratory rodents (Vaughan and Reiter, 1971; McKinney et al., 1975), an effect that is mediated largely by a reduction in lymphocytes (Maestroni et al., 1986). Additionally, both cell-mediated and humoral immune function are impaired by pinealectomy, an effect that is blocked by exogenous melatonin (Vermeulen et al., 1993; Yellon et al., 1999b). Exogenous melatonin enhances such diverse immune responses as lymphocyte proliferation, antibody-dependent cellular cytotoxicity, antigen presentation, and cytokine production; evidence suggests that these effects are mediated at least in part by interaction with endogenous opioids (Maestroni et al., 1987; Vermeulen et al., 1993; Guerrero and Reiter, 2002; Hotchkiss and Nelson, 2002). Further, melatonin is particularly effective in enhancing immune function in immunosuppressed organisms and appears to antagonize some of the immunosuppressive effects of glucocorticoids (Maestroni et al., 1988).

In photoperiodic rodents, however, exogenous melatonin tends to recapitulate photoperiodic differences in immune function rather than being universally immunoenhancing. As discussed above, short day lengths and the lengthened melatonin rhythm are associated with suppression of some immunological parameters such as humoral immunity and cytokine-mediated sickness behaviors in Siberian hamsters (Bilbo et al., 2002b; Drazen et al., 2001). Melatonin can mimic these suppressive effects (Bilbo and Nelson, 2002).

Photoperiodic adjustments in the mammalian immune system require alterations in melatonin secretion as pinealectomy blocks photoperiod-induced enhancement of humoral immunity and exogenous melatonin can recapitulate short day lengths



(Demas and Nelson, 1998a; Yellon et al., 1999a, 2005; Bilbo and Nelson, 2002; Yellon, 2007). Further, melatonin and photoperiod share similar temporal characteristics. For instance, photoperiodic rodents typically become refractory to the suppressive effects of short day lengths on the reproductive system and will spontaneously regrow their testes after 20–24 weeks in photoperiod treatment (Prendergast et al., 2002a). Immunological adjustments associated with short day lengths also revert to the long-day pattern once animals become reproductively refractory to short days (Prendergast and Nelson, 2001). Melatonin does not alter immune function either in vivo or in vitro in refractory animals (Prendergast et al., 2002b).

Latitude of origin is an important determinant of reproductive responses to day length (animals from lower latitudes tend to be less responsive to day length than those from higher latitudes) (Bronson, 1985). Similarly, immunological adjustments to both day length and exogenous melatonin were linked to reproductive responses to photoperiod (Demas et al., 1996). Said another way, individuals that did not regress their gonads in short days also did not adjust cell-mediated or humoral immune function in response to changes in day length or exogenous melatonin.

Although lengthened melatonin rhythms are necessary for photoperiod changes in the immune system, it is difficult to parse direct effects of melatonin on immune cells and tissues from indirect immunomodulatory effects of melatonin that occur via changes in other neuroendocrine axes. To test the hypothesis that melatonin directly altered proliferation responses, we treated cultured prairie vole lymphocytes with melatonin and observed an enhanced proliferative response (Drazen et al., 2000a; Kriegsfeld et al., 2001). Indirect immune effects of melatonin on the reproductive or hypothalamic-pituitary-adrenal (HPA) axes are not possible as those tissues were no longer present. Siberian hamsters reduce lymphocyte proliferation following exposure to short day lengths; addition of melatonin to lymphocyte cultures suppresses proliferative responses in long but not short days (presumably because endogenous melatonin has already suppressed proliferative responses) (Prendergast et al., 2001b). Importantly, in house mice, the melatonin 2 (mt2), but not mt1, receptor is required for enhancement of lymphocyte proliferation (Drazen and Nelson, 2001). However, Siberian hamsters lack a functional mt2 receptor, and this may underlie the divergent effects of photoperiod on the hamster immune system as compared to other photoperiodic rodents (Weaver et al., 1996). These data indicate that at least some aspects of immune photoperiodic modulation of the immune system are mediated directly by acute changes in circulating melatonin. In most cases, however, chronic long-duration melatonin treatment is necessary for the expression of short-day-like patterns of immune activity (Bilbo and Nelson, 2002; Drazen et al., 2002). Taken together, these data indicate that melatonin is necessary for the expression of photoperiod changes in immune activity, and to a large extent, it is also sufficient even in the absence of other neuroendocrine cues.

### *Sex Steroid Hormones*

One of the key downstream targets of long-duration melatonin is the neuroendocrine reproductive axis. As sex steroids are also potent immunomodulators, it was reasonable to hypothesize that melatonin acted on the immune system at least in

part by modulation of gonadal steroids. In general, females have stronger immune systems and are less prone to infection than males (Klein, 2000). This sex difference is mediated largely by sex steroids with androgens being generally immunosuppressive (Folstad and Karter, 1992), whereas estrogens tend to have the opposite effect (Klein, 2004); although many counterexamples are available (Roberts et al., 2004). Yet, it has been known since the nineteenth century that prepubertal castration of rabbits increased thymic mass (Calzolari, 1898), and important ecological theories have been based on the idea that the support of secondary sex characteristics by androgens obligately suppresses immune activity in males (e.g., Folstad and Karter, 1992).

Short day lengths decrease circulating androgens and enhance many aspects of immune function in *Peromyscus* (Blom et al., 1994). This set of findings led to an early hypothesis that short day lengths enhanced immune activity by removing (disinhibiting) the suppressive effects of androgens (Nelson and Demas, 1996). A further prediction of that hypothesis is that if estrogens are immunoenhancing and short day lengths suppress estrogen release, then short days should suppress immune activity in females but enhance immune activity in males. However, this explanation has fallen out of favor because photoperiod differences in immune activity are relatively similar in both sexes (Demas and Nelson, 1998b) and gonadectomy (or androgen replacement) does not significantly alter photoperiod differences in immune activity in either sex (Demas and Nelson, 1998b; Prendergast et al., 2005). Nonetheless, sex steroids may have some lesser modulatory role that is typically masked by the larger effects of melatonin (Bilbo and Nelson, 2001; Prendergast et al., 2002b, 2008).

Further evidence against sex steroid mediation of the immune system comes from a series of studies where reproductive and immunological responses to day length can be dissociated. First, castrated animals exhibit the expected photoperiod differences in immune function (Demas and Nelson, 1998b; Prendergast et al., 2005), with only relatively small contributions of sex steroids to photoperiod differences in the immune system (Prendergast et al., 2008). Second, intermediate and perinatal photoperiods can dissociate reproductive and immunological responses to day length (Prendergast et al., 2004a; Weil et al., 2006c) such that regression of the reproductive tract can occur in the absence of enhanced immune function. Housing Siberian hamster males with ovariectomized females in short days suppresses both immune activity and the reproductive tract (Weil et al., 2007b). Finally, photoperiodic nonresponders (animals that fail to respond to day length with regression of the reproductive tract; Prendergast et al., 2001a) enhance immune function (Drazen et al., 2000b). Taken together, these data suggest that the immune and reproductive systems are both affected by photoperiod, but there is minimal direct interaction between the two systems, in a seasonal context, at least when food is available ad libitum.

### *Glucocorticoids*

The HPA axis is another immunomodulatory system that is regulated by day length (Nelson and Demas, 1996; Ronchi et al., 1998). Glucocorticoids are the end product,

primary effectors, and principal negative regulators of the HPA axis and are intimately and bidirectionally connected with the immune system. Glucocorticoids receptors are present on most types of immune cells (Smith et al., 1977; Armanini et al., 1988; Miller et al., 1998). Cytokines produced by cells of the innate immune system are a potent driver of HPA activity (Turnbull and Rivier, 1995); the resulting glucocorticoids then feedback to inhibit cytokine production and modulate other aspects of leukocyte physiology. Glucocorticoids have thus been conceptualized as brakes on the neuroendocrine-immune circuit that have evolved to prevent runaway inflammation (McEwen et al., 1997; Sapolsky et al., 2000; Sapolsky, 2002). The downside of this putative adaptation is that chronic elevation or high doses of glucocorticoids can suppress the immune system generally, and inflammatory responses specifically (McEwen et al., 1997; Padgett and Glaser, 2003).

HPA axis physiology (basal glucocorticoid concentrations, stress-evoked concentrations, negative feedback dynamics, and receptor distribution, etc.) is altered by day length (Ronchi et al., 1998; Pyter et al., 2007); the effects of photoperiod on HPA physiology are not nearly as large as those on the reproductive axis (Pyter et al., 2007), and the direction of these adjustments varies across experimental procedures and species (Ronchi et al., 1998; Bilbo et al., 2002a; Pyter et al., 2005a). Thus, the stressors associated with harsh winter conditions and concomitant adjustments in HPA axis dynamics could suppress immune function and therefore may have led to the evolution of photoperiod-mediated mechanisms for prophylactic enhancement of immune activity (Demas and Nelson, 1996; Nelson and Demas, 1996).

Basal circulating corticosteroids do not appear to mediate photoperiod differences in immune activity. For example, photoperiod differences in immune activity have been reported in rodents that do not differ in circulating corticosteroids (e.g., Demas and Nelson, 1998b; Weil et al., 2006a). Furthermore, many studies have reported enhanced immune activity in short days in animals with *higher* basal glucocorticoids (Bilbo et al., 2002a; Pyter et al., 2005c; Weil et al., 2006c). Such studies, however, do not fully consider the importance of 24-h dynamics of glucocorticoids, potential differences in receptor distribution, or downstream signaling events. Studies on animals with clamped HPA axes will be necessary to fully uncover the role of basal glucocorticoids in photoperiod differences in immune activity.

In recent years there has been a partial reconceptualization of glucocorticoid-immune interactions. Although chronic exposure to or high doses of glucocorticoids can certainly be immunosuppressive, there is mounting evidence that glucocorticoids can also induce redistribution rather than death of immune cells (Dhabhar and McEwen, 1997). Acutely stressing laboratory rats leads to a marked reduction in circulating leukocytes; the cells are not dying, but rather are trafficking to the front lines of immune defense in the skin, gut, and lymphatics to mediate enhanced immune activity in these tissues (Dhabhar et al., 1995; Dhabhar and McEwen, 1999). This effect is mediated by acute elevation of glucocorticoids (Dhabhar et al., 1996) and is sensible from an evolutionary perspective as acute stressors would

often have been associated with wounding and potential infection; thus, it would not be adaptive for acute increases in glucocorticoids to suppress the immune system. Short day lengths enhanced both basal and stress-induced trafficking of immune cells and skin immune function; the increase in immune cell trafficking was associated with higher glucocorticoid concentrations both prior to and after restraint stress (Bilbo et al., 2002a). Future studies will address whether short-day patterns of exogenous glucocorticoids can produce the short-day magnitude of leukocyte trafficking in long-day hamsters. We predict that this experiment would yield leukocyte-trafficking responses intermediate between the typical patterns of stress-induced trafficking of long- and short-day hamsters. In sum, these results suggest that although glucocorticoids may modulate immune activity differentially at different times of the year, glucocorticoids are probably not the key proximate mediator of photoperiod differences in immune activity.

### *Prolactin*

Prolactin is a protein hormone produced in the anterior pituitary with many and varied roles in the regulation of growth, reproduction, development, and water and electrolyte balance, as well as immune function (Goffin et al., 1999; Yu-Lee, 2002). Prolactin receptors are expressed in the cells and tissues of the immune system (Leite De Moraes et al., 1995), and adenohipophysectomy (removal of the anterior pituitary) leads to thymic involution and deficits in cell-mediated and humoral immunity that can be blocked with exogenous prolactin (Smith, 1930; Reber, 1993). Consistent with these effects on immune tissue, prolactin generally enhances immune responses in laboratory animals. For instance, prolactin increases lymphocyte proliferation (Matera et al., 1992), although these studies typically use doses outside of the physiological range. High concentrations of the hormone can increase immune activity to the point of autoimmunity, and prolactin release inhibitors are being used clinically in cases of organ rejection and autoimmunity (Vera-Lastra et al., 2002). Prolactin antagonizes glucocorticoid-induced lymphocyte apoptosis and may be part of a network that maintains immunological homeostasis (Fletcher-Chiappini et al., 1993; Dorshkind and Horseman, 2001).

A reduced concentration of prolactin in short day lengths is one of the most consistent findings in mammalian physiology (Goldman and Nelson, 1993). Despite the generally immunoenhancing effects of prolactin in laboratory model species, prolactin concentrations are generally elevated in long days and are associated with blunted immune responses. This is likely related to variations in experimental procedures (e.g., doses and the use of highly domesticated laboratory animals). In more recently captive outbred animals such as deer mice, treatment with the chemical carcinogen dimethylbenzanthracene causes squamous cell carcinomas to develop only if the animals are housed in long day lengths; no short-day mice developed the tumor. However, when long-day mice were treated with the prolactin release inhibitor bromocriptine, the incidence of tumors declined nearly 50% (Nelson and Blom, 1994), although the role of immunological processes in the development of these tumors is relatively small. In steers, short photoperiods increase lymphocyte proliferation

and neutrophil chemotaxis. However, exogenous prolactin, administered to increase short-day animals to long-day levels, blocked the short-day enhancement of these responses (Auchtung and Dahl, 2004). More data are required, but it seems possible that reduced circulating prolactin in short days may be necessary for photoperiodic plasticity in the immune system.

### *Leptin*

As described, leptin is an adipocyte-derived peptide hormone that was originally described as the product of the *OB* gene (Zhang et al., 1994). *OB* mice are obese due to both overeating and decreased energy expenditure (Coleman, 1978). Leptin serves at least in part as a signal to various tissues of adiposity and energy availability. Immune cells express leptin receptors, so leptin can interact directly with them, and the leptin receptor shares many signaling properties with the cytokine receptor interleukin-6R (Baumann et al., 1996). However, immunomodulatory effects of leptin may be, at least in part, mediated by changing the availability of metabolic fuels. Leptin-deficient mice have impaired wound healing, fewer T-cells, and impaired macrophage responses (Lord et al., 1998). These effects can be recapitulated in wild-type mice by severe food restriction and reversed in both starved and leptin-deficient mice with exogenous leptin (Lord et al., 1998). In wild-type animals, leptin increases phagocytosis and T-cell proliferation (Baumann et al., 1996).

No consistent relationship exists between day length and leptin concentrations across species. This probably reflects the various strategies that different species utilize in terms of winter energy storage (i.e., weight loss or weight gain). Photoperiodic differences have been reported in Siberian hamsters and woodchucks (*Marmota monax*) (Klingenspor et al., 1996b; Concannon et al., 2001). Siberian hamsters reduce antibody production in short day lengths. Treatment with exogenous leptin increased antibody production in short-day animals but had no effect on long-day animals (Drazen et al., 2001). This effect was mediated by increased food intake in leptin treated hamsters (Drazen et al., 2001). Surgical removal of body fat decreases humoral immune function (Demas et al., 2003), and this effect can be antagonized by exogenous leptin (Demas and Sakaria, 2005). Leptin appears to provide information to the immune system about energy stores and thus may regulate the expression of energetically costly immune responses (Demas, 2004; Demas and Sakaria, 2005).

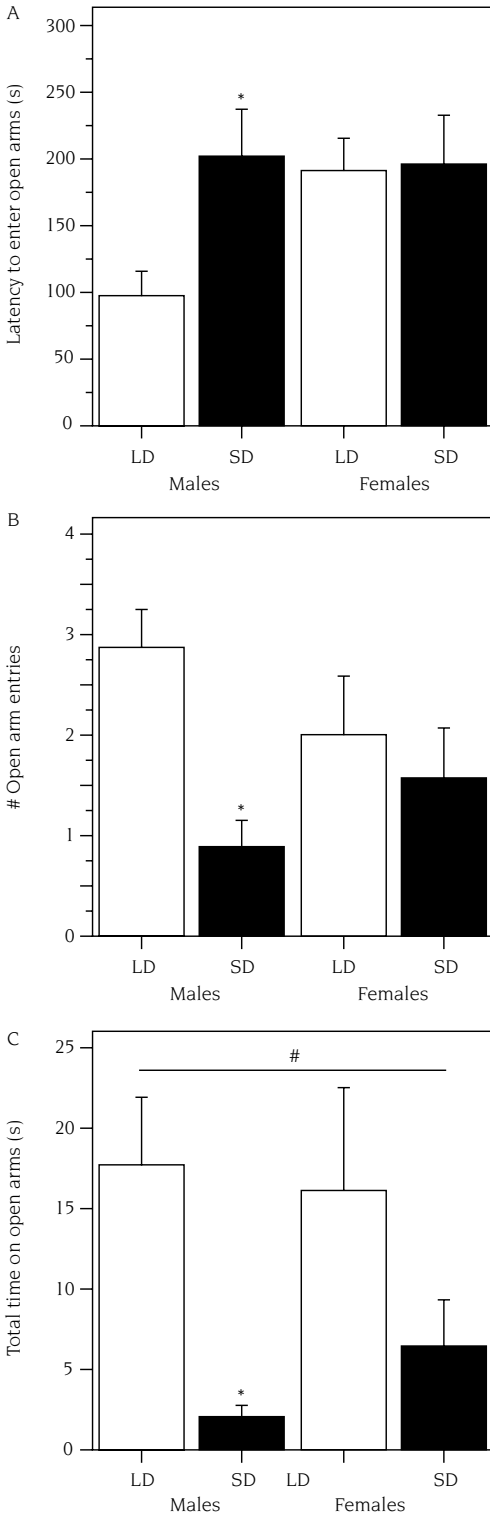
## **PHOTOPERIODIC REGULATION OF BEHAVIOR**

### **Affective Behaviors**

Higher mental functions in human and nonhuman animals can be divided into cognition, behavior, and affect/mood (Rubin et al., 2002). Affect is defined as the “observable emotional state of individuals,” and affective disorders as “abnormal states of feeling, primarily excessive sadness or elation” (Rubin et al., 2002).

Although traditionally considered to be maladaptive in humans, behaviors similar to symptoms of depression and anxiety disorders persist in other species and may be adaptive under specific environmental circumstances, including those associated with the changing seasons. For example, symptoms of affective disorders, such as lethargy, altered food intake, loss of sexual motivation, and fearfulness may actually conserve energy during the winter, a time when many organisms experience marked energetic bottlenecks (Nesse and Williams, 1996; Nesse, 2000; Wehr et al., 2001). Affective behaviors, and the neuroanatomical and neurochemical regulation of these behaviors, may be modified by seasonal information. Studies examining the effects of photoperiod on affective behaviors in nonhumans, however, are limited. One study conducted in laboratory rats (*Rattus rattus*) demonstrated that exposure of animals to long days reduced depressive-like behavior (Molina-Hernandez and Tellez-Alcantara, 2000). In addition, short day lengths decreased neophobia in two strains of *Mus musculus* (Kopp et al., 1999). Lastly, a recent study in rats demonstrated that rats housed in short days displayed higher levels of anxiety-like behavior on both open-field and elevated-plus tests (Benabid et al., 2008). Short-day-exposed rats that received a light pulse in the middle of the dark phase, however, did not display comparable increases in anxiety (Benabid et al., 2008). Interestingly, both house mice and laboratory rats have traditionally been considered nonresponsive to photoperiod, at least with respect to reproduction.

A recent study in a photoperiodic rodent reported elevated depressive- and anxiety-like peripubertal Siberian hamsters (Prendergast and Nelson, 2005). Short-day-exposed male hamsters spent less time in exposed areas of an elevated-plus maze relative to sheltered areas and exhibited behavioral despair more frequently in the Porsolt forced-swim test relative to long-day males (figure 19.4). These behaviors were seen after only two weeks of short-day exposure, well before changes in gonadal steroids and reproductive function. Subsequently, the effects of both perinatal and postweaning photoperiod on affective behavior were examined in Siberian hamsters (Pyter and Nelson, 2006). Hamsters exposed to short days (LD 8:16) perinatally displayed more anxiety-like behavior as adults in an elevated-plus maze test but displayed less anxiety-like behavior in the open-field and marble burying tests compared with hamsters born in long days (LD 16:8) (Pyter and Nelson, 2006). Hamsters exposed to short days postweaning displayed more anxiety-like behavior as adults in the elevated-plus maze and open-field tests and more depressive-like behavior in the Porsolt forced-swim test compared with those exposed to long days. A similar study was conducted in collared lemmings housed in long (LD 22:2), intermediate (LD 16:8) or short day lengths (LD 8:16) for nine weeks (Weil et al., 2007a). Specifically, lemmings housed in long days reduced anxiety-like responses in the elevated-plus maze. Depressive-like behaviors were decreases in animals housed in the intermediate photoperiod relative to both long- and short-day-housed lemmings (Weil et al., 2007a). Collectively, these results support the hypothesis that affective behaviors are organized early in life and can be maintained throughout adulthood. In addition, both anxiety- and depressive-like behavioral responses can be modulated by the postnatal environment, suggesting that photoperiodic



**FIGURE 19.4.**

Results of an elevated-plus maze test of male and female Siberian hamsters exposed to either long days (LD) or short days (SD) beginning at weaning (day 18). (A) Latency to first enter an exposed arm. (B) Number of entries onto exposed arms. (C) Total amount of time exploring exposed arms. Modified from Prendergast and Nelson (2005).

changes in human affective behaviors and disorders may reflect adaptive responses to a seasonally changing environment. The precise neuroendocrine mechanisms explaining these changes in behavioral state are not known at this time but may reflect seasonal/photoperiodic changes in HPA activity. In support of this idea, it has recently been demonstrated that exposure to short days increases corticosterone responses to environmental stressor and enhances glucocorticoid negative feedback of the HPA axis in white-footed mice (Pyter et al., 2007). Regardless of the precise mechanisms, the adaptive need for behavioral and affective adjustments to the seasonal environment suggests that resulting photoperiodic modifications of physiology and behavior are critical for survival.

## **Aggressive Behaviors**

Aggression is a highly complex behavior displayed by virtually all living organisms that serves a wide range of adaptive functions. The possibility for aggressive behavior exists whenever the interests of two or more individuals are in conflict, typically involving limited resources (e.g., food, territories, and mates). Despite its importance, aggression is a notoriously nebulous concept that has been defined and categorized in a multitude of ways over the years. Aggression has traditionally been defined as overt behavior with the intention of inflicting physical damage upon another individual or “goal entity” (Moyer, 1971).

### *Photoperiod*

Several studies have indirectly examined the role of testosterone (T) in aggression by manipulation of photoperiod. Many nontropical rodent species are seasonal breeders, maintaining reproductive function during summer and curtailing breeding during the winter. Ambient day length (photoperiod) is the proximal environmental cue used by individuals within these species to coordinate their reproduction to the appropriate season (Goldman, 2001). For example, reproductive function (and high levels of circulating T) is maintained during long “summerlike” days (>12.5 h of light per day), whereas reproductive regression, including virtual collapse of the gonads and marked decreases in T, occurs during the short “winterlike” days (<12.5 h/day) (Goldman, 2001). Interestingly, maintaining male Syrian hamsters in short days *increases* resident-intruder aggression compared with long-day hamsters (Garrett and Campbell, 1980). Specifically, adult male Syrian hamsters housed in short days for nine weeks display approximately twice the amount of aggression in a resident-intruder test compared with long-day controls when tested 4 h before dark, despite gonadal regression (Garrett and Campbell, 1980). After prolonged maintenance in short days (>15 weeks), hamsters typically undergo spontaneous gonadal recrudescence (i.e., increased testicular mass and circulating T), despite continued maintenance in short days. The short-day increase in aggressive behavior largely disappear in animals undergoing spontaneous recrudescence, returning to long-day levels of aggression by 21 weeks (Garrett and Campbell, 1980). More



recently, short-day increases in aggression in male Syrian hamsters have been confirmed (Jasnow et al., 2002; Caldwell and Albers, 2004). For example, Syrian hamsters housed in short days (LD 10:14) for 10 weeks displayed a significantly greater number of attacks and a longer duration of attacks than did long-day hamsters when tested using a resident-intruder test (Jasnow et al., 2002). Furthermore, timed daily melatonin injections mimicking short-day patterns of the hormone in long-day, pineal-intact animals will produce short-day-like increases in aggression. Because these injections occurred for only 10 days, gonadal mass and circulating levels of T are unaffected, supporting the idea that photoperiodic changes in aggression are not mediated by changes in gonadal steroids in this species (Jasnow et al., 2002). In contrast, these results suggest that levels of aggressive behavior are mediated by changes in the pattern of melatonin secretion.

Photoperiodic changes in aggression have been demonstrated in females of at least one species, Syrian hamsters (Fleming et al., 1988; Badura and Nunez, 1989). Female hamsters were housed in long (LD 14:10) or short days (LD 6:18) for 12 weeks, and then both offensive and defensive aggression were tested (Fleming et al., 1988). Female hamsters maintained in short days displayed significantly less defensive aggression compared with long-day animals and thus had a higher ratio of offensive to defensive aggression than did long-day animals.

### *Melatonin*

In virtually all mammals, photoperiodic responses are mediated by changes in the pineal indoleamine melatonin. Melatonin is secreted in abundance during darkness, whereas daylight inhibits pineal melatonin secretion (Goldman, 2001). Thus, changes in ambient day length result in changes in the pattern of secretion of melatonin. In this manner, it is the precise pattern of melatonin secretion, and not the amount of hormone per se, that provides the biochemical “code” for day length (Goldman, 2001).

Pinealectomy, which eliminates melatonin secretion and renders animals physiologically “blind” to day length, prevents the short-day increase in aggression in female Syrian hamsters, whereas treatment of long-day hamsters with exogenous short-day-like melatonin increases aggression in female Syrian hamster (Fleming et al., 1988). Ovariectomy, in contrast, has no effect of aggression. This finding suggests that photoperiodic changes in aggression are independent of changes in gonadal steroids in female Syrian hamsters (Fleming et al., 1988). A subsequent study in female Syrian hamsters confirmed these findings and provided further support for a role of pineal melatonin in mediating photoperiod changes in aggression. Specifically, a higher percentage of female hamsters housed in short days (LD 6:18) showed aggressive behavior compared with long-day-housed (LD 16:8) hamsters (Badura and Nunez, 1989). Consistent with previous findings, short-day aggression was attenuated by pinealectomy, but treatment with exogenous estradiol (alone or in combination with progesterone) had no effect on aggression. These results support the hypothesis that photoperiodic changes in aggression are mediated by pineal melatonin, but independent of gonadal steroids, at least in female Syrian hamsters.

In Syrian hamsters, unlike most rodent species, females are more aggressive than males (Marques and Valenstein, 1977; Ciaccio et al., 1979). Few studies have examined the role of photoperiod on male aggression in rodents displaying typical male-dominant aggression. Unlike Syrian hamsters, male Siberian hamsters display significantly more aggression than do females. It has been demonstrated that short-day male Siberian hamsters are significantly more aggressive than were long-day animals (Jasnow et al., 2000, 2002), consistent with previous studies in Syrian hamsters. Specifically, male Siberian hamsters housed in short days (LD 8:16) for 10 weeks display a greater number of attacks during a resident-intruder test and have a lower latency to initial attack, relative to long-day (LD 16:8) animals. As previously reported for many rodent species, prolonged maintenance on short days (i.e., 20 weeks) resulted in spontaneous reproductive recrudescence in which the gonads, and thus T, returned to normal long-day levels (Jasnow et al., 2000). Gonadally recrudescenced hamsters displayed less aggression than gonadally regressed animals even though both groups experienced the same photoperiod and melatonin signal; levels of aggression in recrudescenced hamsters were generally indistinguishable from long-day hamsters (Jasnow et al., 2000). These results support previous findings in male Syrian hamsters (Garrett and Campbell, 1980). When short-day Siberian hamsters were implanted with Silastic capsules containing T (to achieve long-day-like levels), aggression actually *decreased* compared with short-day control animals (Jasnow et al., 2000), suggesting that short-day increases in aggression may be inversely related to serum T concentrations.

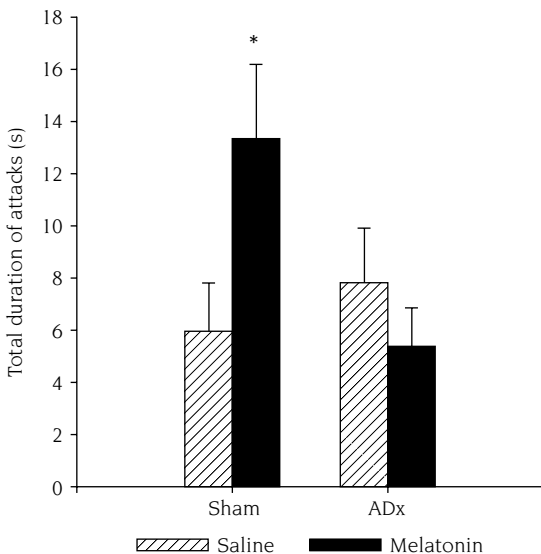
Despite growing evidence that short-day increases in aggression are independent of (or inversely related to) circulating levels of T, much less is known about the precise neuroendocrine mechanisms underlying seasonal aggression in rodents. As previously described, several studies have implicated changes in the pineal hormone melatonin in mediating short-day aggression. More recent research in male Siberian hamsters (Demas et al., 2004) confirms previous findings that treatment of long-day animals with short-day-like levels of melatonin mimics photoperiodic changes in aggression; long-day hamsters given daily timed injections of melatonin 2 h before lights out to mimic short-day levels of the hormone displayed elevated aggression in a resident-intruder test compared with control animals. As with previous studies, these results were not likely due to changes in gonadal steroids, as serum T was unaffected by this injection protocol.

The effects of melatonin on aggression in rodents may be due to direct actions of this hormone on neural substrates mediating aggression (e.g., hypothalamus, limbic system). Alternatively, melatonin-induced aggression may be indirectly due to changes in HPA activity, as adrenal hormones have been implicated in aggressive behavior (Haller and Kruk, 2003). In support of the latter hypothesis, changes in both the size and function of the adrenal gland are associated with changes in aggression (Paterson and Vickers, 1981). In addition, male house mice housed in a LD 12:12 photoperiod and treated with melatonin display increased territorial aggression, but decreased adrenal masses compared to saline-treated animals (Paterson and Vickers, 1981). The increases in aggression displayed by melatonin-treated

animals, however, can be blocked by adrenalectomy (Paterson and Vickers, 1981). Experimental reductions of both adrenomedullary catecholamines, as well as adrenocortical glucocorticoids, are associated with decreased aggression in rodents (Paterson and Vickers, 1981; Haller and Kruk, 2003) and reductions of glucocorticoids via pharmacological blockade of adrenocorticotrophic hormone release can attenuate melatonin-induced increases in aggression in mice (Paterson and Vickers, 1981). Thus, exogenous melatonin, despite reducing adrenal mass, appears to increase aggression by stimulating adrenocortical steroid release. These results are particularly intriguing given that house mice have traditionally been assumed to be photoperiodically nonresponsive (Nelson, 1990).

### Adrenal Steroids

More recently, research has implicated changes in adrenocortical hormones in mediating melatonin-induced and possibly short-day-induced aggression in Siberian hamsters. As described previously, long-day hamsters treated with short-day-like levels of melatonin displayed increased aggression, comparable to levels seen in short-day animals (Demas et al., 2004; figure 19.5). Melatonin-induced aggression could be blocked by bilateral adrenalectomy, consistent with previous results in house mice (Paterson and Vickers, 1981). Adrenal demedullation, which eliminates adrenal catecholamines (i.e., epinephrine) but leaves adrenocortical steroid release (i.e., cortisol, dehydroepiandrosterone [DHEA]) intact, had no effect on melatonin-induced aggression (Demas et al., 2004). Collectively, these results support the hypothesis that the effects of exogenous melatonin on aggression are mediated by the effects of this hormone on adrenocortical steroids. However, it is



**FIGURE 19.5.**

Mean (+ standard error) total duration of attacks in adult male Siberian hamsters that received bilateral adrenalectomies (ADx) or sham operations and subsequently treated with either melatonin or control (saline) injections \* $p < 0.05$ . Modified from Demas et al. (2004).

currently not known which class of steroid hormones may mediate this effect, as adrenal androgens (e.g., DHEA) and glucocorticoids (e.g., cortisol) have both been implicated in aggression in rodents (Schlegel et al., 1985; Haller and Kruk, 2003). In laboratory rats and mice, corticosterone is the predominant adrenal glucocorticoid, and these species secrete little to no adrenal DHEA. In contrast, in hamsters, as in humans, cortisol is the primary adrenal glucocorticoid, and both hamsters and humans secrete measurable amounts of DHEA and its sulfated form, DHEA-S (Pieper and Loboeki, 2000; Mellon and Vaudry, 2001).

Evidence in avian species suggests that aggression in the nonbreeding season (i.e., winter) may be mediated by changes in DHEA (Soma et al., 2000). Although similar evidence suggesting a role for DHEA in mediating photoperiodic changes in aggression in rodents is lacking, studies in mice suggest that exogenous melatonin can stimulate DHEA production from cultured adrenal glands (Haus et al., 1996). Behavior was not examined in this study; however, these results are consistent with the hypothesis that short-day increases in melatonin may increase adrenal production of DHEA and thus affect aggression.

Several field studies published to date support the laboratory data discussed above suggesting differential dependence on gonadal steroids for animals during breeding season versus when they are not breeding. Specifically, male ratlike hamsters (*Cricetus triton*) in the field display elevated aggression during the winter nonbreeding season, despite low levels of plasma T (Zhang et al., 2001). Seasonal changes in aggression appear independent of seasonal changes in circulating T in wild wood rats (*Neotoma fuscipes*) (Caldwell et al., 1984). Pronounced seasonal changes in aggression are seen in male wood rats, with high levels during midbreeding season and low levels during the nonbreeding season. Despite differences in circulating T levels at these two time points, castration has no effect on aggression, suggesting an independence of seasonal aggression from circulating levels of T (Caldwell et al., 1984). More recently, seasonal changes in aggressive encounters have been examined in free-living arctic ground squirrels (*Spermophilus parryii*) (Buck and Barnes, 2003). The effects of challenges by conspecific males on circulating T levels varied seasonally, with challenges by male intruders eliciting significant increases in circulating T during the spring breeding season, but similar challenges failed to trigger increase in androgen at the end of the summer after the breeding season. These results suggest that androgens play a more important role during the breeding season than during the nonbreeding season (i.e., late summer). Collectively, these studies fail to support the simple notion that all forms of aggression are mediated by circulating T by providing salient examples of T-independent aggression, at least with respect to circulating levels of the hormone. Unlike other forms of aggression, however, very little is known regarding the neuroendocrine mechanisms underlying seasonal changes in aggression in mammals.

#### *Gonadal Steroids*

In contrast to inbred laboratory mice, outbred *Peromyscus* mice show different patterns of aggression. In male California mice (*Peromyscus californicus*), aromatase

activity in the bed nucleus of the stria terminalis is negatively correlated with aggression (Trainor et al., 2004). Moreover, an aromatase inhibitor increases aggressive behavior (Trainor et al., 2004). Similarly, in male beach mice (*Peromyscus polionotus*) in long days, estradiol reduces aggression, but under short-day photoperiods, estradiol increases aggression. These effects are mediated by estrogen receptor- $\alpha$  as specific agonists for that receptor recapitulate the effect of estradiol. Photoperiod alters the expression of estrogen receptors in the beach mouse limbic system but the opposite effects of estrogen receptor activation on aggressive behavior appears to be independent of differential receptor expression (Trainor et al., 2007b). Rather, a photoperiod-mediated shift between genomic and nongenomic mechanisms of estrogen receptor signaling appears to mediate photoperiod differences in responses to estrogenic compounds. Microarray analysis indicated that a greater number of estrogen-responsive genes were up-regulated in the bed nucleus of stria terminalis in long-day mice relative to short-day animals. Importantly, estrogen rapidly increased (within 15 min) aggressive behaviors in short- but not long-day mice. Such rapid effects of steroid hormones are unlikely to occur via genomic mechanisms given the very short time scale (Trainor et al., 2007a).

#### *Nitric Oxide*

Nitric oxide (NO) is an endogenous gas that has the biochemical properties of a free radical and was initially identified as regulator of blood vessel tone (Moncada and Higgs, 1993). Since its initial characterization, NO has also been identified as an important neuronal messenger in the CNS and peripheral nervous system (Dawson and Snyder, 1994). NO is labile, with a half-life of approximately 5 s; consequently, many studies have manipulated NO indirectly by affecting its synthetic enzyme, NO synthase (NOS), involved in the transformation of arginine into citrulline and NO. Three distinct isoforms of NOS have been discovered in rodents: eNOS is located in endothelial tissue of blood vessels, nNOS is localized in neurons, and an inducible form (iNOS) is found in macrophages. Male nNOS<sup>-/-</sup> mice display a marked decrease in behavioral inhibition and display persistent fighting despite submissive displays by other mice (Nelson et al., 1995). Female nNOS<sup>-/-</sup> mice, in contrast, do not display elevated aggressiveness. Similar increases in aggression are seen in normal wild-type mice following pharmacological blockade of nNOS (Demas et al., 1997c). Castration of nNOS<sup>-/-</sup> mice results in a marked reduction in aggression and testosterone replacement restores aggression to precastration levels, suggesting that testosterone is necessary, but not sufficient, to elevate aggression in nNOS<sup>-/-</sup> mice (Kriegsfeld et al., 1997). More recently, the role of NO in mediating seasonal aggression has been explored (Wen et al., 2004). Specifically, Siberian hamsters housed in short days displayed increased aggression and significantly fewer nNOS-immunoreactive cells in several amygdala regions compared to long-day animals. Interestingly, these short-day changes in aggression and NO staining were also seen in short-day “nonresponders” (i.e., the subset of animals that fail to inhibit their reproductive systems in short days), suggesting that these changes are independent of changes in T (Wen et al., 2004).

Much of research on the neuroendocrine mechanisms of aggression focused on the role of gonadal steroid hormones, and predominantly testosterone, as the primary factor regulating aggression. Recent studies examining seasonal changes in aggression described in this chapter, however, have demonstrated that aggression is more complex than a single hormonal mechanism. Furthermore, studies of seasonal aggression point out that the same behavior (i.e., aggression) can have markedly different underlying mechanisms depending on differences in environmental conditions.

## **PHOTOPERIODISM AS A “MODEL SYSTEM” FOR THE STUDY OF NONREPRODUCTIVE TRAITS**

The primary goal of this chapter is to consider some of the exciting areas of research in which pronounced photoperiodic changes in physiology and behavior have been recently reported, including changes in immune function, energetics, and aggression/affective behavior. As discussed at the beginning of this chapter, the majority of research with respect to photoperiodism has traditionally focused on reproductive cycles. This is understandable, given the robust and reliable effects of changing day length on reproductive physiology and behavior, and its importance for reproductive success, in a large majority of mammalian species.

The considerable attention given to seasonal cycles in reproduction, however, has likely limited investigations of other interesting areas in which photoperiod may exert important effects on physiological and behavior. For example, considerable progress has been made in the last decade in understanding the neuroendocrine mechanisms regulating energy balance. Consistent with these findings, a large number of peptides have been identified within the last decade that appear to play a major role in the regulation of energy balance. Not surprisingly, these peptides, several of which were discussed above, are involved in the photoperiodic regulation of body weight in seasonally breeding animals. Thus, photoperiodic animals that display marked cycles in body fat on an annual basis serve as excellent animal models to uncover fundamental physiological mechanisms that regulate changes in adiposity. Further, an appreciation of these basic physiological mechanisms should contribute to the understanding and treatment of human metabolic disorders, including obesity.

Marked photoperiodic changes in immune function and sickness have also been demonstrated. The precise nature of these changes and whether they correlate with changes in other physiological systems (e.g., reproduction) or with changes in specific hormones (e.g., gonadal steroids, leptin, and melatonin) have provided important insights into the interactions among the endocrine, nervous, and immune systems. These studies have also changed the way we view epidemiological patterns of disease. By recognizing that seasonal changes in underlying immune responses, in addition to seasonal changes in pathogen prevalence, contribute to seasonal

changes in disease prevalence, more effective approaches to the treatment of a variety of illnesses can be developed.

Lastly, more recent investigations have demonstrated photoperiodic changes in aggression and affective behaviors. For example, some species of animals demonstrate marked aggression during short days despite low levels of testosterone, suggesting that the display of aggression is highly context specific and likely mediated by several neuroendocrine systems in addition to gonadal steroids. Similarly, photoperiodic changes in anxiety and depression have also been reported. As discussed above, these changes likely evolved as adaptive mechanisms to limit specific behaviors to the appropriate times of the year. Importantly, understanding the nature of these behavioral changes in nonhuman models will help us understand the physiological bases of clinical conditions such as seasonal affective disorder (SAD).

Despite the considerable progress that has been made in our understanding of the photoperiodic regulation of physiology and behavior, there are still important research questions that need to be addressed. For example, studies of photoperiodic changes in physiology and behavior have traditionally focused on specific characteristics (e.g., reproduction, immunity, energetic, aggression). Rather than focusing on specific systems in isolation, studies that employ an integrated approach to the study of photoperiodic changes in physiology and behavior will provide a more functional approach. For example, it is becoming increasingly clear that there are important links between seasonal changes and reproduction, energy balance, and immune function. In fact, recent evidence suggests the existence of important energetic trade-offs between reproduction and immune function such that one physiological system is curtailed while the other is up-regulated during times when energy is limited (i.e., winter). Furthermore, animals display increased depression-like changes in behavior during this same energetic bottleneck. These findings suggest that animals undergo a suite of coordinated adaptations, both behavioral and physiological, that maximize the chances for survival. Our understanding of these integrative responses would be considerably limited if each response was studied in isolation. In contrast, an integrative approach to understanding the coordinated photoperiodic responses of animals provides a deeper, more meaningful analysis of both the adaptive functions and the neuroendocrine mechanisms driving these responses. Lastly, photoperiodic species that display marked changes in physiology and behavior serve as ideal model systems to study naturally occurring changes in physiology and behavior in an environmentally relevant manner. In this era of translational research, the findings from these studies will contribute to our understanding and treatment of a wide range of clinical disorders, including obesity, autoimmune disorders, and SAD.

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