SEASONAL PLASTICITY OF PHYSIOLOGICAL SYSTEMS, BRAIN, AND BEHAVIOR

DISSertation

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by

Leah M. Pyter, B.S., M.S.

Ohio State University
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Dissertation Committee:

Professor Randy J. Nelson, Advisor
Professor Bennet S. Givens
Professor Karl Obrietan

Approved by

________________________
Advisor
Neuroscience Graduate Studies Program
ABSTRACT

Seasonal adaptations have evolved in animals and are believed to promote survival and reproduction through yearly changes in the environment. These seasonal responses are physiological, morphological, and behavioral and are coordinated by day length (photoperiod) information in non-tropical rodents. In the laboratory, short days promote a winter phenotype, whereas long days promote a summer phenotype. This dissertation was designed to examine the effects of photoperiod on adult plasticity of physiology, brain, and behavior in male white-footed mice (*Peromyscus leucopus*) that may represent seasonal adaptations to the changing environment.

Recent evidence suggests that significant plasticity occurs in adult systems including the central nervous system. The first studies examined the effects of photoperiod on angiogenesis in the periphery and the brain. Short days altered expression of angiogenesis genes in testes and brain and decreased cerebral blood flow compared with long days. The next set of studies was based on the finding that short days decreased brain mass and impaired spatial learning and memory compared with long days. The hippocampus is involved in spatial learning and memory and is one of the few brain regions associated with significant adult plasticity. Short days
decreased hippocampal volume, altered hippocampal dendritic spine density, modified the effects of learning experience on neurogenesis, and dampened long-term potentiation compared with long days.

Photoperiod alters concentrations of various hormones, some of which affect the hippocampal morphology and function. Testosterone reversed the short-day impairment of spatial learning and memory indirectly of hippocampal steroid receptors, but did not affect long-day performance. Corticosterone altered spatial learning and memory depending on photoperiod and duration of exposure and short days altered the hypothalamic-pituitary-adrenal axis compared with long days.

The effects of photoperiod on the other brain region characterized by significant adult plasticity, the olfactory bulbs, and olfaction was examined. Neurogenesis increased in the caudal olfactory bulbs of short-day mice. Finally, I tested the effects of photoperiod and testosterone on depressive- or anxiety-like behaviors and observed few differences. Taken together, photoperiod appears to coordinate adult plasticity within the endocrine and nervous system that result in behavioral changes hypothesized to be adaptive to a seasonally-changing environment.
DEDICATION

To my Dad, a role model in profession and in life.
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VITA

May 6, 1978……………………………..Born – Madison, Wisconsin, USA
1999……………………………………..B.S. Biology, University of Illinois at Urbana
2001……………………………………..M.S. Biology, University of Illinois at Urbana
2001 – present…………………………...Graduate Research and Teaching Associate, Ohio State University

PUBLICATIONS

Research Publication


**FIELDS OF STUDY**

Major Field: Neuroscience
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INTRODUCTION

Photoperiodism and Seasonal Changes in Energy Balance

Adaptations have evolved in organisms to promote survival and reproduction in seasonally-changing (non-tropical) environments. The harsh conditions of winter (i.e., low temperatures and scarce food availability) impose an energetic threat for individuals compared with the mild conditions of summer (i.e. mild temperatures and relatively abundant food). Therefore, vertebrates display seasonal changes in morphology, physiology, and behavior that presumably compensate for seasonal disruptions in energy homeostasis (Nelson, 1999; Piersma, 1997). Seasonal reproduction is a well-characterized example of a seasonal adjustment that is ultimately honed by energetic constraints in the environment (Bronson, 1989).

Most nontropical mammals breed seasonally (Bronson & Heideman, 1994). In seasonal environments, relatively short annual periods of mild environmental conditions confine energy-costly activities (such as reproduction) to brief seasons. Therefore, breeding, gestational, and lactational intervals often represent the best fit with optimal conditions, and thus typically coincide with mild seasons. However, morphological, physiological, and behavioral adjustments that are appropriate for particular seasons require time to develop. Animals use environmental cues such as
rainfall, temperature, and photoperiod to predict seasonal changes in the environment. Photoperiod, or day length, is determined precisely by the earth’s annual revolution around the sun and the tilt of the planet on its axis. Therefore, compared to other proximate cues (e.g., rainfall and temperature), photoperiod is the most consistent environmental predictor of the time of year. Whether day lengths are increasing or decreasing is the other essential component to accurate time-of-year predictions. For example, in Columbus, Ohio, there are approximately 12 h of light/day on March 17th and September 26th. To accurately distinguish between these two dates, information about the pattern of previous day lengths (i.e., decreasing or increasing successive day lengths) is used (Gorman & Zucker, 1995).

Exposure to varying photoperiods in the laboratory is sufficient to trigger many of the seasonal modifications observed in wild animals. For example, adult male white-footed mice (*Peromyscus leucopus*) maintained in short photoperiods (<12 h light/day), which are indicative of impending harsh conditions of winter (non-breeding season), undergo regression of their reproductive systems (Whitaker, 1940). Testosterone inhibition causes involution of the testes and inhibits reproductive behaviors among other anti-reproductive responses. The dramatic short-day inhibition of the reproductive system and the prevalence of seasonally-confined breeding seasons among species suggest that the energetic cost of maintaining a reproductive system during the non-breeding season is high (Bronson & Heideman, 1994). White-footed mice exposed to short days also lose body mass. The energetic cost of maintaining a smaller body size is lower than maintaining a large body size. Energy that is conserved from inhibition of the reproductive system and reduction in body mass during the non-breeding season may be
shunted to processes to promote mechanisms necessary for surviving the harsh environmental conditions such as thermoregulation and immune function (Nelson, 2004). Indeed, although rodents regress their reproductive systems in response to short days, some immune responses are enhanced (Bilbo et al., 2002a; Demas & Nelson, 1998; Nelson, 2004). The simultaneous inhibition of reproduction and enhancement of some components of immune function in short-day rodents may represent a shift in energetic priorities in preparation for the harsh conditions of winter. Individuals that fail to undergo these seasonal modifications to balance energetic demands with energy resources may not survive (Prendergast, Kriegsfeld, & Nelson, 2001).

**Melatonin**

Melatonin, an indoleamine hormone, transduces environmental day length information throughout the body in most vertebrates. Melatonin synthesis in the pineal gland is inhibited by light perceived through the retina (Malpaux, Migaud, Tricoire, & Chemineau, 2001). In the absence of light, inhibition of a multi-synaptic pathway linking specialized ganglion cells in the retina to noradrenergic afferents synapsing on the pineal gland is released and melatonin is synthesized (Goldman, 2001). Therefore, the duration of melatonin release is indirectly proportionate to day length (Illnerova, 1988). Because the circadian rhythm in melatonin secretion translates day length changes across the year, melatonin communicates seasonal information (Nelson & Demas, 1997; Prendergast, Nelson, & Zucker, 2002) in addition to circadian information (Goldman, 2001; Illnerova, 1988). Many of the effects of photoperiodic manipulation on physiology, morphology, and behavior can be replicated by daily melatonin infusions. For example, long durations
of melatonin secretion induce short-day-like adjustments. The inverse relationship between melatonin secretion and day length persists in all mammals, including humans (Lewy, Wehr, Goodwin, Newsome, & Markey, 1980; Smale, Lee, & Nunez, 2003). However, melatonin rhythms have been selectively “bred out” or weakened in strains of domestic laboratory rodents (Bronson, 1979). Therefore, the study of seasonal adaptations in rodents requires the use of wild-caught or outbred models derived from high-latitude (seasonally-changing) environments.

**Brain Energetics**

Based on the assumption that energetic homeostasis is challenged by seasonal changes in the environment, it is important to consider the major energy sinks for individuals. In addition to the energy used for survival behaviors, a portion of energetic resources is consumed by the maintenance of physiological systems, such as the nervous system. Although the brain comprises a small fraction of body mass, it consumes more energy per tissue mass than any other tissue in the body (Attwell & Laughlin, 2001; Peters et al., 2004). In fact, a theory termed “Selfish Brain Theory” uses evidence that the brain outcompetes the periphery for energy to explain some of the pathogenesis associated with disease (Peters et al., 2004). As previously discussed, short days trigger changes that decrease the metabolic costs in rodents such as reproductive tract involution, reduced body mass, hibernation, and reduced foraging. Similarly, any reduction in brain mass during the winter might confer considerable energetic savings. Functional consequences of reduced brain mass, such as reduced behaviors may confer even larger energetic savings than the cost of maintaining brain tissue itself (Jacobs, 1996a).
Adult Brain Plasticity

Until recently, it was believed that structural changes in the brain were limited to the developmental period and that the healthy adult brain was structurally immutable. This dogma was first challenged several decades ago, but was not rigorously contested until the 1970’s and 1980’s. Joseph Altman first reported neuronal proliferation in adult rats (*Rattus rattus*) in 1962 and Edward Bennett and colleagues published their findings on changes in cortical width of rats following environmental enrichment two years later (Altman, 1962; Bennett, 1964). Before these reports, only indirect evidence hinted that the adult brain may have the potential for plasticity (Lugano, 1906; Allen, 1912). Adult “plasticity” is operationally defined as the capacity of organisms to vary in phenotypic characteristics (e.g., anatomy or behavior) according to varying environmental conditions. Intricate changes to neurons within specific regions of the adult brain followed these reports. Alterations in neuronal dendritic complexity following environmental enrichment were first reported in the visual cortex of rats in the early 1970’s (Volkmar & Greenough, 1972). Spine density in the cerebral cortex was impacted by experience in adult rats (Globus et al., 1973). Later, seasonal birth of new neurons was observed in adult bird brains (Nottembohm, 1981). Subtle changes in inter-neuronal connections that may be considered examples of adult brain plasticity such as long term potentiation (LTP) and depression (LTD) were also described in the rat cortex by Hebb (Hebb, 1947). Following these pivotal contributions to the field of adult brain plasticity, numerous examples of extrinsic and intrinsic stimuli that alter adult brain structure have been reported.
Dendritic arborization, or complexity, is believed to represent the number of interactions (or synapses) between neurons. Therefore, complex dendrites, with numerous branches are capable of forming numerous synapses with other cells and enhancing interneuronal communication. Changes in adult dendritic complexity within the hippocampus, cerebral cortex, and olfactory bulbs of adult vertebrates have been reported in addition to earlier reports of changes in dendritic complexity within the visual cortex (Kolb & Whishaw, 1998; Volkmar & Greenough, 1972). These dendritic changes have been induced by learning and memory experience (Kavaliers, Ossenkopp, Galea, & Kolb, 1998; Kolb & Whishaw, 1998), environmental enrichment (Leggio et al., 2005; van Praag, Kempermann, & Gage, 2000), temperature (Popov, Bocharova, & Bragin, 1992), and sex hormones (McEwen & Woolley, 1994; Woolley, 1998).

Dendritic spines are short protrusions on dendrites that receive the majority of excitatory input from axon terminals (Hering and Sheng, 2001). Therefore, the number or density of spines on a dendrite represents the number of synapses between neurons (Sorra & Harris, 2000). Spines on pyramidal cells of the hippocampus and cortex and Purkinje cells of the cerebellum are well-characterized. The number and shape of spines on dendrites in adult brains change rapidly following exposure to learning and memory experiences (Moser, Trommald, & Andersen, 1994), hormones (McEwen & Woolley, 1994; Rasia-Filho, Londero, & Achaval, 1999; Shors, Chua, & Falduto, 2001a), temperature (Popov et al., 1992) fluctuation, and certain types of synaptic activity (Engert & Bonhoeffer, 1999).

Structural changes in the vascular network supporting the brain also occur in adults. Angiogenesis is the term used to define the sprouting of new blood vessels from
existing blood vessels in adults (Klagsbrun & Moses, 1999; Risau, 1997). Many growth factors regulate the process of angiogenesis. The majority of research on adult angiogenic plasticity in the brain focuses on cardiovascular insult and pathological growth (Zagzag & Capo, 2002). However, exercise (Black, Isaacs, Anderson, Alcantara, & Greenough, 1990; Isaacs, Anderson, Alcantara, Black, & Greenough, 1992), stressors (Heine et al., 2005), and environmental enrichment (Sirevaag & Greenough, 1987) also affect adult brain angiogenesis.

In stark contrast to the outdated belief that the adult brain contains a fixed number of cells, many recent studies demonstrate that neural stem cells derived from the hippocampus and subventricular zone can differentiate into neurons and glia in adult vertebrate brains (Cayre, Malaterre, Scotto-Lomassese, Strambi, & Strambi, 2002; Kempermann, 2005). Newly generated neurons migrate from the subgranular to the granular zone of the hippocampus and from the subventricular zone to the olfactory bulbs. These new neurons establish functional synaptic contacts with other cells (Van Praag, 2002; Carlen et al., 2002; Belluzzi, et al., 2003). Adult neurogenesis is affected by synaptic activity (LTP) (van Praag, Christie, Sejnowski, & Gage, 1999a), hormones (McEwen, 1994; Ormerod, Lee, & Galea, 2004), environmental enrichment (Brown et al., 2003), exercise (van Praag, Kempermann, & Gage, 1999b), learning and memory (Pham, McEwen, Ledoux, & Nader, 2005; Shors et al., 2001b; Shors, Townsend, Zhao, Kozorovitskiy, & Gould, 2002), and stressors (McEwen, 1999)(e.g. dietary restriction, social environment or odor deprivation). Changes in neurogenesis are often coincident with changes in brain angiogenesis (Louissaint, Rao, Leventhal, & Goldman, 2002; Palmer, Willhoite, & Gage, 2000), affected by similar stimuli, and regulated by growth
Seasonal Changes in the Adult Brain

Seasonal changes in adult brain anatomy were first identified in male songbirds (Nottebohm, 1981). Specific regions of the canary (*Serinus canaria*) brain that controls
song production are larger in volume in the spring, when males are producing song to defend their territories and attract mates, compared to the fall, when reproductive behaviors have subsided. Since the 1980’s, significant progress has been made to elucidate the mechanisms underlying this phenomenon in songbirds (Ball et al., 2004). Other models of seasonal brain plasticity in birds include the seasonal change in hippocampal volume of food-caching (Smulders, Sasson, & DeVoogd, 1995) and brood parasitic (Sherry, Forbes, Khurgel, & Ivy, 1993) bird species. The hippocampal region of the brain is involved in spatial learning and memory. In general, species with a larger relative hippocampal volume display enhanced and more frequent spatial learning and memory behaviors (Sherry, Jacobs, & Gaulin, 1992). Hippocampal size is reduced during the winter in these bird species when spatial learning and memory performance associated with food storing (Barnea & Nottebohm, 1994; Barnea & Nottebohm, 1996) and nest depositing of brood (Clayton, 1997; Sherry et al., 1993) is reduced. In mammals, however, little investigation of seasonal plasticity of the brain has been reported. Winter reduction of brain mass in grey squirrels (Sciurus carolinensis) (Lavenex, Steele, & Jacobs, 2000b) and ferrets (Mustela putorius) (Weiler, 1992) has been reported, as well as, a winter reduction in brain mass and masses of specific brain regions (e.g., hippocampus) in common shrews (Sorex araneus) and bank voles (Clethrionomys glareolus) by the Carnegie Museum of Natural History (Yaskin, 1984). However, hippocampal volume did not vary with season in grey squirrels (Lavenex et al., 2000b). Seasonal changes in hippocampal morphology have been reported in hibernating ground squirrels (Citellus undulatus) and meadow voles (Microtus pennsylvanicus) (Galea & McEwen, 1999; Popov et al., 1992). In European hamsters (Cricetus cricetus)
and sheep (Xiong, Karsch, & Lehman, 1997), seasonal changes in the innervation of the brain have been observed (Buijs et al., 1986). Finally, seasonal changes in hypothalamic expression of vasopression have also been reported in humans (Hofman & Swaab, 1995).

Several studies have isolated the important role of photoperiod in seasonal brain changes (Hofman & Swaab, 2002). Many of the results from the bird models of seasonal brain plasticity have been replicated using long or short photoperiods (Dawson, King, Bentley, & Ball, 2001; Krebs, Clayton, Hampton, & Shettleworth, 1995). In mammals, reports of photoperiod-induced brain changes are rare. Exposure to short days throughout weaning decreased brain mass in male meadow voles (Dark, Dark, & Zucker, 1987). No differences in cell proliferation were observed in Siberian hamster brains (Huang, DeVries, & Bittman, 1998) or grey squirrels (Lavenex et al., 2000b), although, experimental procedures from these studies may mask potential differences. However, the motoneurons controlling the penile muscles, neuromuscular junctions, and somas are smaller in adult male Siberian hamsters (*Phodopus sungorus*) or white-footed mice exposed to short days compared with animals exposed to long days (Forger & Breedlove, 1987; Hegstrom & Breedlove, 1999). Adult male deer mice (*Peromyscus maniculatus*) maintained in short days had smaller brains and decreased corrected hippocampal volume relative to those maintained in long days (Perrot-Sinal, Kavaliers, & Ossenkopp, 1998). Finally, hypothalamic expression of neural cell adhesion molecule and polysialic acid was also affected by photoperiod in Siberian hamsters (Lee, Watanabe, & Glass, 1995).
Photoperiod and Angiogenesis

To date, descriptions of seasonal alterations in brain vasculature is limited to birds. Increased circulating testosterone concentrations that are characteristic of the breeding season in adult male canaries induced mitotic angiogenesis, vascular endothelial growth factor (VEGF; a pro-angiogenesis protein) production, and microvascular expansion in the enlarged regions of the brain that regulate song production (Louissaint et al., 2002). Hypoxia caused by the increased oxygen demand of growing tissue stimulates blood vessel growth in order to maintain an adequate supply of oxygen and growth factors to the growing cells (Klagsbrun & Moses, 1999; Risau, 1997). Based on evidence that seasonal adjustments in brain anatomy occur and that bird brain vasculature is altered, it is reasonable to predict that the vasculature supporting brain tissue also changes in adult mammals.

There are several examples of seasonal angiogenesis occurring in regions of the body outside of the mammalian brain. For example, blood volume within antlers of red deer (*Cervus elaphus*) increases in the winter when the antlers begin regrowth (Muir et al., 1988). Additionally, testicular blood flow is reduced during the non-breeding season compared to the breeding season in dormice (*Glis glis*), ferrets (*Mustella furo*), fox (*Vulpes vulpes*), and mink (*Mustela vison*) (Joffre & Joffre, 1973; Pelletier, 1986). Molecular analyses demonstrate a seasonal pattern of growth factors associated with angiogenesis in testes of roe deer (*Capreolus capreolus*) (Wagener, et al., 2000).

Photoperiod manipulation is sufficient to induce similar changes in the vasculature of seasonal mammals. Long-day exposure following reproductive quiescence triggers increased blood vessel volume and density in testes of Syrian hamsters.
(Mesocricetus auratus) (Mayerhofer, et al., 1989). Short days reduce expression of VEGF in testes of adult white-footed mice (Young & Nelson, 2000). Given the relationship between tissue renovation and the vasculature, it is possible that photoperiod may also affect angiogenesis in seasonally-changing brains.

Seasonal Changes in Behavior

The functional consequences of seasonal changes in the brain are seasonal changes in behavior. There are numerous changes in behavior associated with changing seasons (Prendergast et al., 2002). Similar to the physiological and morphological changes induced by photoperiod, these behavioral changes are thought to be adaptive for specific seasons by promoting reproduction and survival. In vertebrates, seasonal changes in territorial aggression and mating behavior promote reproduction, whereas hibernation, food hoarding, and huddling promote winter survival. Numerous other season-dependent behavioral modifications occur in vertebrates (Jacobs, 1996a).

Many seasonal changes in behavior can be induced by photoperiodic manipulation and have been rigorously studied in rodents. Photoperiod affects a broad range of behaviors including: reproduction (Goldman, 1999), aggression (Jasnow, Huhman, Bartness, & Demas, 2000), analgesia (Kavaliers & Galea, 1995), sickness (Bilbo, Drazen, Quan, He, & Nelson, 2002b), affect (Prendergast & Nelson, 2005), feeding (Dark & Zucker, 1984), and learning and memory (Galea, Kavaliers, Ossenkopp, Innes, & Hargreaves, 1994a).
Photoperiod and Learning and Memory

The significant effects of photoperiod on learning and memory are supported by a theory based on reproductive strategy (Gaulin & FitzGerald, 1986). Spatial learning and memory performance is often positively correlated with hippocampal size (Jacobs, Gaulin, Sherry, & Hoffman, 1990; Sherry et al., 1992). In many polygynous rodents, both hippocampal size and spatial learning and memory performance are sexually dimorphic (Gaulin & FitzGerald, 1989; Jacobs, 1996b). Polygynous male rodents outperform females in spatial tasks and have larger hippocampi (Galea, Kavaliers, & Ossenkopp, 1996; Galea et al., 1994a; Gaulin, FitzGerald, & Wartell, 1990; Jacobs et al., 1990). This difference is believed to represent an adaptation that allows polygynous males to spatially locate multiple mates and navigate within their large home range during of the breeding season (Gaulin & FitzGerald, 1986; Gaulin & FitzGerald, 1989; Jacobs et al., 1990). It is important to note that these sexually dimorphic differences in brain and behavior are only reported during the breeding season (long days). Under non-breeding conditions, home range size diminishes, reproductive behavior is greatly reduced, and no sexually dimorphic differences in spatial learning and memory performance are observed (Galea et al., 1996; Galea et al., 1994a). This adaptive theory is also supported by evidence that monogamous pine voles (*Microtus pinetorum*), which pair with a single mate, do not display the sexually dimorphic differences in hippocampal size or spatial learning and memory performance that are characteristic of polygynous meadow voles (Gaulin & FitzGerald, 1986).
Hormones and Changes in Brain and Behavior

*Melatonin.* In addition to manipulating photoperiod to induce seasonal-like changes in brain and behavior, studies have described similar seasonal results by manipulating a physiological signal of photoperiod: melatonin. Melatonin clearly affects circadian rhythms of behavior (Golombek, Pevet, & Cardinali, 1996), but also provides seasonal information throughout the body. Melatonin receptors are distributed within the rodent brain (Drew et al., 2001; Dubocovich, Rivera-Bermudez, Gerdin, & Masana, 2003). Administration of melatonin durations comparable to long or short days induces photoperiod-appropriate changes in behavior and pinealectomy impairs photoperiodic responses in rodents (Golombek et al., 1996; Prendergast et al., 2002).

For example, long melatonin durations increase aggressive behavior in male Syrian and Siberian hamsters similar to short days (Demas, Polacek, Durazzo, & Jasnow, 2004; Jasnow, Huhman, Bartness, & Demas, 2002). Shortening the duration of melatonin exposure with melatonin antagonist administration counteracts the short-day-induced anxiolytic-like behavior in C3H/H3 mice (*Mus musculus*) (Kopp et al., 1999). Constant exposure to melatonin reduces food intake in meadow voles (*Microtus pennsylvanicus*) similar to short days (Dark, Zucker, & Wade, 1983). Melatonin injections induce, and pinealectomy inhibits, daily torpor in white-footed mice (Lynch, Sullivan, & Gendler, 1980) and treatment with a melatonin antagonist reduces short-day-induced hibernation time in Syrian hamsters (Pitrosky, Delagrange, Rettori, & Pevet, 2003). Although it has not been tested for all behavioral responses, the strong relationship between melatonin and day length suggests that melatonin likely mediates many seasonal and photoperiodic modifications in behavior, including learning and
memory (see El-Sherif, Tesoriero, Hogan, & Wieraszko, 2003). The only evidence that melatonin alters brain structure similar to photoperiod treatment is in the songbird model. Long durations of melatonin secretion reversed the long-day-induced volume increases in song production regions of songbird brains (Bentley, Van't Hof, & Ball, 1999). Further investigation of the role of melatonin on seasonal changes in brain and behavior, especially in mammals, are warranted.

**Testosterone.** Circulating testosterone concentrations decrease rapidly in response to the dampening effects of short days on gonadotropin releasing hormone (GnRH) secretion in male rodents (Glass, 1986). Therefore, short-day male rodents display lower circulating testosterone concentrations compared with long-day male rodents. Because testosterone modulates numerous behaviors (in addition to reproduction) and can affect brain structure and function, it is another potential mediator of seasonal adjustments in the brain. Androgen receptors are located in regions of the mammalian brain that regulate seasonal behaviors (Ball et al., 2004; Bittman & Krey, 1988).

Examples of testosterone affecting seasonal behaviors include the combined effect of testosterone and photoperiod on locomotor activity in male Syrian hamsters (Ellis & Turek, 1983) and the reinstatement by exogenous testosterone of female odor preference in castrated male meadow voles maintained in long or short days (Ferkin & Gorman, 1992). Testosterone replacement prevents increased food intake in castrated male deer mice (Blank, Korytko, Freeman, & Ruf, 1994) and restores aggressive behaviors in Syrian hamsters maintained in short days (Jasnow et al., 2000). No correlation was reported between testosterone concentrations and spatial learning and
memory in male meadow voles (Galea, Kavaliers, Ossenkopp, & Hampson, 1995), but short days reduce testosterone, hippocampal volume, and spatial learning in deer mice (Perrot-Sinal et al., 1998). However, direct manipulation of testosterone and the potential interaction between photoperiod and testosterone has not been investigated in learning and memory paradigms. Testosterone increases the volume of song production brain regions in songbirds (Ball et al., 2004) and alters neurochemistry (Bittman, Tubbiola, Foltz, & Hegarty, 1999) and neuroanatomy (Gomez & Newman, 1991) in photoperiodic rodents. For example, testosterone increases the number of cells expressing the proopiomelanocortin gene in the hypothalamus (Bittman et al., 1999) and restores neuronal branching and morphology within the amygdala (Gomez & Newman, 1991) of castrated Syrian hamsters.

Corticosteroids. The primary glucocorticoid is corticosterone in most rodents and cortisol in humans and hamsters. Corticosteroids are involved in metabolism and are often associated with stress responses (Nelson, 2005). A physiological marker of a stress response is increased circulating glucocorticoid concentrations (Selye, 1956). Seasonal changes in basal corticosteroid concentrations differ among species. Short-day white-footed mice display lower basal concentrations of corticosterone than long-day mice (Pyter, Neigh, & Nelson, 2005a), whereas short-day meadow voles (Pyter et al.2005b), sheep (Ovis aries) (Tucker, Petitclerc, & Zinn, 1984) and Siberian hamsters (Bilbo & Nelson, 2003) display higher basal corticosteroid concentrations. However, no photoperiodic differences in basal corticosteroid concentrations have been reported in deer mice and collared lemmings (Dicrostonyx groenlandicus) (Demas & Nelson, 1998; Gower, Nagy, & Stetson, 1996).
In addition to basal differences in corticosteroid concentrations, photoperiodic differences in corticosteroid response to stressors may mediate adult plasticity of behavior. For example, stress-induced corticosteroid responses modulate Porsolt forced swim performance in rats (Kelliher et al., 2000) and locomotor activity in white-crowned sparrows (*Zonotrichia leucophrys gambelii*) (Breuner et al., 2003; Breuner & Wingfield, 2000). Photoperiod and stress-induced corticosteroid concentrations modulate physiological binding globulin levels in dark-eyed juncos (*Junco hyemalis*) (Deviche, Breuner, & Orchinik, 2001) and immune responses in Siberian hamsters (Bilbo et al., 2002a).

The possible link between photoperiodic effects on basal or stress-induced corticosterone and brain plasticity has not been studied in mammals. However, one study in white-crowned sparrows reported seasonal changes in neuronal corticosteroid receptors (Breuner & Orchinik, 2001). Outside of photoperiodic research, corticosteroids have been implicated in several types of brain plasticity including: neurogenesis, dendritic arborization, and LTP (reviewed in McEwen, 1994; McEwen, 1999). Glucocorticoid-induced plasticity is possible in the hippocampus due to the large concentration of corticosteroid receptors in this brain region (Joels, 2001). Often these changes within the hippocampus coincide with modification of learning and memory performance (Roozendaal, 2000).
CHAPTER 1

PHOTOPERIODIC MODULATION OF ANGIOGENESIS IN TESTES

Angiogenesis is the process by which new blood vessels are formed from existing vessels. In adult mammals, angiogenesis is principally confined to the processes of wound healing, tumorigenesis, and physiological changes associated with female reproductive cycles (Smith, 2001; Tomanek & Schatteman, 2000). Some evidence suggests, however, that adult male reproductive tissues are also capable of angiogenesis. Rodents residing in environments that undergo dramatic seasonal changes display marked morphological changes in order to adapt to the fluctuating environment (Bronson, 1985). These rodents often use day length (photoperiod) for temporal information to initiate and terminate seasonally-appropriate morphological, physiological, and behavioral adaptations that maximize survival and reproductive success (Prendergast et al., 2001). Short day lengths trigger regression of the reproductive tract and cessation of reproductive behaviors in many rodents, an adaptation thought to shift energy reserves to survival functions (Nelson, Demas, Klein, & Kriegerfeld, 2002). Regrowth of the reproductive tract commences after prolonged exposure to short day lengths so that full reproductive development
precedes the onset of the mating season (Gram, Heath, Wichman, & Lynch, 1982). In the laboratory, seasonal changes can be triggered in many seasonally-responsive rodents solely by manipulation of photoperiod (Prendergast & Nelson, 2001; Smale, Dark, & Zucker, 1988); photoperiod information is transduced into a physiological signal via the pineal hormone, melatonin (Goldman, 2001).

The mass of the reproductive tract of male white-footed mice (*Peromyscus leucopus*) decreases following 6-12 weeks of exposure to short photoperiods (Lynch, Heath, & Johnston, 1981). Testicular cells (primarily spermatids and spermatocytes) undergo apoptosis in short days, which may contribute to decreased tissue mass (Young & Nelson, 2001; Young, Zirkin, & Nelson, 2000). Seasonal changes in testicular capillary blood flow (Joffre & Joffre, 1973), permeability of blood-testis barrier (Pelletier, 1986), and volume and density of testicular microvasculature (Mayerhofer, Sinha Hikim, Bartke, & Russell, 1989) have been reported in several species that breed seasonally. The molecular mechanisms that regulate vascular development and demolition in response to environmental cues remain unspecified. However, the collapse and regrowth of the testes positively correlates with vascular endothelial growth factor (VEGF) protein expression (Young & Nelson, 2000).

VEGF is a growth factor that is present during blood vessel growth of tumors, wound healing, and uterine vascularization (Neufeld, Cohen, Gengrinovitch, & Poltorak, 1999).

The photoperiod-driven cycle of VEGF protein levels in the testes of white-footed mice is evidence that changes in tissue mass may be accompanied by changes in the supporting vasculature. Tissue growth may precede growth of the vascular bed.
and tissue demise may precede vascular regression or vice versa. For example, excessive tumor growth can be inhibited by prevention of angiogenesis suggesting that tissue growth precedes angiogenesis (Brem, 1976). However, seasonal changes in testicular blood vessel volume precede measurable changes in testes morphology in Syrian hamsters (*Mesocricetus auratus*), suggesting that altered angiogenesis precedes adjustments in tissue morphology (Mayerhofer et al., 1989). Also, seasonal increase in testicular blood flow preceded the presence of spermatozoa in testes of dormice (*Glis glis*), but not ferrets (*Mustello furo*) and foxes (*Vulpes vulpes*) (Joffre & Joffre, 1973). Regardless of the timing of angiogenesis, the process consists of four main steps: 1) degradation of epithelial layer of existing vessel by matrix metalloproteinases, 2) recruitment of growth factors, 3) proliferation and recruitment of new epithelial cells, 4) stabilization of new vessel by smooth muscle cells. (Klagsbrun & Moses, 1999). To maintain vasculature, many blood-born factors are involved and are expressed in a balance between promoting and inhibiting angiogenesis (Klagsbrun & Moses, 1999). When the signal to expand the vasculature is initiated, this balance swings in favor of the pro-angiogenic factors. I hypothesized that expression of angiogenesis genes would differ in the testes between long and short photoperiod-treated white-footed mice and that these differences would correspond to the pattern of testicular regression and regrowth.

**Materials and Methods**

**Animals.** One hundred sixteen male adult (>55 days of age) white-footed mice (*Peromyscus leucopus*) from our breeding colony were used in this study.
Animals were housed individually in polypropylene cages (27.8 x 7.5 x 13 cm) with a constant temperature and humidity of 21 ± 5° C and 50 ± 5%, respectively, and *ad libitum* access to food (Harlan Teklad 8640 rodent diet, Indianapolis, IN) and filtered tap water. Mice were either housed in reverse long (16 h/light/day; lights illuminated at 2300 Eastern Standard Time [EST]) photoperiods (n = 36), or in short (8 h/light/day; lights illuminated at 700 h EST) photoperiods (n = 80 males). Mice exposed to short days that failed to regress their reproductive tract (approximately 35%) were included in the study as a separate group described as “short-day non-responsive” mice below. Mice were maintained in their respective photoperiod for 7, 14, 21, or 34 weeks. All studies were conducted after approval by the Ohio State University Institutional Animal Care and Use Committee and were in compliance with all US federal animal welfare requirements.

**Experimental Procedures.** Body mass and reproductive responses to photoperiod were assessed bi-weekly under light isoflurane anaesthetization. Reproductive status was assessed by external measurement of the left testis with calipers. The product of testis length times testis width squared provides a measure of estimated testis volume (ETV) that is highly correlated with testis weight (Gorman & Zucker, 1995). After 7, 14, 21 and 34 weeks of photoperiod treatment, 4-34 male mice (from each photoperiod) were killed via rapid cervical dislocation and tissues were collected.

**Tissue collection.** Right testis, epididymides, epididymal fat pads, and seminal vesicles were collected from mice under aseptic conditions and submerged in 10 volumes (10 µl per mg tissue) of RNALater RNA Stabilization reagent (Qiagen,
Valencia, CA) and stored at 4° C for 24 h. All surgical instruments were washed consecutively with 70% alcohol, RNAse Away (Fisher) and sterile deionized water between mice. Seminal vesicle fluid was expressed prior to storage. Following 24 h of RNAlater submergence, tissues were blotted briefly, weighed, and frozen at -70° C until RNA processing. Prior to weighing, connective tissue and fat were cleaned from epididymides. The left testis was collected from each mouse and fixed in 10% neutral buffered formalin for at least 2 weeks until processed for histology. The mean testes mass for long-day (LD) mice was determined and all short-day males with testes mass equal or greater than 1.5 standard deviations below this mean were considered reproductively responsive to short days (SDR), whereas those mice that did not differ from the LD mean were considered non-responsive (SDNR).

**Histology.** Testes were fixed in 10% neutral buffered saline, paraffin embedded, and cut into 5 μm sections on a rotary microtome. Sections were progressively stained with eosin Y and Harris hematoxylin and cover slips were affixed with Permount. Diameters of 10 seminal vesicles chosen semi-randomly using a fixed line across the section were visualized with light microscopy at 20X or 30X power and measured on 5 randomly selected sections of each testes using SpotBasic software (Diagnostic Instruments, Inc., Sterling Heights, MI). Diameters were measured only if their length and width differed by <25%. Seminiferous tubule measurements of SDNR mice at 21 weeks were excluded due to improper embedding processing.

**RNA extraction.** Total RNA was extracted from ≤ 30 mg of individual testes using a rotor-stator homogenizer with an RNeasy Mini Kit according to the
manufacturer’s protocol (Qiagen). Extracted RNA was suspended in 30-50 µl RNase-free water and RNA concentration was determined by spectrophotometer. For gene array analysis, aliquots of equal mass (e.g. 1 µg) from individual RNA samples at the 14 week time point were pooled to create a single pooled sample representative of each photoperiod treatment group. Individual samples with concentrations < 100 µg/ml were excluded from the pooled sample (n = 1, LD at week 21). For qPCR, individual RNA samples were used. All RNA samples were stored at -80° C until gene expression analysis.

**Microarrays.** As a preliminary screen for differences in angiogenesis gene expression, microarrays were hybridized with testicular RNA after 14 weeks of photoperiod treatment. Non-Radioactive Mouse Angiogenesis GEArray™ Q Series microarrays with the GEArray RT-labeling method (SuperArray, Frederick, MD) were used according to the manufacturer’s protocol with a few modifications. Briefly, 4 µg of each pooled RNA sample was reverse transcribed to cDNA and used for probe synthesis and the reverse transcription step was extended to 4 h. Hybridization of labeled cDNA to the microarray occurred at 59° C for 24 h. On a sheet of plastic wrap, the chemiluminescent substrate was incubated on the array for 4 min while covered by a piece of Parafilm (Fisher) to equalize the substrate distribution. Immediate, side-by-side chemiluminescent imaging of the microarray from each treatment was made possible by a CCD camera imaging system and detection was stopped upon pixel saturation. Quantity One software (Applied Biosystems, Foster City, CA) was used to capture images and measure relative density of gene spots after background subtraction. Individual gene spot density was
quantified following normalization to the density of the \textit{Gapdh} spot. We plotted the distribution of the fold differences in gene expression against the number of genes and designated a cut-off point based on the mean fold difference (mean = 5.7). Therefore, genes with expression levels that differed $\geq 6$ fold among treatment groups were examined throughout testicular regression and recrudescence via qPCR.

\textbf{Sequencing.} In order to design species-specific primers and probes for quantitative PCR, a portion of each gene of interest was sequenced. Semi-degenerate primers were designed based on conserved regions between \textit{Mus musculus} and \textit{Rattus norvegicus} gene sequences using PrimerExpress software (Applied Biosystems; \textbf{Table 1.1}). Forty cycles of PCR were conducted on 1µl cDNA from pooled \textit{P. leucopus} RNA with Taq DNA Polymerase enzyme (Invitrogen) according to the manufacturer’s protocol in a thermocycler with melting temperatures of 59-60°C. Gene product amplification was visualized on 2% TAE-agarose gels containing ethidium bromide using a CCD camera. To verify amplification of the correct gene, PCR products of the expected molecular size were purified (Centricon-100, Millipore, Billerica, MA), and sequenced at the Plant-Genomics Center at Ohio State University. Resulting amplicon sequences were $> 90\%$ homologous to the \textit{Mus} gene of interest and therefore assumed to be the correct \textit{P. leucopus} gene of interest.

\textbf{Quantitative RT-PCR.} We examined and quantified potentially functional gene expression differences of \textit{Hif1a}, \textit{Tgfb3}, \textit{Serpine1}, and \textit{Tnf} using quantitative PCR (qPCR). Expression of these genes was quantified for individual animals of each photoperiod group at all four time points in duplicate or triplicate. Individual RNA samples ($n = 4$/photoperiod group/time point) were reverse transcribed into
cDNA with MMLV Reverse Transcriptase enzyme (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol. Primers and probes for quantitative PCR were designed based on the attained sequencing information using PrimerExpress. Primers and probes were synthesized as follows, with probes labeled with 6-FAM and MGB (non-fluorescent quencher) at the 5’ and 3’ ends, respectively: *Hif1α* forward: 5’-CTGTGATGAAAGAATTACTGAGTTGATG-3’, *Hif1α* reverse 5’-CATAAATTGAGCGGCCCAAA-3’, *Hif1α* probe 5’-TATGAGCCAGAAGAAC-3’; *Tgfβr3* forward 5’-CAGGACCAGCTCGATGGAA-3’, *Tgfβr3* reverse 5’-TACCAGGAAGGTTGGTTATACA-3’, *Tgfβr3* probe: 5’-CATCACCTTCAACATGG-3’; *Serpine1* forward 5’-CTCACCAGCATCTTGAGATGCT-3’, *Serpine1* reverse: 5’-CGAGGCAGTCTGGTCATGTTC-3’, *Serpine1* probe: 5’-CTCATCCGACAATGGAA-3’; *Tnf* forward: 5’-GAGCCAGCGTGCGAATG-3’, *Tnf* reverse: 5’-AGCTGGTGGTCCTTGAGAGACAT-3’, *Tnf* probe: 5’-CCCAACAACCTCC-3’. A TaqMan 18S Ribosomal RNA primer and probe set (labeled with VIC; Applied Biosystems) was used to as the control gene for relative quantification. Ninety-six well PCR plates were composed of duplicate or triplicate wells for all 5 genes of each individual sample. On each plate, batches of samples were randomized for photoperiod treatment and time point and sample identification was encoded to eliminate potential experimental bias. Amplification was performed on an ABI 7000 Sequencing Detection System by using Taqman® Universal PCR Master Mix. The universal two-step RT-PCR cycling conditions used were: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min.
Relative gene expression was calculated by comparison to relative standard curve consisting of serial dilutions of pooled *P. leucopus* cDNA (1, 1:10, 1:100, 1:1000) followed by normalization to *18S rRNA* gene expression.

**Statistical analysis.** ANOVA repeated measure tests were used to compare all repeated measures among time points. Two-tailed Student’s *t*-tests compared treatment groups of interest. Data with unequal variances were log transformed and compared by ANOVA (within week comparisons for PCR data) or raw data were compared using nonparametric Kruskal-Wallis test (among week comparisons for PCR data). All comparisons were considered significant when *p* < 0.05. StatView software was used for all analyses (Cary, NC).

**Results**

**Body Mass.** SDR mice weighed less than LD mice after 10, 12, 14, and 17 weeks of photoperiod treatment (**Figure 1.1**; *p* < 0.05 in all cases). SDR mice also weighed less than SDNR mice after 2, 4, 10, 12, and 14 weeks of photoperiod treatment (**Figure 1.1**; *p* < 0.05 in all cases).

**Estimated Testicular Volume.** SDR mice displayed decreased ETV as compared to LD mice after 6, 8, 10, 12, 14, 17, 19, 21, and 29 weeks of photoperiod treatment (**Figure 1.2**; *p* < 0.05 in all cases). SDR mice displayed decreased ETV as compared to SDNR mice after 0, 6, 8, 10, 12, 14, 17, 19, 21, and 23 weeks of photoperiod treatment (**Figure 1.2**; *p* < 0.05 in all cases).

**Tissue Masses.** Secondary reproductive tissue absolute masses and masses relative to body mass (absolute mass/body mass x 1000) are shown in **Table 1.2**.
Testes and epididymides absolute masses decreased in SDR mice as compared to LD mice after 7, 14, and 21 weeks of photoperiod treatment, whereas absolute seminal vesicles mass decreased after 7, 14, 21, and 34 weeks (p < 0.05 in all cases). Calculation of masses of these tissues after adjusting for body mass yielded similar statistically significant differences except for epididymides mass at 7 weeks and testes mass at 14 weeks (see Table 1.2; p < 0.05). Absolute and relative epididymal fat pad mass also decreased in SDR mice as compared to LD mice, but only after 14 weeks of treatment (p < 0.05). Additionally, relative seminal vesicles and epididymides masses were decreased in SDR mice after 34 weeks as compared to long-day mice (p < 0.05). Both absolute and relative testes and epididymides masses of SDR mice also decreased as compared to SDNR mice after 7, 14, and 21 weeks of photoperiod treatment (p < 0.05 in all cases). Absolute seminal vesicle mass also decreased in SDR as compared to SDNR at all time points (p < 0.05 in all comparisons). Both absolute and relative epididymal fat pad mass decreased in SDR as compared to SDNR, but only after 14 weeks of treatment (p < 0.05).

**Histology.** Average seminiferous tubule diameters decreased in SDR mice after 7 and 14 weeks of photoperiod treatment as compared to LD mice (Figure 1.3a-d; p < 0.05 for both time points). SDR mice had significantly reduced seminiferous tubule diameters as compared to SDNR mice at 14 weeks (Figure 1.3a-d; p < 0.05). Tubule diameter did not differ between LD and SDNR mice at any time point (p > 0.05 in all cases). Additionally, stained nuclei found in the centre of seminiferous tubules of LD and SDNR mice depicted active spermatogenesis in these groups (Figure 1.3a and 1.3c).
Microarrays. Microarray expression differences of at least 2-fold are summarized in Table 1.3. Based on the distribution of the fold-differences, genes with ≥ 6 fold differences among photoperiod treatments were examined throughout testicular regression and recrudescence using qPCR.

Quantitative RT-PCR. All gene expression data were log transformed due to unequal variances. Relative gene expression of Hif1α, Tgfb3, Serpine1, and Tnf was increased in all short-day mice at week 7 as compared to long-day mice (Figure 1.4a-d; p < 0.05). At week 14, only SDR had increased Hif1α, Tgfb3, and Tnf gene expression (Figure 1.4a, b, and d; p < 0.05), whereas expression levels of SDNR were similar to LD mice (p < 0.05). However, the Serpine1 gene expression relationship among photoperiod groups reversed at 14 weeks such that expression in LD mice was numerically higher than SDNR, although this difference was not statistically significant (Figure 1.4c; p = 0.17). At week 21, this increase in Serpine1 expression in LD mice was significantly greater than all short-day mice (Figure 1.4c; p < 0.05). Also at week 21, SDR maintained their heightened expression level of Tnf, although it was only significantly higher than SDNR (Figure 1.4d; p < 0.05). SDR and LD mice had similar Tgfb3 expression levels by week 21, although the SDNR level was significantly decreased (Figure 1.4b; p < 0.05). Finally, LD mice displayed an increase in Tgfb3 expression after 34 weeks of photoperiod treatment (Figure 1.4b; p < 0.05). No differences in Hif1α, Serpine1, or Tnf expression existed among groups at 34 weeks (Figure 1.4a, c, and d; p > 0.05). There were significant
effects of week on $Tgf\beta r3$, $Serpine1$, and $Tnf$ expression in LD, SDR, and SDNR mice ($p < 0.05$ in all cases). Additionally, there was a significant effect of week on $Hif1a$ expression in LD only ($p < 0.05$).

**Discussion**

The present study confirms and extends previous results that short days decrease reproductive tract mass, body mass, and seminiferous tubule diameter in adult male white-footed mice (Glass, 1986; Johnston & Zucker, 1980; Whitaker, 1940). Additionally, expression of genes involved in angiogenesis differs in testes of long- and short-day mice and corresponds to the photoperiod-driven pattern of regression/recrudescence of the reproductive tract. Specifically, short-day mice display high expression levels of $Hif1a$, $Tgf\beta r3$, and $Serpine1$ during testicular regression and high expression levels of $Tnf$ during testicular recrudescence. These data are consistent with the hypothesis that photoperiod alters expression of angiogenesis genes in adult males.

Angiogenesis in adult mammals has been studied in the context of tumor growth, wound healing, and the cyclical changes of the female reproductive tract. Studies of angiogenesis specific to adult males are sparse and limited to models of seasonal breeders including dormice ($G. glis$), ferrets ($M. furo$), and foxes ($V. vulpes$) (Joffre & Joffre, 1973). Dormice and foxes increase capillary blood flow in the testes during the breeding season. Short days decrease blood vessel volume and transfer to long days increase vascular permeability in the testes of Syrian hamsters ($M. auratus$) (Mayerhofer et al., 1989). Similarly, testicular blood vessel volume of adult male red
stags (*Cervis elaphus*) and camels (*Camelus dromedaries*) was greater when measured in the breeding season than during the non-breeding season (Hochereau-de Reviers & Lincoln, 1978; Zayed, Hifny, Abou-Elmagd, & Wrobel, 1995). In mink (*Mustela vison*), the blood-testis barrier competency changes across season, although the pattern of competency does not completely match the seasonal progression of spermatogenesis (Pelletier, 1986). These studies provide evidence for structural changes in the testicular vasculature of seasonally breeding adult males.

The mechanisms by which testicular vasculature varies across seasons have not been established. However, *Vegf* may be implicated in this process based on the positive relationship between VEGF protein expression and testes mass in short- and long-day treated white-footed mice (Young & Nelson, 2000). No differences (≥ 2-fold) in *Vegf* gene expression were found in this study. However, the time points at which gene expression were assessed are “snapshots” of a 34-week process of regression and recrudescence. It is possible that differential gene expression necessary for morphological changes in the testes was relatively transient, and therefore missed by our sampling schedule. Additionally, gene transcription and translation of gene products may not overlap in time, and therefore, VEGF protein expression may be high at particular time points whereas, *Vegf* transcription may have already returned to basal levels. Studies using models in which more abrupt changes in testes mass are necessary to examine changes in angiogenesis gene expression over a more concentrated window of time. Our study implicates several other genes in the process of seasonal angiogenesis of the testes.
Directed by our preliminary microarray findings after 14 weeks of photoperiod treatment (the nadir of testicular regression), I investigated expression patterns of four genes involved in angiogenesis. *Hif1α* is an oxygen-regulated subunit of the HIF transcription factor that has been implicated in both angiogenesis and apoptosis (Piret, Mottet, Raes, & Michiels, 2002). The overlap of the timing of testicular apoptosis in short-day white-footed mice and the expression pattern of *Hif1α* suggests that expression of *Hif1α* in short-day mice be an indicator of apoptosis associated with testicular regression (Young, Zirkin, & Nelson, 2001). *Tgfβr3*, also known as betaglycan, in its soluble form, is a potent antagonist of TGFβ, a promoter of angiogenesis. Additionally, expression of the soluble extracellular domain of TGFβr3 inhibits angiogenesis *in vivo* and *in vitro* (Bandyopadhyay et al., 2002). Therefore, it is likely that the initial increase in *Tgfβr3* expression in short-day mice represents an inhibition of testicular angiogenesis. The effect of *Serpine1* on tumor growth and angiogenesis depends on the level of expression. High SERPINE1 doses inhibit tumor growth in angiogenesis *in vivo*, whereas, low doses increase angiogenesis (McMahon et al., 2001). Consistent with this bi-modal function of SERPINE1, short-day mice display high expression levels of *Serpine1* at week 7 during regression, which suggests an inhibitory role of *Serpine1* on angiogenesis. Lastly, *Tnf* promotes proliferation and differentiation of mesenchymal cells into pericytes or smooth muscle cells that support newly formed vessels during angiogenesis (Distler et al., 2003). The late increase in *Tnf* in short-day testes suggests that *Tnf* may promote new vessel stabilization during testicular
recrudescence. Overall, the timing of the expression of these four genes in short-day responsive mice suggests that $Hif1\alpha$, $Tgf\beta3$, and $Serpine1$ are involved in testicular regression, whereas, $Tnf$ is involved in testicular recrudescence.

The effect of time (weeks) on gene expression in long-day testes was unexpected. I predicted that the vasculature in LD animals would be stable. However, many genes (including the ones examined in the present study) have multiple functions and it is possible that the changes measured in LD testes may be associated with extra-angiogenic processes. Alternatively, the trend of increasing gene expression peaking around week 21 in LD mice may be due to a fluctuation in the overall body growth (including the testes). Between 14 and 21 weeks, the long-day animals display a slight drop in body mass followed by a recovery that may account for slight changes in testicular gene expression at this time.

In this study, microarrays designed for *Mus musculus* were used for *P. leucopus* which may have resulted in false positive and negative findings based on possible sequence differences between the species. Therefore, the microarray analysis was solely used as a screening procedure whereas the specificity and sensitivity of qPCR enabled precise examination of specific genes of interest. Common laboratory animals that have been selectively bred to ignore seasonal cues such as *Mus musculus* and *Rattus norvegicus* and for which molecular tools are readily available, are not suitable for this type of comparative study. Because of the genomic homology among species, however, it is possible to work around some of the molecular roadblocks and be able to address ecologically relevant questions in genetically heterogeneous populations.
The SDNR group represents the population of mice that fails to regress their gonads in response to short day lengths. This population is not merely a laboratory anomaly because winter breeding has been reported in field studies and is thought to confer fitness advantages during mild winters (Prendergast et al., 2001). I compared the SDNR animals to the LD and SDR phenotypes. Body mass, testes size, and testes morphology of the SDNR group were more similar to long- as compared to short-day mice. With respect to gene expression of our four candidate genes, the SDNR profile initially (Figure 1.4a-d; week 7) was similar to that of the SDR group. However, by week 14, SDNR expression of all four genes switched to resemble that of the long-day mice. These data suggest that there is some breakdown between the transcription of the gene and the function of the gene product. Potential differences between SDNR and SDR post-transcriptional pathways may exist. However, in SDNR mice the uncoupling between large, sperm-filled testes (characteristic of LD animals) and their initial SDR-like gene expression profile suggests that testosterone is not the only factor influencing angiogenesis gene expression in the testes. Additionally, long durations of melatonin secretion do not appear sufficient to induce SDR-like angiogenesis expression profiles in the testes because SDNR and SDR white-footed mice display the same long melatonin durations (Carlson, Zimmermann, & Lynch, 1989) but different gene expression patterns.

The present study is among the first to describe seasonal plasticity of angiogenesis in adult testes on a molecular level. Changes in angiogenesis gene expression differ between testes of long- and short-day white-footed mice and correspond to changes in testicular morphology. The gene expression profile of
SDNR mice initially resembled that of SDR, but subsequently became similar to LD mice. Future studies are necessary to determine the intervening role of testosterone and melatonin in photoperiod-induced changes in testicular angiogenesis gene expression. It is likely that seasonal melatonin secretion exerts its effects on testicular angiogenesis via an indirect central mechanism that may implicate testosterone.

Melatonin receptors have not been localized in the testes in this species and melatonin does not affect testosterone secretion from testicular cells in vitro (Knotts, Bruot, & Glass, 1988). However, binding of melatonin to receptors in the hypothalamus does modulate reproductive hormone secretion (Glass & Dolan, 1988). Therefore, melatonin-induced changes in testosterone secretion may regulate seasonal angiogenesis (or possibly GnRH, LH, FSH, or some combination of these hormones). Because non-pathological angiogenesis in adults is rare (particularly in males) understanding the mechanisms underlying this model of seasonal angiogenesis may prove to have therapeutic value.
Figure 1.1. Effect of photoperiod treatment on body mass (mean ± SEM).

Weeks 0 - 7: LD (n = 38), SDR (n = 53), SDNR (n = 28); Weeks 8 - 14: LD (n = 29), SDR (n = 43), SDNR (n = 19); Weeks 15 - 21: LD (n = 11), SDR (n = 9), SDNR (n = 8); Weeks 22 - 34: LD (n = 4), SDR (n = 4), SDNR (n = 4). * p < 0.05 between LD and SDR; + p < 0.05 between SDR and SDNR.
Figure 1.2. Effect of photoperiod treatment on reproductive status (mean ± SEM).

Weeks 0 - 7: LD (n = 38), SDR (n = 53), SDNR (n = 28); Weeks 8 - 14: LD (n = 29), SDR (n = 43), SDNR (n = 19); Weeks 15 - 21: LD (n = 11), SDR (n = 9), SDNR (n = 8); Weeks 22 - 34: LD (n = 4), SDR (n = 4), SDNR (n = 4). * p<0.05 between LD and SDR; + p < 0.05 between SDR and SDNR. Time points of tissue collections (▲).
**Figure 1.3** Influence of 14 weeks of photoperiod treatment on seminiferous tubule diameter in testes (mean ± SEM).

* p<0.05 between long-day and short-day responders; + p < 0.05 between short-day responders and non-responders. Representative morphology of seminiferous tubules in LD (B), SDR (C), and SDNR (D) mice. LD: n = 6; SDR: n = 8; SDNR: n = 6. Arrowheads point at stained spermatocyte nuclei.
Figure 1.4. Effect of 7, 14, 21, and 34 weeks of photoperiod treatment on Hif1α (A), Tgfβ3 (B), Serpine1 (C), and Tnf (D) gene expression relative to 18S rRNA expression in the testes.

* p < 0.05 among all photoperiod groups of a single time point unless specified by a bar. n = 4/photoperiod group/time point.
<table>
<thead>
<tr>
<th>GENE</th>
<th>PRIMER SEQUENCES FOR SEQUENCING</th>
<th>Tm</th>
<th>ACCESSION NUMBER CREATED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoxia-inducible factor 1α (Hif1α)</td>
<td>forward: AGAAACCRCYATGACGTGC&lt;br&gt;reverse: CCACCTCTTTTTKGAAGCAT</td>
<td>60</td>
<td>AY591916</td>
</tr>
<tr>
<td>Plasminogen activator inhibitor 1 (Serpine1)</td>
<td>forward: ACRTCSTGGAAGCTGCCTAC&lt;br&gt;reverse: GAGCTGCTCCTTGTGCGAAA</td>
<td>60</td>
<td>AY591915</td>
</tr>
<tr>
<td>Transforming growth factor β receptor 3 (Tgfβr3)</td>
<td>forward: GCACCAGGAAGGTCTGGTT&lt;br&gt;reverse: ATGGGTTTGAAGCATGGAGTCA</td>
<td>59</td>
<td>AY591914</td>
</tr>
<tr>
<td>Transforming growth factor (Tnf)</td>
<td>forward: GGAGGCAGCTCCAAAAG&lt;br&gt;reverse: AGCCTTGGCCCTGGAAGAG</td>
<td>59</td>
<td>AY608911</td>
</tr>
<tr>
<td>18S rRNA</td>
<td>forward: CACGGCCGGTGACAGAAAC&lt;br&gt;reverse: AGTTGATAGGGCAGCGTTGCA</td>
<td>60</td>
<td>AY591913</td>
</tr>
</tbody>
</table>

**Table 1.1.** Sequencing information for genes differentially expressed >6-fold in microarray analysis.
<table>
<thead>
<tr>
<th>TISSUE MASS</th>
<th>7 WEEKS</th>
<th>14 WEEKS</th>
<th>21 WEEKS</th>
<th>34 WEEKS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LD (9)</td>
<td>SDR (10)</td>
<td>SDNR (9)</td>
<td>LD (18)</td>
</tr>
<tr>
<td>TESTES Abs.</td>
<td>221.0±16.1</td>
<td>118.6±20.9</td>
<td>*204.7±24.3</td>
<td>221.0±12.4</td>
</tr>
<tr>
<td>Rel.</td>
<td>10.0±0.7</td>
<td>5.8±1.1</td>
<td>*8.3±0.9</td>
<td>9.1±0.4</td>
</tr>
<tr>
<td>SEMINAL VESICLES Abs.</td>
<td>117.9±13.8</td>
<td>52.9±13.1</td>
<td>*117.8±16.7</td>
<td>142.7±16.0</td>
</tr>
<tr>
<td>Rel.</td>
<td>5.2±0.5</td>
<td>2.7±0.7</td>
<td>*4.7±0.6</td>
<td>6.1±0.6</td>
</tr>
<tr>
<td>EPIDIDY. Abs.</td>
<td>48.5±4.1</td>
<td>34.2±2.6</td>
<td>*46.0±2.6</td>
<td>52.5±3.5</td>
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<tr>
<td>Rel.</td>
<td>2.2±0.2</td>
<td>1.7±0.2</td>
<td>*1.9±0.1</td>
<td>2.2±0.1</td>
</tr>
<tr>
<td>E. FAT PAD Abs.</td>
<td>184.0±35.3</td>
<td>201.4±56.7</td>
<td>324.2±74.7</td>
<td>207.1±54.2</td>
</tr>
<tr>
<td>Rel.</td>
<td>8.0±1.2</td>
<td>9.5±2.1</td>
<td>11.8±2.3</td>
<td>7.7±1.6</td>
</tr>
</tbody>
</table>

Table 1.2. Mean values (±SEM) of absolute and relative male secondary reproductive tissue masses (mg) after 7, 14, 21, and 34 weeks of photoperiod treatment.

* = p < 0.05 between SDR and both LD and SDNR
# = p<0.05 between SDR and LD
+ = p<0.05 among all groups
Sample sizes are indicated in parentheses. EPIDIDY = epididymides; E. FAT PAD = epididymal fat pad; Abs. = absolute; Rel. = relative
Angiopoietin 2

Hypoxia-inducible factor 1α (Hif1α)

eNOS

Plasminogen activator inhibitor 1 (Serpine1)

Secreted acidic cysteine rich glycoprotein

TGFβ receptor 3 (Tgfβr3)

Tenascin C

Tumor necrosis factor (Tnf)

<table>
<thead>
<tr>
<th>GENE</th>
<th>14 WEEKS</th>
<th>LD</th>
<th>SDR</th>
<th>SDNR</th>
<th>FOLD (X)</th>
<th>RT-PCR</th>
<th>FUNCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiopoietin 2</td>
<td></td>
<td>↑</td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td>vessel remodeling and sprouting</td>
</tr>
<tr>
<td>Hypoxia-inducible factor 1α (Hif1α)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>19.4</td>
<td>*</td>
<td>pro-angiogenic or pro-apoptotic</td>
</tr>
<tr>
<td>eNOS</td>
<td></td>
<td>↑</td>
<td></td>
<td></td>
<td>5.1</td>
<td></td>
<td>vessel permeability</td>
</tr>
<tr>
<td>Plasminogen activator inhibitor 1 (Serpine1)</td>
<td></td>
<td>↑</td>
<td></td>
<td></td>
<td>6.4</td>
<td>*</td>
<td>matrix remodeling; pro- or anti- angiogenic</td>
</tr>
<tr>
<td>Secreted acidic cysteine rich glycoprotein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.2</td>
<td></td>
<td>cell detachment, matrix remodeling</td>
</tr>
<tr>
<td>TGFβ receptor 3 (Tgfβr3)</td>
<td></td>
<td>↑</td>
<td></td>
<td></td>
<td>69.4</td>
<td>*</td>
<td>inhibit formation of new vessels by TGFβ</td>
</tr>
<tr>
<td>Tenascin C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.5</td>
<td></td>
<td>cell adhesion and detachment</td>
</tr>
<tr>
<td>Tumor necrosis factor (Tnf)</td>
<td></td>
<td>↑</td>
<td></td>
<td></td>
<td>20.9</td>
<td>*</td>
<td>differentiate cells to support new vessels</td>
</tr>
</tbody>
</table>

**Table 1.3.** Two-fold or greater angiogenesis gene expression differences in the testes after 14 weeks of photoperiod treatment.

FOLD (X) = Expression difference between LD and SDR at 14 weeks

* = examination of gene expression by RT-PCR
CHAPTER 2

PHOTOPERIODIC MODULATION OF ANGIOGENESIS IN BRAIN

Previous studies have established that adult angiogenesis is associated with pathology and changes within the reproductive tract. However, seasonal changes in the vasculature of red deer (Cervus elaphus) antlers (Muir, Sykes, & Barrell, 1988) suggest that the effects of season on angiogenesis are not confined to the reproductive system. Evidence that suggests that the adult brain is also capable of seasonal cycles of angiogenesis in response to changes in the environment has recently emerged.

Similar to short day-provoked deterioration of the testes, short days also decrease brain mass and hippocampal volume in rodents (Dark et al., 1987; Perrot-Sinal et al., 1998; Yaskin, 1984; Chapter 3). Winter retrogression of brain tissue is thought to confer energetic savings and result in behavioral consequences that may be adaptive (Jacobs, 1996a; Chapter 3). Because the vasculature is responsible for providing oxygen and other trophic factors to tissues, I predicted that the vasculature supporting seasonally-changing brain tissues would fluctuate similarly to the testicular vasculature during seasonal testicular regression and regrowth.
Angiogenesis in the adult brain is traditionally considered pathological (e.g., associated with tumor growth) or “reactive” angiogenesis (i.e., in response to neurotrauma) (Greenberg & Jin, 2005). However, environmental influences also alter adult brain angiogenesis. In rodents, exercise, environmental enrichment, and motor activity increase angiogenesis in the cerebellum (Isaacs et al., 1992) or cortex (Black et al., 1990; Sirevaag & Greenough, 1987), and in songbirds, breeding season-typical concentrations of testosterone induced angiogenesis in the higher vocal center of the brain (Louissaint et al., 2002). However, the effects of photoperiod on adult brain angiogenesis have not been reported in mammals. Angiogenesis also appears to be coordinated with adult mammalian neurogenesis in the subventricular zone of the lateral ventricles and subgranular zone of the dentate gyrus (Greenberg & Jin, 2005; Palmer et al., 2000) and adult neurogenesis may be modified by season or photoperiod (Barnea & Nottebohm, 1994; Galea & McEwen, 1999; Louissaint et al., 2002).

Based on the evidence that photoperiod alters angiogenesis in the testes (and other peripheral tissues) and brain mass and the association between angiogenesis and neurogenesis, I hypothesized that photoperiod affects expression of genes involved in angiogenesis in adult rodent brains.

Materials and Methods

Animals. One hundred and one adult (>55 days of age) male white-footed mice (*Peromyscus leucopus*) from our breeding colony were used in these experiments. Animals were housed individually in polypropylene cages (27.8 x 7.5 x
13 cm) with a constant temperature and humidity of 21 ± 5°C and 50 ± 10%, respectively, and *ad libitum* access to food (Harlan Teklad 8640 rodent diet, Indianapolis, IN) and filtered tap water. Mice were either housed in reversed long days (LD; 16 h light/day; lights illuminated at 2300 Eastern Standard Time [EST]), or in short days (SD; 8 h light/day; lights illuminated at 700 h EST). Siblings were pseudo-randomly distributed among all groups.

**Experiment 1: Effects of photoperiod on cerebral blood flow.** Fourteen mice (n = 7/photoperiod) were used for this experiment. Mice were exposed to 14 weeks of photoperiod treatment prior to laser-Doppler measurement (Moor Instruments Limited, Devon, UK) of cerebral blood flow. Laser-Doppler technology measures the number of red blood cells passing the laser over time. After mice were anesthetized with halothane vapors, a shallow indentation was made in the temporal muscle (2 mm posterior, 3 mm lateral to Bregma) for the placement of the brain laser probe. A 2 mm incision was also made in the scrotal skin over the left testis for direct placement of the testicular probe. Thin oil interfaces were applied to the tips of the probes and the probes were stabilized within 1 mm of the middle cerebral artery and the testicular artery. Once the probes were stabilized, the laser-Doppler signal was recorded semi-continuously (< every 5 sec) and averaged over 5 min for comparison between photoperiod groups.

**Experiment 2: Effects of acute and chronic photoperiod treatment on angiogenesis gene expression in the brain.** Eighty-seven mice were used for this experiment. All mice were exposed to long or short photoperiod conditions for 14 weeks (to induce long- and short-day phenotypes determined by maximum testicular
regression in short-day mice; Chapter 1) and then transferred to the opposite photoperiod for 0, 2, 7, 21, or 49 days. Mice that were transferred into the opposite photoperiod for 0 days following 14 weeks in their original photoperiod were considered to have had “chronic” photoperiod exposure and those that were transferred to the opposite photoperiod for 2 - 49 d were considered “acute” photoperiod treatments. At these time points, mice were rapidly decapitated, brains and testes were removed, and the olfactory bulbs, hippocampus, and hypothalamus were dissected out and stored in RNALater solution (Qiagen, Valencia, CA) at -70°C until RNA processing. Body mass was recorded bi-weekly and at the time of tissue collection.

**RNA extraction, gene sequencing, and qPCR.** Total RNA was extracted from ≤ 30 mg of individual testes and regions of the brain and cDNA was transcribed as described in Chapter 1. Vegf was sequenced as previously described (Chapter 1) and primers and a probe were synthesized with the probe labeled with 6-FAM and MGB (non-fluorescent quencher) at the 5’ and 3’ ends, respectively: Vegf forward 5’- GAGCAACATCACCATGCAGATC-3’, Vegf reverse 5’- CATCTCTCCTATGTGCTGGCTTT-3’, Vegf probe 5’-TGCGGATCAAACCT-3’-TGC GGATCAACCT-3’. The primers and probes used for Hif1α and 18S Ribosomal RNA and qPCR conditions were previously described in Chapter 1.

**Statistical analyses.** ANOVA tests were used for within photoperiod comparisons over time and two-tailed student’s t-tests were used to compare between photoperiod at specific times (Keppel & Wickens, 2004). Data with unequal
variances were compared using nonparametric Mann-Whitney tests. All comparisons were considered statistically significant when p < 0.05. StatView software was used for all analyses (v. 5.0.1, Cary, NC).

Results

Experiment 1: Effects of photoperiod on cerebral blood flow. Fourteen weeks of short days reduced cerebral blood flow compared with long days (Figure 2.1) (p < 0.05). No differences in testicular blood flow were observed between photoperiods (p > 0.05). Short days significantly decreased testes mass (mean ± SEM) compared with long days (LD: 301.5 ± 14.7, SD: 190.8 ± 23.3 mg) (p < 0.05).

Experiment 2: Effects of acute and chronic photoperiod treatment on angiogenesis gene expression in the brain. After 14 weeks, short-day mice weighed less than long-day mice (Figure 2.2a) (p < 0.05). Short-day mice transferred to long days for 7 days remained significantly lighter than those transferred to short days (p < 0.05), however, by 21 and 49 days there were no differences between short- and long-day mice (Figure 2.2a) (p > 0.05). This likely reflects the increase in body mass observed in short-day mice following 21 and 49 days after transfer to long days relative to body masses of those only transferred for 0, 2, or 7 days (Figure 2.2a) (p < 0.05 in all cases). Body mass of mice transferred from long to short days did not differ (Figure 2.2a) (p > 0.05 in all cases).

After 14 weeks, relative testes mass of short-day mice was significantly decreased relative to long-day mice (Figure 2.2b) (p < 0.05). Testes mass of short-day mice transferred to long days for 2 or 7 days remained lower than that of long-
day mice (Figure 2.2b) (p < 0.05 in both cases), however by 21 and 49 days after transfer into long days, testes mass did not differ between long- and short day mice (p > 0.05 in both cases). Within short-day mice that were transferred to long days, 7, 21, and 49 days of long days significantly increased relative testes mass relative to 0 days (p < 0.05 in all cases). Within long-day mice that were transferred to short days, testes mass significantly decreased following 49 days of short days (p < 0.05).

Based on the testicular responses to photoperiod, gene expression data of mice transferred from long into short days were analyzed in two groups: 0-21 and 49 d in short days; gene expression data of mice transferred from short into long days were analyzed in two groups: 0-7 and 21-49 d in long days. These divisions estimate when potential photoperiodic changes in the brain may occur based on photoperiodic changes in the gonads (Figure 2.2b).

Overall, hypothalamic Vegf expression was greater than hippocampal, testicular, and olfactory bulb expression (Figure 2.3) (p < 0.05). Relative Vegf expression in the testes decreased after 21-49 d of long days relative to 0-7 d (Figure 2.3a) (p < 0.05). No differences in testicular Vegf were observed in the mice transferred to short days or between photoperiod treatments within days (p > 0.05 in all cases). In the hypothalamus, Vegf expression did not differ between photoperiods or over time (Figure 2.3b) (p > 0.05). In the hippocampus, Vegf expression decreased in long-day 21-49 d mice relative to long-day 0-7 day mice and increased in short-day 49 d mice relative to short-day 0-21 day mice (Figure 2.3c) (p < 0.05 in all cases). Additionally, short-day 49 d mice displayed more hippocampal Vegf expression than all other long-day groups (Figure 2.3c) (p < 0.05). Olfactory bulb
Vegf expression decreased in the long-day 21-49 d mice compared with long-day 0-7 d mice (Figure 2.3c) (p < 0.05). Short-day 49 d olfactory bulb Vegf expression was greater than long-day 21-49 d expression (Figure 2.3c) (p < 0.05).

Hif1α expression in the testes and hypothalamus was higher than Hif1α expression in the hippocampus and olfactory bulbs (Figure 2.4) (p < 0.05 in all cases). Hif1α expression in the testes decreased after 21-49 d of long days relative to 0-7 d (Figure 2.4a) (p < 0.05). Testicular Hif1α expression was lower in 49 d short-day mice relative to 0-7 d long-day mice (p < 0.05) (Figure 2.4a). In the hypothalamus, Hif1α expression did not differ between photoperiods or over time (Figure 2.4a) (p > 0.05). In the hippocampus, short days decreased Hif1α expression, such that mice exposed to 2 d of short days had greater Hif1α expression than all other times (Figure 2.4b) (p < 0.05). No differences in hippocampal Hif1α expression were observed in the long-day mice (p > 0.05). With the exception of decreased Hif1α expression in mice exposed to 49 d of long-days compared with 49 d of short days (p < 0.05), there were no differences in olfactory bulb Hif1α expression (Figure 2.4b) (p > 0.05).

Discussion

The present study is the first to describe photoperiod-evoked changes in adult brain angiogenesis in mammals. Short days reduced cerebral blood flow, but not testicular blood flow in adult mice. Acute transfer from long to short days slowly decreased testicular mass, increased testicular Vegf expression, and decreased Hif1α expression in the hippocampus. Acute transfer from short to long day lengths
increased body and paired testes mass, decreased testicular, hippocampal, and olfactory bulb Vegf expression and testicular Hif1a expression. Acute photoperiodic differences in Vegf were observed in the hippocampus and olfactory bulbs and in Hif1a expression in the testes and olfactory bulbs. Taken together, these results suggest that photoperiod modifies the vascular network supporting the brain which may reflect photoperiodic changes in brain tissue morphology or neurogenesis.

The short-day decrease in cerebral blood flow described in the present study is novel and important, and has not been previously reported. Decreased blood flow may represent decreased oxygen consumption or metabolic activity in brains of short-day mice compared with long-day mice. Previous studies in birds and rodents suggest that photoperiodic brain plasticity may have functional consequences in adults including changes in behavior (Louissaint et al., 2002; see Chapter 3). Additional changes in brain vasculature plasticity including changes in blood vessel volume or density, as well as determining specific regions of the brain in which vasculature is affected by photoperiod remain to be tested. In contrast to our prediction that blood flow would decrease in short-day testes relative to long-day testes, no differences were observed. However, other landmarks of testicular circulation that were not measured (e.g., vessel diameter, density, or volume) may be reduced during short-day induced gonadal regression and be more representative of vascular deterioration within the testes.

Angiogenesis is regulated by a sensitive balance between numerous pro- and anti-angiogenesis factors. VEGF is one of the most well-characterized pro-angiogenesis factors and VEGF cellular signaling is considered to be a rate-limiting
step in angiogenesis (Ferrara, 2004). Vegf gene expression was higher in the hypothalamus than any other tissue examined in this study. Given the portal vascular connection between the hypothalamus and the pituitary gland, these tissue differences in Vegf expression are not unexpected. On the other hand, I expected that Vegf expression would remain elevated in testes of mice transferred from short into long throughout testicular growth. This prediction was based on a previous study in white-footed mice in which VEGF protein decreased after 2-10 weeks of short days (Young & Nelson, 2000) and the present evidence that testicular growth was increasing by 7-49 d of long-day exposure. In contrast, testicular Vegf expression decreased following 21 or 49 d of long days. These discrepancies may reflect differences in mRNA versus protein expression, as well as the timing of Vegf gene expression preceding changes in testicular tissue mass and then tapering off throughout the final stages of testicular regrowth. These results are similar to those reported in testes of roe deer (Capreolus capreolus) (another seasonal breeder) in which peak Vegf expression proceeded, but did not coincide, with maximum testicular volume (Wagener, Blottner, Goritz, & Fickel, 2000). A similar decrease in Vegf was observed in the hippocampi and olfactory bulbs of mice exposed to prolonged periods (21 – 49 d) of long days. Vegf expression in the hippocampus of long-day mice transferred to short days displayed an opposite pattern. The opposing patterns of expression following acute photoperiod treatments in the hippocampus suggest that photoperiod may tightly regulate angiogenesis (and possibly neurogenesis) in this brain region, as opposed to other tissues in which one photoperiod treatment appears to trigger changes in gene expression while expression from tissue in the other
photoperiod remains static. According to the proposed relationship between neurogenesis, angiogenesis, and learning and memory, I predicted that short days (characterized by impaired hippocampal learning and decreased hippocampal volume; see Chapter 3) would decrease hippocampal pro-angiogenesis factors. However, the relationship between the timing of testicular regression and hippocampal regression has not been established and may not be concurrent. The initially elevated hippocampal Vegf expression in the mice transferred to long days may reflect either regrowth of hippocampal tissue resulting from the previous prolonged short-day treatment or an acute angiogenic reaction to long-day exposure. The former conclusion is supported by the increased hippocampal Vegf expression following prolonged exposure to short days (49 d) in the other group of mice. In support of the latter hypothesis, however, Vegf mRNA expression in song bird brains was increased within 4 d of testosterone exposure (Louissaint et al., 2002).

The expression of Vegf, and ultimately angiogenesis, is evoked by hypoxia in tissue. Hypoxia triggers expression of Hif gene expression following release of HIFα degradation (Maxwell, 2005). Relative to the hippocampus and olfactory bulbs, Hif1α expression was higher in the testes and hypothalami of these mice. Differences in relative Hif1α expression may represent enhanced sensitivity to changes in oxygen level among tissues. It is possible that the testes and hypothalamus are more vulnerable to the consequences of hypoxia or the effects of hypoxia on these tissues are more detrimental than in other regions of the brain. A previous report demonstrated an increase in testicular Hif1α expression following 49 and 98 d of short days (Chapter 1). The present findings that 49 d of short days or 0 d of long
days (preceded by 98 d of short days) increase testicular Hif1α corroborate these previously reported results. Complementary, prolonged exposure (21 or 49 d) to long days reduced testicular Hif1α expression. As previously described, maximum testicular regression, is shortly followed by testicular recrudescence (Chapter 1). It is likely that increased Hif1α expression following prolonged short-day exposure proceeds subsequent vascular development in recrudescing testes. In contrast, maintainence of large, long-day typical testes may not require constant transcription of Hif1α. Prolonged exposure to short days decreased Hif1α expression in the hippocampus. Decreased hippocampal Hif1α and therefore, possibly angiogenesis, support previous findings of reduced hippocampal volume in response to short days in this species (see Chapter 3). The only significant effect of photoperiod on Hif1α expression in the olfactory bulbs was the relative decrease in long-day mice compared with short-day mice following 49 d of photoperiod treatment. The effects of photoperiod on olfactory bulb mass, neurogenesis, and function remain untested, and therefore it is possible that several weeks of short days may actually increase olfactory bulb mass which would support the delayed difference in angiogenesis factor gene expression (see Chapter 8). Similar to photoperiod-evoked changes in testicular mass in this species, changes in olfactory bulb mass may not be evident until several weeks of photoperiod treatment.

In sum, these results suggest that photoperiod alters cerebral blood flow and angiogenesis gene expression in the hippocampus and olfactory bulbs of adult mammals. These changes in vasculature may accompany changes in brain structure
morphology and possibly function (i.e., behavior). Studying naturally occurring, non-pathological adult angiogenesis may provide insight into mechanisms for potential treatment of neurological diseases and neurotrauma.
Figure 2.1. Effects of 14 weeks of photoperiod on laser-Doppler blood flow in the brain and testes.

LD = long-day; SD = short-day. * p < 0.05 between photoperiod treatments
Figure 2.2. Effects of acute transfer (0, 2, 7, 21, or 49 d) to the opposite photoperiod following 14 weeks of original photoperiod treatment on body mass (A) and relative testes mass (B).

LD = long-day; SD = short-day. * p < 0.05 between photoperiod treatments within time; letters and other symbols represent p < 0.05 differences within photoperiod over time.
Figure 2.3. Effects of acute transfer (0, 2, 7, 21, or 49 d) to the opposite photoperiod following 14 weeks of original photoperiod treatment on relative Vegf gene expression in the testes (A), hypothalamus (B), hippocampus and olfactory bulbs (C).

LD = long-day; SD = short-day. * p < 0.05 between photoperiod treatments within time; # p < 0.05 within photoperiod over time; † p < 0.05 between photoperiod treatments over time.
Figure 2.4. Effects of acute transfer (0, 2, 7, 21, or 49 d) to the opposite photoperiod following 14 weeks of original photoperiod treatment on relative Hif1α gene expression in the testes, hypothalamus (A), hippocampus and olfactory bulbs (B).

LD = long-day; SD = short-day. * p < 0.05 between photoperiod treatments within time; # p < 0.05 within photoperiod over time; † p < 0.05 between photoperiod treatments over time.
Photoperiod (day length) is a reliable cue by which organisms determine the time of year and coordinate adaptations to seasonal changes in their environment (Goldman, 2001). In nontropical environments, energy-conserving, adaptive adjustments occur among individuals in response to decreasing day lengths that are believed to promote survival during the harsh conditions of winter (Bronson, 1985). For example, short days decrease reproductive system size and function in white-footed mice (*Peromyscus leucopus*) (Ruf, Korytko, Stieglitz, Lavenburg, & Blank, 1997; Whitaker, 1940).

In addition to reproductive involution, individuals of several species also decrease brain mass or volume of specific brain regions in winter (non-breeding season) compared to summer (breeding season) (Smith, Brenowitz, & Wingfield, 1997; Tramontin & Brenowitz, 2000; Tramontin, Smith, Breuner, & Brenowitz, 1998; Yaskin, 1984). The brain uses far more energy per mass than other tissues and any reduction would confer significant energetic savings, especially during the winter
In songbirds, seasonal changes in size and morphology of the brain circuitry mediating song is well-established (Brenowitz, Margoliash, & Nordeean, 1997; Smith et al., 1997; Tramontin, Perfita, Wingfield, & Brenowitz, 2001). However, studies of seasonal plasticity of mammalian brain structure and function are sparse (reviewed in Hofman & Swaab, 2002). Much of the work in rodents has focused on sexual dimorphisms of specific brain regions (Gaulin & FitzGerald, 1989; Jacobs, 1996b). Reduced hippocampal mass or volume have been reported in a few rodent species trapped during the winter or exposed to short day lengths (Galea & McEwen, 1999; Perrot-Sinal et al., 1998; Yaskin, 1984). Furthermore, deficits in spatial learning and memory (behaviors that require an intact hippocampus) (Morris, Garrud, Rawlins, & O'Keefe, 1982) have been reported in short-, relative to long-day, rodents (Perrot-Sinal et al., 1998). Spatial memory performance may be decreased during the non-breeding season when home range size is smaller and roaming to find mates is decreased compared to the breeding season (Stickel, 1968).

Photoperiod was hypothesized to trigger morphological changes in the brain that underlie seasonal adjustments in behavior to promote energetic savings. This hypothesis is supported by spatial learning and memory research in food-storing and brood-parasitic birds and other species (Healy, de Kort, & Clayton, 2005; Oberlander, Schlinger, Clayton, & Saldanha, 2004; Sherry et al., 1992).

The hippocampus is among the most plastic sites in adult mammalian brains (Breedlove & Jordan, 2001; McEwen, 1999). Changes within the hippocampus include fluctuations in dendritic complexity, spine density, soma size, neurogenesis, and apoptosis and may be associated with altered learning and memory performance.
The goal of this study was to test the hypothesis that short days would decrease hippocampal dendritic complexity and spine density in addition to specifically impair spatial learning and memory in rodents.

In the present study, short days reduced brain size and hippocampal volume and our results confirmed previously reported short-day deficits in spatial learning and memory. Additionally, photoperiod treatment affected neuronal spine density in the hippocampus. Overall, these data suggest that the functional learning and memory deficits exhibited by short-day mice are associated with structural changes in the hippocampus, and reveal that adult mammalian brains display seasonal plasticity.

**Materials and Methods**

**Experiment 1. Animals.** Twenty adult male (>55 days of age) white-footed mice (*P. leucopus*) from a breeding colony maintained at Ohio State University were used in this study. Breeder mice were originally obtained from the *Peromyscus* Genetic Stock Center at the University of South Carolina (Columbia, SC). All mice were housed in polypropylene cages (27.8 x 7.5 x 13 cm) with a constant temperature and humidity of 21 ± 5° C and 50 ± 10%, respectively, and *ad libitum* access to food (Harlan Teklad 8640 rodent diet, Indianapolis, IN) and filtered tap water. Mice were housed in either long photoperiods (LD: n = 7) with a reverse 16 h light : 8 h dark cycle (lights on at 2300 h Eastern Standard Time [EST]) or in short photoperiods (SD: n = 13 males) with an 8 h light : 16 h dark cycle (lights on at 0700 h EST) for 14 weeks. Mice were undisturbed except for routine cage changes and bi-weekly body
mass measurement, until week 10 when behavioral testing commenced. Mice that failed to regress their gonads in response to short days were dropped from the study (n = 4). All studies were conducted with approval of the Ohio State Institutional Animal Care and Use Committee approval and were conducted in compliance with all US federal animal welfare requirements.

Sensory tests. Mice were examined for potential photoperiod-induced differences in sensory discrimination prior to water maze training. Visual placing was assessed by suspending mice by their tail and noting the first observable extension of forearms when lowering mice onto a wire grid from a height of 30 cm (Hatcher et al., 2001). Mice were scored from 0-4 for forearm extension (0 = none, 1 = upon nose contact, 2 = upon vibrissae contact, 3 = before vibrissae contact, 4 = early vigorous extension). Tactile discrimination was determined by the presence or absence (scored as 1 or 2, respectively) of a directed head movement in response to brushing a cotton-tip applicator on the underside of the whiskers. Olfactory and auditory discrimination was tested after water maze training at the beginning of the dark phase (1500-1800 h). For the olfactory testing, a modified “find the cookie” paradigm was used. Individual mice were placed in a clean cage for 3 min while a 1 cm piece of fruit (orange) was buried in the corner of the home cage. Mice were then returned to their home cages and latency to uncovering the fruit was recorded for up to 300 sec. For auditory discrimination, acoustic startle was tested using a single ventilated startle chamber (SR-Lab, San Diego Instruments, San Diego, CA). Mice were placed inside a clear, nonrestrictive Plexiglas cylinder within the chamber that was hooked up to an electrostatic sensor that measured the amplitude (mV) of
displacement caused by the animal’s movement. The mice were subjected to a series of acoustic startle trials (120 dB pulse for duration of 40 msec) with an average of 15 sec between each trial. The average amplitude (mV) transduced by the animal’s movement was used to determine the acoustic startle response. Before onset of the testing session mice were allowed to acclimatize to the apparatus for 10 min.

*Learning and memory tests: Water maze.* The water maze was used to test long-term spatial learning and memory (Morris, 1984). Testing occurred during the light phase (between 0700 – 1100 h). The maze consisted of a white tank (1.3 m diameter) filled with 27°C water to a depth of 47.5 cm. The water was made opaque with white non-toxic tempera paint. The maze was divided into four equal quadrants and release points were designated at each quadrant as N, E, S, and W. Fixed extra-maze cues in the form of large black geometric shapes surrounded the tank. A tracking video camera was suspended from the ceiling above the pool and 2020 PLUS tracking software (HVS Image, Buckingham, UK) was used. Mice were handled using a small fishing net to avoid the stress of direct handling. On day 1, mice were allowed to swim freely for 60 sec without a platform to acclimate to the pool. On days 2-9, a platform (9 cm diameter) was hidden 0.5 cm below the water surface in one quadrant. Mice were given 60 sec to locate the hidden platform from random release points around the pool to “escape” from the water 3 times/day (one block of trials). Upon reaching the platform or after 60 sec, mice were placed on the platform for 10 sec and then returned to the home cage. The inter-trial intervals were 15 sec during which the pool was skimmed of debris. Latency to reach the platform, the distance of the mouse’s path, and swim speed were recorded by the system for
each trial to assess acquisition of the spatial task. After the third trial of the day, each mouse received a piece of tissue paper in their cage to expedite drying. On day 10, the platform was removed and a 60 sec probe trial was run to examine retention of spatial memory. The percent time spent in each quadrant (including the quadrant in which the platform had been removed) was recorded. To evaluate reversal learning, the platform was repositioned in a different quadrant and retraining to the new location on days 11-15 was completed as previously described. A second probe trial followed reversal training on day 16. On day 17, a single 60 sec visible platform trial was run to determine general visual acuity of the mice in this paradigm. The visual platform (9 cm diameter) was raised 0.5 cm above water level and was encircled with a black rim. Latency to reach the platform was recorded.

*Spontaneous alternation in Y-maze.* Upon completion of the water maze task, spontaneous alternation behavior was recorded to assess working memory performance with a spatial component during the dark phase (Crawley, 2000). The maze was made of black Plexiglas and covered with clear Plexiglas to prevent escape during testing. Each arm was 35 cm and converged at an equal angle. Mice were placed in one arm and allowed to move freely through the maze during a 3-min session. The series of arm entries was recorded. An alternation was defined as entries into all three arms on consecutive occasions. The percent alternation was calculated using the following formula: (actual alternations/maximum possible alternations) x 100. The maximum possible number of alternations was calculated as the total number of arm entries minus 2.
Passive avoidance. Twenty-four h after spontaneous alternation testing, mice were tested for cued and contextual conditioning memory (non-spatial) using passive avoidance during the dark phase. On day 1 of testing, mice were placed in one chamber of a dark 2-chambered box separated by a door (San Diego Instruments). After 30 sec of acclimation, a house light in the chamber in which the mouse resided was turned on and the door opened. To escape the light, mice entered the other chamber (which remained dark) where they received a 0.06 mA electrical shock (1 sec duration) from the grid floor. Mice were then returned to their home cage. On day 2, the same procedure was followed except the shock was removed. In general, mice that learn to avoid the location of the aversive shock are slow to enter the dark chamber. Latency to enter the dark chamber was recorded to a maximum of 300 sec.

Tissue collection and histology. Twenty-four h following the completion of all behavioral testing, mice were rapidly decapitated. Paired testes were removed and weighed to determine reproductive responsiveness to photoperiod treatment. The average testes mass for LD mice was determined, and SD males with testes mass greater than two standard deviations below this mean were considered reproductively non-responsive to short days and excluded from the study (n = 3). Brains were removed and processed for Golgi staining according to the manufacturer’s protocol (FD Rapid GolgiStain™ Kit, FD Neurotechnologies, Ellicott City, MD). Throughout the hippocampus, brains were cut coronally in 80 µm sections on a cryostat and mounted on 3% gelatin-coated slides before staining. Sections were also counterstained with cresyl violet. Granule cells in the dentate gyrus (n = 10/mouse) and pyramidal cells in the CA1 and CA3 region (n = 5-10/mouse) were traced using a
camera lucida at 400X magnification (Neurolucida, MicroBrightField, Williston, VT). Neurons chosen for tracing met the following criteria: 1) completely impregnated with Golgi stain 2) not obscured by other impregnated neurons or precipitant and 3) all dendrites were visible within the plane of focus. The hemispheric location of the traced neurons was chosen randomly. Additionally, dendritic spines were traced on five 10 µm distal dendritic segments of each neuron at 1000X magnification of randomly chosen granule cells (throughout the stratum moleculare) and pyramidal cells (throughout the stratum oriens [basilar] and the stratum lacunosum-moleculare [apical]) that had at least one branch point. Using the accompanying software (NeuroExplorer, MicroBrightField) dendritic complexity (via Sholl analysis), dendritic length, cell body area, and spine density was calculated. The Sholl analysis defines dendritic complexity by the number of dendritic branch points at fixed intervals from the cell bodies (Sholl, 1956). All samples were number-coded and the experimenter was blind to the treatments.

**Experiment 2.** Sixteen adult male white-footed mice were housed in either LD (n = 8) or in SD (n = 8) for 14 weeks. Mice were undisturbed except for routine cage changes and bi-weekly body mass measurement. At week 14, mice were deeply anesthetized with sodium pentobarbital (Nembutal®; Abbott Laboratories, North Chicago, IL) and transcardially perfused with 50 ml saline followed by 75 ml 10% phosphate buffered formalin. Brains were removed and post-fixed in formalin for at least 24 h at room temperature and then transferred to 15% sucrose in formalin at 4°C overnight. Finally, brains were transferred to 30% sucrose in 0.1 M phosphate buffered saline at 4°C until permeated. Brains were frozen on dry ice and punctured
with three stick pins on a rostral-caudal plane through the medial ventral area of the brain. These pin-holes were used as reference points to orient the traced sections for volume reconstruction. Brains were then stored at -70°C until being cut in 35 μm sections on a cryostat at 22°C. Every sixth section throughout the hippocampus (from bregma -1.06 to -2.92; Franklin & Paxinos, 1997) was dry-mounted on 3% gelatin coated slides, dried overnight, and then stored at -20°C until they were stained with 2.5% cresyl violet. The orientation and sequence of sections were kept consistent. Slides were then coverslipped with Permount (Fisher, St. Louis, MO). The hippocampus (dentate gyrus + Ammon’s horn) and the outline of the surrounding telencephalon were traced at 40X using a camera lucida. Means (± SEM) of 15 ± 1.07 sections were traced per mouse. Using Neuroexplorer, 3-D representations of the hippocampus and the telencephalon were created by stacking the tracings in order. Volumes of these structures were calculated based on the Cavalieri method (Gundersen & Jensen, 1987). Relative hippocampal volumes were calculated by dividing the absolute hippocampal volume by the volume of the surrounding telencephalon. All samples were number-coded and the experimenter was blind to the treatments.

**Statistical analyses.** Repeated measures ANOVAs were used to compare water maze performance over time. Within days, pairwise comparisons were planned *a priori* in the analysis models and were conducted using two-tailed student’s *t*-tests (Keppel & Wickens, 2004). Student’s *t*-tests were also used for other behavioral and physiological comparisons. Data with unequal variances were compared using nonparametric Mann-Whitney tests to compare photoperiod differences. One-tailed *t*-
tests were used to compare hippocampal volume due to our a priori hypothesis. All comparisons were considered statistically significant when p < 0.05. StatView software was used for all analyses (v. 5.0.1, Cary, NC).

**Results**

**Short Days Decrease Tissue Masses.** Exposure to short days for 13 weeks decreased body mass (LD: 21.3 ± 0.8; SD: 19.5 ± 0.7 g), testes mass (LD: 203.5 ± 11.0; SD: 74.1 ± 9.0 mg), and brain mass (LD: 539.8 ± 6.5; SD: 509.6 ± 16.0 mg) (p < 0.05 in all cases). Photoperiod treatment had no effect on visual, tactile, olfactory, or auditory discrimination (Table 3.1; p > 0.05 in all cases).

**Short Days Impair Morris Water Maze Performance.** Short days increased the latency to reach the hidden platform and the distance traveled to reach the hidden platform after 7 and 8 blocks of trials in the water maze test (Figure 3.1a and b; p < 0.05 in all cases). The overall effect of photoperiod on latency and path length taken to reach the hidden platform were statistically non-significant (p = 0.08 and p = 0.1, respectively). However, all animals displayed some degree of learning because both the latency and distance to reach the platform decreased over time (p < 0.001 in both cases). Swim speed, a possible indication of motivation, did not differ at any time point in the hidden platform trials (data not shown). All mice spent more time in the quadrant in which the platform had been removed for the probe trial (data not shown; p < 0.05); however, there were no differences between long- or short-day treated mice (p > 0.05).

Similar to the initial hidden platform trials, short days increased the latency to reach the hidden platform and the distance swam to reach the hidden platform on
block 3 of the reversal learning training (**Figure 3.2a and b**; p < 0.05 in both cases). All mice displayed reversal learning to some degree because latency and distance to reach the platform decreased over time (p < 0.05 in both cases). Again, swim speed did not differ between photoperiod groups at any time point (data not shown; p > 0.05). Short-day mice also displayed impaired reversal learning during the probe trial following reversal training. Short-day mice spent significantly more time than long-day mice in the quadrant of the pool in which the platform had been hidden during the original hidden platform trials (**Figure 3.2c**; p < 0.05), whereas short-day mice spent less time than long-day mice in the quadrant of the pool in which the platform had been removed from the recent reversal training, although this difference did not reach statistical significance (p = 0.1).

**Short Days Do Not Affect Non-Spatial Learning and Memory.**

Photoperiod treatments did not affect the percent spontaneous alternation or the total number of possible alternations (a measure of locomotor activity; data not shown; p > 0.05 in both cases).

Both long- and short-day mice hesitated before stepping through to the side of the chamber that previously shocked them in the passive avoidance test (day 1: LD: 12.3 ± 4.1, SD: 6.3 ± 0.9 sec; day 2: LD: 43.6 ± 29.2, SD: 24.3 ± 9.9 sec). However, only the short-day mice hesitated to step-over significantly longer on day 2 (p < 0.05).

**Short Days Affect Spine Density.** Photoperiod treatment did not affect dendritic complexity in the CA1, CA3, or dentate gyrus regions (**Figure 3.3a-c**; p > 0.05 in all cases). Raw dendritic length also did not differ between photoperiod-treated groups in any of the hippocampal regions examined (data not shown; p > 0.05.
in all cases). However, short days decreased spine density on the terminal tips (stratum lacunosum-moleculare) of both CA1 (p < 0.05) and CA3 (p = 0.1) pyramidal cells compared to long days (Figure 3.4a and c). On the other hand, short days increased spine density on basilar tips (stratum oriens) of CA3 dendrites (Figure 3.4b; p < 0.05), but had no effect on spine density in basilar dendritic tips (stratum oriens) of pyramidal cells in the CA1 or dendritic tips (stratum moleculare) in the dentate gyrus (p > 0.05). Cell body area did not differ between photoperiods in the CA1, CA3, or dentate gyrus regions (data not shown; p > 0.05 in all cases).

**Short Days Decrease Hippocampal Volume.** Short days decreased both absolute (LD: 4.50E10 ± 9.4E9; SD: 2.86E10 ± 3.3 µm³) and relative (hippocampus volume / telencephalon volume; LD: 1.07 ± 0.7; SD: 0.22 ± 0.1) hippocampal volume relative to long days (p < 0.05 in both cases). There were no differences in volume of the telencephalic region surround the hippocampus between the photoperiod-treated groups (LD: 10.2E10 ± 3.4E10; SD: 14.1E10 ± 1.9E10 µm³; p > 0.05).

**Discussion**

In this study, short days impaired both spatial-specific learning and memory relative to long days without affecting sensory abilities. Specifically, short-day mice required longer to find a hidden platform in the water maze and spent less time in the quadrant with the platform compared to long-day mice. Furthermore, photoperiod treatment induced changes in hippocampal morphology such that short days
decreased apical spine density in the CA1 region of the hippocampus and hippocampal volume and increased basilar spine density in the CA3 region compared to long days.

The hypothesis that in addition to inhibiting the reproductive system, short days trigger morphological changes in the hippocampus that mediate seasonal deficits in learning and memory was tested. The short-day impairment of spatial learning and memory in the water maze supported previous findings in non-breeding deer mice (*P. maniculatus*) (Galea et al., 1994a; Perrot-Sinal et al., 1998). Also, reversal learning, as well as memory for the reversal platform location, was compromised.

**Selective Impairment of Spatial Memory in Short Days.** I also tested whether or not spatial memory is specifically impaired, as compared to other types of memory, based on the hypothesis that spatial memory deficits are derived from the shrinkage of *P. leucopus* home range during the non-breeding season (Stickel, 1968). The selective impairment of long-term spatial learning and memory is supported based on our results from the spontaneous alternation and passive avoidance tests. Additional tests of this hypothesis require experiments using multiple long-term spatial tasks (e.g. radial arm maze). However, controls for photoperiod-induced differences in motivation to eat and locomote are difficult to equalize in these tests that are dependent on food motivation.

**Photoperiodic Differences in Spine Density.** In rats, spine density of the dentate gyrus and CA1 region of the hippocampus was positively correlated with water maze learning and memory performance (Moser et al., 1994; O'Malley et al., 2000). Spines increase the surface area of dendrites, which increase the excitatory
synaptic density and number of connections between neurons (reviewed in Sorra & Harris, 2000). Therefore, hippocampal spine density may be an indication of the efficiency of the synaptic network involved in spatial learning and memory. Similarly, our results indicate that apical spine density decreased in the CA1 pyramidal cells of short-day mice in conjunction with their impaired behavioral performance. In contrast to the impaired water maze performance, however, basilar spine density increased in CA3 of short-day mice. The role of spine density among hippocampal regions and dendritic poles in learning and memory remains unspecified (Sorra & Harris, 2000). Photoperiod-induced changes in specific spine morphology remain to be characterized and may provide insight into the functional significance of the present alterations of spine density.

In addition to photoperiod, spine density in adult rodents is also affected by other environmental factors. For example, environmental enrichment (Diamond, Ingham, Johnson, Bennett, & Rosenzweig, 1976; Rampon et al., 2000) and awakening after hibernation (Popov et al., 1992) increase spine density in the hippocampus, whereas restraint stress decreases hippocampal spine density (Magarinos, Deslandes, & McEwen, 1999). These environment-induced changes in hippocampal spine density occur over varying durations of time (2 h – 25 d). The time course over which the present photoperiod-induced changes in spine density are established needs to be examined. Neurogenesis in the dentate gyrus, another example of adult hippocampal plasticity, may also be affected by photoperiod (Huang
et al., 1998; Lavenex, Steele, & Jacobs, 2000a). Studies that test the hypothesis that differences in hippocampal neurogenesis between long- and short-day mice modulate spatial learning and memory are described in Chapter 4.

**Photoperiodic Differences in Brain and Hippocampal Size.** Gross brain masses were low in voles and shrews trapped during the winter and in juvenile voles exposed to short days from birth (Dark et al., 1987; Yaskin, 1984). In the present study, short days decreased gross brain mass in adult male white-footed mice, both in absolute terms and after correcting for reduced body mass. Additionally, short-day mice had decreased hippocampal volumes which is consistent with the hypothesis that polygynous (having multiple female mates) rodents increase spatial memory during the breeding season to acquire multiple mates, as well as the hypothesis that hippocampal volume is positively correlated with hippocampal function (i.e. spatial learning and memory) (Gaulin & FitzGerald, 1986). Similarly, large seasonal variations in home range size have been described in field studies on *Peromyscus* such that home range sizes are dramatically smaller in the winter (non-breeding season) relative to the summer (Metzgar, 1978). It is important to note that all previously published estimations of hippocampal volume were based on a formula designed to calculate the volume of a truncated cone as opposed to the more sensitive method of volume estimation used in this study (i.e. Cavalieri’s method). In sum, short day-induced deficits in spatial learning and memory were linked with whole brain and hippocampal involution. These data support the concept that the relative size of a brain region represents its functionality and is capable of displaying adaptive plasticity among seasons in adults (Tramontin & Brenowitz, 2000).
Potential Role of Hormones on Photoperiodic Brain Changes. Because the duration of melatonin secretion is directly modulated by photoperiod, and mediates many photoperiod-induced adaptations, melatonin is a likely hormonal candidate for a signal mediating the photoperiod-induced hippocampal modifications described in this report. Melatonin receptors are present in the hippocampal area (entorhinal cortex) of white-footed mice (Weaver, Carlson, & Reppert, 1990). The only model of seasonal plasticity in which the role of melatonin has been examined is in the neural song circuitry of songbirds (Bentley et al., 1999). However, melatonin also affects LTP in hippocampal slices of photoperiod non-responsive animals (El-Sherif et al., 2003). Future study of the role of melatonin in seasonal hippocampal plasticity is warranted.

Decreased circulating testosterone and corticosterone concentrations in short-day white-footed mice (Feist, Feist, & Lynch, 1988) may also modulate morphological hippocampal alterations and result in the learning and memory behavioral differences observed in this study. Although breeding condition has been associated with spatial learning and memory performance, the relationship between adult testosterone concentrations and spatial performance is not clear (Galea et al., 1995; Perrot-Sinal et al., 1998), although there appears to be a positive correlation between testosterone concentration and hippocampal volume (Galea, Perrot-Sinal, Kavaliers, & Ossenkopp, 1999; Perrot-Sinal et al., 1998). Additionally, sustained (i.e. chronic) corticosterone treatment or stress-induced increases in corticosterone inhibit learning and memory (reviewed in Roozendaal, 2000), as well as decrease hippocampal dendritic complexity (reviewed in McEwen & Magarinos, 1997) in
various species. The hypothesis that photoperiodic alteration of testosterone or corticosterone (basal or stress-induced via water maze) concentrations mediate the changes described in the present study were tested and are described in Chapters 6 and 7, respectively.

Taken together, the influence of photoperiod on brain and behavioral plasticity in adults is likely profound in animals that reside in environments with dramatic seasonal changes. Prior to this study, the link between seasonal effects on brain structure and related function was largely confined to the neural song circuitry in birds. The results of this experiment indicate that a parallel mammalian model of adult brain plasticity exists in the hippocampus and affects spatial learning and memory. Specifically, morphological changes in the hippocampus appear to underlie seasonal alterations in learning and memory performance. Future research investigating the mechanisms underlying this seasonal plasticity may be applicable to treatment of seasonal cognitive and affective disorders.
Figure 3.1. Photoperiod-induced differences in spatial learning (via water maze).

A) Latency to reach the hidden platform by block of training. B) Path length to reach hidden platform by block of training. Each block = 3 trials. Long-day (n = 7), Short-day (n = 9). * p < 0.05.
Figure 3.2. Photoperiod-induced differences in water maze reversal learning and memory following relocation the hidden platform.

A, Latency to reach the relocated hidden platform by block of training. B, Path length to reach hidden platform by block of training. C, Time spent in each quadrant of the pool including the quadrant in which the platform was hidden in the original hidden platform trials and quadrant in which the platform was hidden for the reversal trials. Long-day (N = 7), Short-day (N = 9). * p < 0.05.
Figure 3.3. Effects of photoperiod on dendritic complexity (via Sholl analysis) of hippocampal neurons.

A, Pyramidal neuron complexity in CA1 region (n = 5-10 neurons/brain). B, Pyramidal neuron complexity in CA3 region (n = 5-10 neurons/brain). C, Granule neuron complexity in dentate gyrus (n = 10 neurons/brain).
Figure 3.4. Effects of photoperiod on spine density on terminal tips of hippocampal dendrites.

A, Spine density of apical tips of pyramidal dendrites in CA1 and CA3 regions. B, Spine density of basilar tips of pyramidal dendrites in CA1 and CA3 regions and of granule cells in the dentate gyrus. C, Photomicrographs of apical spines in CA1 of long-day (left) and short-day (right) mice; 1000X magnification. Dotted line represents the unipolarity of granule cells in the dentate gyrus. n = 5 ten µm dendritic segments of spines/neuron. * p < 0.05.
<table>
<thead>
<tr>
<th>SENSORY TEST</th>
<th>LONG-DAY</th>
<th>SHORT-DAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual Discrimination</td>
<td>2.1 ± 0.1</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>Vibrissae Discrimination</td>
<td>1.5 ± 0.2</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>Olfactory Latency (sec)</td>
<td>171.3 ± 30.8</td>
<td>172.6 ± 36.4</td>
</tr>
<tr>
<td>Auditory Discrimination (% startle)</td>
<td>45.1 ± 7.6</td>
<td>33.1 ± 3.5</td>
</tr>
</tbody>
</table>

**Table 3.1.** Mean (± SEM) behavioral scores after 10 weeks of long or short days.
Evidence supporting adult mammalian neurogenesis within the hippocampus is rapidly increasing (Kempermann, 2005) despite general pessimism of the earliest reports of this phenomenon (Altman, 1962). Cells that proliferate from the subgranular zone within the dentate gyrus differentiate into neurons, migrate to the granule cell layer, and extend axons to the CA3 region. These cells display electrophysiological characteristics of neurons and synaptic connections to other neurons, and are therefore assumed to functionally contribute to hippocampal processing (Meltzer, Yabaluri, & Deisseroth, 2005; Schinder & Gage, 2004). Other cells differentiate into glia and migrate around the granule cell layer. Adult neurogenesis involves the coordinated action of numerous growth factors including brain-derived neurotropic factor ($Bdnf$) (Hagg, 2005).

Several lines of research have described external influences on the rate of cell proliferation, neurogenesis, or survival of newly born cells within the hippocampus (Kempermann, 2005). Studies that describe changes in cell proliferation measure the numbers of cells that have recently divided, but have not yet differentiated, whereas
those describing changes in neurogenesis measure newly born cells that have differentiated into neurons. Exercise (van Praag et al., 1999a; van Praag et al., 1999b), environmental enrichment (Kempermann, Kuhn, & Gage, 1997), hormones (McEwen, 1994; Ormerod et al., 2004; Shingo et al., 2003), and stressors (Fowler, Liu, Quimet, & Wang, 2002; McEwen, 1999)(e.g. dietary restriction, social environment, or odor deprivation) alter aspects of adult hippocampal cell proliferation or neurogenesis.

In addition to extrinsic cues, hippocampal function (e.g., learning and memory experience) may affect hippocampal neurogenesis (Pham et al., 2005; Shors et al., 2001b; Shors et al., 2002). A reciprocal relationship between neurogenesis and some types of learning and memory performance may exist (Gould, Beylin, Tanapat, Reeves, & Shors, 1999a; Gould, Tanapat, Hastings, & Shors, 1999c; but see Shors et al., 2002; van Praag et al., 1999b). Spatial learning and memory performance (Galea et al., 1994a; Perrot-Sinal et al., 1998; Chapters 3, 6, and 7) and adult brain (e.g., hippocampal) plasticity is affected by photoperiod (day length) (Dark et al., 1987; Hofman & Swaab, 2002; Tramontin & Brenowitz, 2000; Chapters 3 and 7). Several weeks of short-day exposure impairs spatial learning and memory, decreases brain mass and hippocampal volume, and alters hippocampal spine density compared with long days in adult male white-footed mice (Peromyscus leucopus) (Chapter 3). Inhibition of hippocampal size and function in short days coincides with decreased home range area and reduced mate searching in the winter in wild rodents. Therefore, these photoperiod-evoked changes in brain and behavior may be adaptive (Jacobs, 1996a; Sherry et al., 1992).
In this study, we hypothesized that short days also decreases adult hippocampal neurogenesis underlying impaired hippocampal function (i.e., spatial learning) relative to long days. We also tested whether reduced neurogenesis would be characterized by reduced hippocampal brain-derived neurotrophic factor (BDNF) gene expression and whether spatial learning and memory training itself may modulate photoperiodic effects on neurogenesis.

Materials and Methods

Animals. Sixty-five adult male (>55 days of age) white-footed mice (P. leucopus) from a breeding colony maintained at Ohio State University were used in this study. Breeder mice were originally obtained from the Peromyscus Genetic Stock Center at the University of South Carolina (Columbia, SC). All mice were housed in polypropylene cages (27.8 x 7.5 x 13 cm) with a constant temperature and humidity of 21 ± 5° C and 50 ± 10%, respectively, and ad libitum access to food (Harlan Teklad 8640 rodent diet, Indianapolis, IN) and filtered tap water. Mice were housed in either long photoperiods (LD) with a reverse 16 h light/day cycle (lights on at 23.00 h Eastern Standard Time [EST]) or in short photoperiods (SD) with an 8 h light/day cycle (lights on at 07.00 h EST) for 14 weeks. Mice were undisturbed except for routine cage changes and bi-weekly body mass measurement, until week 10 when injections followed by behavioral testing commenced. Learning and memory tests were performed at this time in half of the mice, while the other half of the mice remained undisturbed in order to examine the effects of behavioral experience on cell proliferation and neurogenesis (see Table 4.1 for sample sizes).
All studies were conducted with approval of the Ohio State Institutional Animal Care and Use Committee approval and were conducted in compliance with all US federal animal welfare requirements.

**Experiment 1: Effects of photoperiod and water maze experience on hippocampal cell proliferation and neurogenesis.**

*Injections.* To estimate hippocampal cell proliferation and neurogenesis, all mice were injected with the cell division marker, bromodeoxyuridine (BrdU; Sigma). Subsets of each of the photoperiod x behavioral testing groups were used to examine cell proliferation or neurogenesis (see Table 4.1 for sample sizes and time line). To estimate cell proliferation, a single i.p. BrdU injection (50 mg/kg in 0.1 ml saline) was administered to the mice 1.5-2.5 h prior to perfusion at week 14. To estimate neurogenesis, daily i.p. BrdU injections (50 mg/kg in 0.1 ml saline) were administered to the mice over 6 consecutive days at week 10. The times of injections were randomized to control for circadian differences in cell division among the treatment groups.

*Water maze.* The water maze procedures were similar to those described in Chapter 3 with a few exceptions. On day 1, mice were allowed to swim freely for 60 sec without a platform to acclimate to the pool. On days 2-5, a platform (9 cm diameter) was hidden 0.5 cm below the water surface in one quadrant. Mice were given 2 consecutive blocks of trials per day. Each block consisted of three 60-sec trials during which the mice were trained to locate the hidden platform from random release points around the pool to “escape” from the water. After the last trial of each block, mice received a piece of tissue paper in their cage to expedite drying. On day
6, the platform was removed and a 60 sec probe trial was run to examine retention of spatial memory. To evaluate reversal learning, the platform was repositioned in a different quadrant and retraining to the new location on days 7-8 (a total of 4 blocks of trials) was completed as previously described. A second probe trial followed reversal training on day 9. On day 10, a single 60-sec visible platform trial was run to determine general visual acuity of the mice in this paradigm.

Tissue collection and histology. At week 14, mice were deeply anesthetized with sodium pentobarbital (Nembutal®; Abbott Laboratories, North Chicago, IL) and transcardially perfused with 50 ml saline followed by 75 ml 4% paraformaldehyde. Paired testes were removed and weighed to determine reproductive responsiveness to photoperiod treatment. The average testes mass for LD mice was determined, and SD males with testes mass two standard deviations below this mean were considered reproductively responsive to short days and those that did not meet this criteria were excluded from the study (n = 3). Brains were removed, post-fixed in paraformaldehyde for 3 h at room temperature, transferred into 0.2 M phosphate buffer overnight at 4° C. The next day, brains were transferred to 30% sucrose in 0.1 M phosphate buffered saline until permeated. Brains were then frozen and stored at -70° C until cut into 25 µm sections on a cryostat at 22° C. Four series of every eighth section throughout the hippocampus (from bregma -1.06 to -2.92; Franklin & Paxinos, 1997) was dry-mounted on plus slides, dried overnight, and then stored at -20° C until immunohistochemistry was performed.

Cell proliferation and neurogenesis immunohistochemistry. One series of every eighth section from all mice was triple labeled fluorescently for BrdU, a
neuronal marker, and a glial marker to determine which types of cells were newly born. A mean (± SEM) of 10.1 ± 0.3 sections were used per mouse. Rat BrdU (Accurate Chemical & Scientific Corporation, Westbury, NY), rabbit glial fibrillary acidic protein (GFAP; Sigma-Aldrich, St. Louis, MO), and mouse neuronal nuclei (NeuN; Chemicon International, Temecula, CA) antibodies were used to detect newly-born, glial and neuronal cells, respectively. Secondary antibodies conjugated with three different flurochromes and made in donkey (anti-rat Alexa594, anti-rabbit Alexa647, anti-mouse Alex488) were purchased from Molecular Probes (Carlsbad, CA). Sections on the slides were encircled with tub caulk and allowed to dry for 6 h. Slides were then rinsed in 0.1M TBS for 30 min and then incubated in 2N HCl at 37°C for 15 min to denature DNA. Slides were immediately transferred to 0.1 M borate buffer for 10 min at room temperature. Following 3 rinses in TBS, slides were blocked (3% donkey serum in TBS + 0.5% Triton-X + 0.2% sodium azide) for 4 h at room temperature with constant agitation. A primary antibody mixture (1:200 BrdU, 1:200 NeuN, 1:500 GFAP) was applied to all slides for 24 h at room temperature with constant agitation. Slides were thrice rinsed in TBS and a secondary antibody mixture (1:200 anti-rat, 1:200 anti-mouse, 1:500 anti-rabbit) was applied to all slides for 3 h at room temperature with constant agitation. Slides were rinsed in TBS and then coverslipped with Fluoromount (Fisher Scientific, Pittsburgh, PA). BrdU+ cells were manually counted in each section within the subgranular zone, dentate gyrus cell layer, and hilus of the hippocampus at 200X magnification with a fluorescent microscope. To determine the cellular phenotypes of BrdU+ cells in the neurogenesis brains (6 injections of BrdU + 4 weeks), approximately 60 cells from 12 sections per
animal were analyzed using a confocal laser scanning microscope (Zeiss 510 META, Thornwood, NY) with excitation wavelengths of 488, 543, and 633 nm at the Microscope and Imaging Facility at Ohio State University.

**Experiment 2: Effects of acute and chronic photoperiod treatment on angiogenesis gene expression in the brain.** The 64 mice used in this experiment were previously described in Experiment 2 in Chapter 2 (n = 4-10/group). Briefly, all mice were exposed to long or short photoperiod conditions for 14 weeks (to induce long- and short-day phenotypes determined by maximum testicular regression in short-day mice; Chapter 1) and then transferred to the opposite photoperiod for 0, 2, 7, 21, or 49 days.

**RNA extraction, gene sequencing, and qPCR.** Total RNA was extracted from ≤ 30 mg of olfactory bulbs and cDNA was transcribed as described in Chapter 1. *BDNF* was sequenced as previously described (Chapter 1) and primers and a probe were synthesized with the probe labeled with 6-FAM and MGB (non-fluorescent quencher) at the 5’ and 3’ ends, respectively: *BDNF* forward 5’-AAGGCACTGGAACTCGCAAT - 3’, *BDNF* reverse 5’-CCATAGTAAGCGCCCGAACA - 3’, *BDNF* probe 5’-CCGAACTACCCAGTCGT - 3’. The primers and probes used for 18S Ribosomal RNA and qPCR conditions were previously described in Chapter 1.

**Statistical analyses.** Repeated measures ANOVAs were used to compare water maze performance over time. Within days, pairwise comparisons were planned *a priori* in the analysis models and were conducted using two-tailed student’s *t*-tests (Keppel & Wickens, 2004). Student’s *t*-tests were also used for other comparisons.
Data with unequal variances (e.g., some of the BrdU cell counts, *Bdnf* gene expression) were compared using nonparametric Mann-Whitney tests to compare photoperiod differences. All comparisons were considered statistically significant when \( p < 0.05 \). StatView software was used for all analyses (v. 5.0.1, Cary, NC, USA).

**Results**

**Experiment 1.** Short days decreased both absolute (long-day: 365.0 ± 22.9; short-day: 149.4 ± 16.7 mg) and relative (to body mass) testes mass compared with long days (\( p < 0.001 \) in both cases) ([Figure 4.1](#)).

*Water maze.* Both latency to reach the original hidden platform and path length swam decreased over block of trial in both photoperiods (\( p < 0.05 \)) ([Figure 4.2a,b](#)). Short days increased both the latency to reach the original hidden platform and the path length swam on blocks 5 and 6 (\( p < 0.05 \) in both cases) ([Figure 4.2a,b](#)). Overall swim speed did not differ between the photoperiod groups (\( p > 0.05 \)) ([Figure 4.2c](#)). Both photoperiod groups spent more time in the quadrant of the maze from which the original hidden platform had been removed (Quadrant 1) compared with the other 3 quadrants in the probe trial (\( p < 0.05 \)) ([Figure 4.2d](#)). The latency to reach the reversal hidden platform, path length swam, and swim speed did not significantly change over the 4 blocks of reversal training (\( p > 0.05 \)) (data not shown).

Photoperiod did not significantly affect reversal latency, path length, or swim speed (\( p > 0.05 \)) (data not shown). Long-day, but not short-day, mice spent more time in the quadrant from which the reversal platform had been removed compared with
Quadrant 2 and 4 in the reversal probe trial (p < 0.05) (**Figure 4.2e**). No photoperiodic differences in latency to reach the visible platform were observed (p > 0.05) (data not shown).

*Immunohistochemistry: Neurogenesis versus cell proliferation.* Mice treated to examine neurogenesis (6 BrdU injections + 4 wks) displayed more BrdU+ cells per section examined within the subgranular zone, granule cell layer, and hilus of the dentate gyrus compared with mice treated to examine cell proliferation (1 BrdU injection + 2 h) (p < 0.05 in all cases) (**Figure 4.3a**). Specifically, neurogenesis mice had significantly more BrdU+ cells within the medial and ventral portions of the granule cell layer and hilus and within the ventral subgranular zone compared with cell proliferation mice (p < 0.05 in all cases) (data not shown). Total numbers of BrdU+ cells were greater in the entire granule cell layer, hilus, and whole hippocampus of neurogenesis mice compared with cell proliferation mice (p < 0.05 in all cases) (data not shown). In the neurogenesis mice, more BrdU+ cells were observed per section and per hippocampus in the subgranular zone and granule cell layer compared with the hilus (p < 0.05 in both cases) (data not shown). In the cell proliferation mice, more BrdU+ cells were observed per section and per hippocampus in the subgranular zone than the granule cell layer and hilus and more cells were observed in the granule cell layer than the hilus (p < 0.05 in all cases) (data not shown).

*Among neurogenesis groups.* Fewer BrdU+ cells were observed in the ventral granule cell layer of long-day mice compared with short-day mice (p < 0.05) (**Table 4.2**). The interaction between water maze groups and photoperiod for the mean
number of BrdU+ cells per section within the granule cell layer and the total numbers of BrdU+ cells within the granule cell layer and the entire hippocampus were significant (p < 0.05 in all cases), such that water maze exposure reduced the number of BrdU+ cells in long-day mice and increased the number of BrdU+ cells in short-day mice (Table 4.2). Within long-day groups, water maze training decreased the average number of BrdU+ cells/section within the granular cell layer and hilus (Figure 4.3b). Specifically, within long days, water maze training decreased the number of BrdU+ cells/section in the dorsal, medial, and ventral portions of the granule cell layer and the dorsal hilus, and within the entire granule cell layer and hippocampus (p < 0.05 in all cases) (Table 4.2). No differences in BrdU+ cell number were observed between water maze treatments in short-day mice (p > 0.05) (Figure 4.3b and Table 4.2).

Among cell proliferation groups. Short days increased the number of BrdU+ cells within the dorsal subgranular zone (p < 0.05) (Table 4.3). The interaction between water maze groups and photoperiod for the number of BrdU+ cells within the medial hilus was significant (p < 0.05), such that water maze exposure increased the number of BrdU+ cells in long-day mice and decreased the number of BrdU+ cells in short-day mice (Table 4.3). Photoperiod and water maze experience did not affect the average number of BrdU+ cells/section within the subgranular, granule cell layer, and hilus (p > 0.05 in all cases) (Figure 4.3c). However, within short-day groups, water maze training decreased the average number of BrdU+ cells/section within the ventral granule cell layer and medial hilus (p < 0.05 in both cases) (Table 4.3).
BrdU cellular phenotype. More BrdU+ cells were double-labeled with NeuN than GFAP or neither NeuN or GFAP and more BrdU+ cells were double-labeled with GFAP than neither NeuN or GFAP regardless of photoperiod or water maze treatments (p < 0.05) (Figure 4.4a-c). No differences in the percent of BrdU-labeled cells that were NeuN+ or GFAP+ were observed among photoperiod and water maze groups (p > 0.05) (Figure 4.4a). The raw number of BrdU+ cells that were not co-labeled with NeuN or GFAP was greater in mice exposed to water maze regardless of photoperiod compared with mice without water maze experience (p < 0.05) (data not shown), however, this statistical difference disappeared following normalization as a percent of the total number of cells examined (p > 0.05) (Figure 4.4a).

Experiment 2. Mice transferred from short to long days for 0-7 d displayed greater hippocampal Bdnf expression than those transferred from long to short days for 0-7 d (p < 0.05) (Figure 4.5).

Discussion

We examined the potential for photoperiod to coordinate adult hippocampal neurogenesis that may functionally affect spatial learning and memory. Although increased neurogenesis or cell proliferation was not correlated with enhanced spatial learning and memory performance, photoperiod appeared to modulate the effects of learning and memory experience on hippocampal neurogenesis. However, neither photoperiod nor water maze experience significantly affected the phenotypic outcome (i.e. neuronal or glial) of BrdU+ cells. Finally, gene expression of a growth factor involved in hippocampal neurogenesis (Bdnf) increased in the hippocampi of mice.
acutely exposed to long days as opposed to short days. Taken together, these results suggest that initial changes in photoperiod may trigger a cascade of molecular events underlying changes in adult hippocampal neurogenesis, but that these photoperiod-evoked differences are transient.

This is the first study in mammals to test the effects of photoperiod on hippocampal neurogenesis based on photoperiodic differences in hippocampal-based behavior. In contrast to our predictions, the effects of photoperiod on hippocampal cell proliferation were only significant by interaction with water maze experience. In general, long days reduced and short days increased the number of BrdU labeled cells. Similarly, previous studies in mammals or food-caching birds (i.e., another group of animals in which spatial learning is seasonally-based) that examine the effects of season or photoperiod on adult neurogenesis in males report either no effect (Hoshooley & Sherry, 2004; Lavenex et al., 2000b) or increased neurogenesis in short days (Huang et al., 1998). In contrast, long-day male meadow voles (*Microtus pennsylvanicus*) display increased hippocampal neurogenesis, but not cell proliferation (Ormerod & Galea, 2003), and in songbirds (*Serinus canarius*), seasonal peaks in cell proliferation within a song production brain region corresponds to peak song production (Kirn, O'Loughlin, Kasparian, & Nottebohm, 1994). Thus it appears that in adult male rodents, photoperiodic effects on hippocampal cell division do not relate to spatial learning and memory performance. However, the possibility remains that photoperiodic effects on hippocampal neurogenesis in this species may be underestimated under laboratory conditions (Barnea & Nottebohm, 1994) and may require testing with wild-caught mice at different times of the year. Although
photoperiodic differences in spatial learning and memory may not be related to changes in neurogenesis, other types of photoperiod-evoked adult hippocampal plasticity are compatible (Chapters 3 and 7).

No photoperiodic differences in the percent of BrdU+ cells that displayed neuronal or glial phenotypes were observed. Although, the ratio of NeuN to GFAP co-labeled BrdU+ cells is consistent with previous studies on adult hippocampal neurogenesis (Kempermann, 2005). Additionally, more BrdU+ cells were located in the granule cell layer of neurogenesis-treated mice compared with cell proliferation-treated mice, supporting the hypothesis that newly born cells migrate to the granule cell layer over time. In mice used to study cell proliferation, most BrdU+ cells remained in the subgranular zone, the origin of dividing cells in the adult hippocampus.

We replicated the previously reported spatial learning impairment in short-relative to long-day mice (Chapters 3 and 6). The out-performance in the water maze of long-day male mice compared to short-day males is hypothesized to reflect a seasonal adaptation promoting spatial orientation of territories and mates during the breeding season (long days) compared with the non-breeding season (short-days) (Jacobs, 1996a; Chapter 3).

In the present study, water maze experience either decreased or did not affect hippocampal neurogenesis or cell proliferation in the granule cell layer of both long- and short-day mice. Experience in the water maze also decreased cell proliferation in the medial hilus of short-day mice. These results corroborate previous studies that report either no change in neurogenesis following water maze exposure (van Praag et
al., 1999b) and other types of learning (Gould et al., 1999a) or decreased neurogenesis following fear-related learning (Pham et al., 2005). Similarly, inhibiting hippocampal cell proliferation with a toxin did not affect water maze performance, but reduced fear in trace fear conditioning (Shors et al., 2001b; Shors et al., 2002). In contrast, one study reported increased neurogenesis following water maze training (Gould et al., 1999a). Our results support the hypothesis that learning and memory experience may not relate directly to hippocampal neurogenesis or cell proliferation.

In support of our hypothesis, acute exposure to long days increased hippocampal Bdnf expression. BDNF is a growth factor implicated in the promotion of neuron survival and differentiation during adult neurogenesis (Hagg, 2005). BDNF may also promote hippocampal learning and memory via its effects on hippocampal plasticity (Hall, Thomas, & Everitt, 2000; Ma, Wang, Wu, Wei, & Lee, 1998; Mizuno, Yamada, Olariu, Nawa, & Nabeshima, 2000; Tyler, Alonso, Bramham, & Pozzo-Miller, 2002). Our results suggest that acute exposure to long days (i.e., Experiment 2) may increase neurogenesis and Bdnf, but these changes in the hippocampal cellular and molecular environment may be resolved following extensive long-day exposure (i.e., Experiment 1). Future studies are necessary to examine more acute photoperiod treatments on adult neurogenesis.

In conclusion, photoperiod may modulate behavioral effects on adult neurogenesis and initial changes in photoperiod may affect hippocampal plasticity transiently. However, functional (i.e., behavioral) effects of photoperiod on hippocampal plasticity may not include changes in hippocampal neurogenesis or cell
proliferation. The potential for seasonal regulation of adult mammalian neurogenesis has clinically-relevant implications (Emsley, Mitchell, Kempermann, & Macklis, 2005; Johansson, 2004; Taupin, 2005) and further investigation is warranted.
Figure 4.1. Photoperiod-induced differences in relative testes mass. * p < 0.05.
Figure 4.2. Photoperiod-induced differences in spatial learning (via water maze).

A) Latency to reach the original hidden platform by block of training. B) Path length to reach the original hidden platform by block of training. C) Swim speed by block of original hidden platform training. D) Time spent in each quadrant of the pool during the memory probe test following the original hidden platform training. E) Time spent in each quadrant of the pool during the memory probe test following the reversal hidden platform training. Each block = 3 trials. * p < 0.05.
**Figure 4.3.** Number of BrdU+ cells in the subgranular zone (SG Zone), granule cell layer (GC Layer), and hilus of the hippocampus.

A) Number of BrdU+ cells following BrdU treatment to test neurogenesis (6 BrdU injections + 4 weeks) or cell proliferation (1 BrdU injection + 2 h). *p < 0.05 between neurogenesis and cell proliferation groups. B) Number of BrdU+ cells in all groups of mice treated to test neurogenesis. MWM = Morris water maze experience, NO = no water maze experience. C) Number of BrdU+ cells in all groups of mice treated to test cell proliferation. *p < 0.05 within photoperiod groups.
Figure 4.4. Effects of photoperiod on the ratio of differentiated cell phenotypes of BrdU+ cells in the hippocampus of mice treated to study neurogenesis.

A) Percent of BrdU+ cells co-labeled with neuronal (NeuN) and glial (GFAP) markers. MWM = Morris water maze experience, NO = no water maze experience.  
B) Confocal photomicrograph of a BrdU+ cell co-labeled with NeuN in the granule cell layer of the dentate gyrus.  
C) Confocal photomicrograph of 3 BrdU+ cells co-labeled with GFAP around the granule cell layer of the dentate gyrus. Arrowheads point to labeled cells. 400X magnification.
Figure 4.5. Effects of acute transfer (0, 2, 7, 21, or 49 d) to the opposite photoperiod following 14 weeks of original photoperiod treatment on relative Bdnf gene expression in the hippocampus. LD = long-day; SD = short-day.
<table>
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<th>Week 12-14</th>
<th>Week 14</th>
</tr>
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<tr>
<td><strong>NO WATER MAZE</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(n=6-9/gp)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LONG-DAY</td>
<td>Neurogenesis</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Proliferation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHORT-DAY</td>
<td>Neurogenesis</td>
<td><strong>++++</strong></td>
<td></td>
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<tr>
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<td></td>
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<tr>
<td><strong>WATER MAZE</strong></td>
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<td>WATER MAZE</td>
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<tr>
<td>(n=7-8/gp)</td>
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</tr>
<tr>
<td>LONG-DAY</td>
<td>Neurogenesis</td>
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<td></td>
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<tr>
<td></td>
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<tr>
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<td></td>
</tr>
<tr>
<td></td>
<td>Proliferation</td>
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</table>

* = BrdU injection (i.p. 50 mg/kg)

**Table 4.1.** Sample sizes and time line of all treatments.
Table 4.2. Mean (±SEM) number of BrdU+ cells per section in 3 dorsal-ventral regions of hippocampus of mice used to measure neurogenesis. MWM = Morris water maze experience, NO = no water maze experience.

<table>
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<tr>
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<th>Short-Day, MWM</th>
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<td>Dorsal SG Zone</td>
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<td>Dorsal GC Layer</td>
<td>0.6 ± 0.4</td>
<td>5.8 ± 2.3 *</td>
<td>4.2 ± 1.5</td>
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<tr>
<td>Dorsal Hilus</td>
<td>0 ± 0</td>
<td>0.8 ± 0.4 *</td>
<td>1 ± 0.4</td>
<td>0.3 ± 0.3</td>
</tr>
<tr>
<td>Medial SG Zone</td>
<td>4.6 ± 0.8</td>
<td>13.3 ± 3.9</td>
<td>11.5 ± 3.2</td>
<td>11.1 ± 2.7</td>
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<tr>
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<td>10.0 ± 3.1 *</td>
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<td>3.1 ± 1.1</td>
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<tr>
<td>Ventral SG Zone</td>
<td>5.0 ± 0.6</td>
<td>11.5 ± 3.1</td>
<td>14.3 ± 3.5</td>
<td>9.6 ± 4.3</td>
</tr>
<tr>
<td>Ventral GC Layer</td>
<td>3.6 ± 0.9</td>
<td>8.4 ± 1.5 *</td>
<td>12.0 ± 1.7</td>
<td>10.4 ± 4.8</td>
</tr>
<tr>
<td>Ventral Hilus</td>
<td>1.5 ± 0.6</td>
<td>2.3 ± 0.8</td>
<td>3.3 ± 1.2</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td>Entire SG Zone</td>
<td>11.6 ± 1.5</td>
<td>29.7 ± 9.1</td>
<td>29.5 ± 7.6</td>
<td>19.9 ± 6.6</td>
</tr>
<tr>
<td>Entire GC Layer</td>
<td>5.1 ± 1.6</td>
<td>23.2 ± 6.3 *</td>
<td>25.1 ± 3.6</td>
<td>17.4 ± 7.7</td>
</tr>
<tr>
<td>Entire Hilus</td>
<td>2.0 ± 0.5</td>
<td>4.9 ± 1.1</td>
<td>7.4 ± 2.6</td>
<td>3.7 ± 1.3</td>
</tr>
<tr>
<td>Entire Hippocampus</td>
<td>18.8 ± 3.5</td>
<td>66.5 ± 14.6 *</td>
<td>71.3 ± 10.6</td>
<td>48.1 ± 14.2</td>
</tr>
</tbody>
</table>

*p < 0.05 within photoperiod
Table 4.3. Mean (±SEM) number of BrdU+ cells per section in 3 dorsal-ventral regions of hippocampus of mice used to measure cell proliferation. MWM = Morris water maze experience, NO = no water maze experience.

<table>
<thead>
<tr>
<th>Region</th>
<th>Long-Day, MWM</th>
<th>Long-Day, NO</th>
<th>Short-Day, MWM</th>
<th>Short-Day, NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsal SG Zone</td>
<td>2.3 ± 0.8</td>
<td>1.6 ± 0.6</td>
<td>3.3 ± 1.0</td>
<td>4.8 ± 1.7</td>
</tr>
<tr>
<td>Dorsal GC Layer</td>
<td>2.6 ± 0.6</td>
<td>1.7 ± 0.6</td>
<td>1.9 ± 0.7</td>
<td>2.9 ± 1.1</td>
</tr>
<tr>
<td>Dorsal Hilus</td>
<td>0.1 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.5 ± 0.5</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>Medial SG Zone</td>
<td>8.8 ± 2.8</td>
<td>6.6 ± 1.2</td>
<td>6.6 ± 1.7</td>
<td>10.0 ± 0.5</td>
</tr>
<tr>
<td>Medial GC Layer</td>
<td>4.1 ± 1.3</td>
<td>3.2 ± 0.5</td>
<td>1.8 ± 0.7</td>
<td>4.6 ± 1.9</td>
</tr>
<tr>
<td>Medial Hilus</td>
<td>0.6 ± 0.3</td>
<td>0.5 ± 0.2</td>
<td>0.5 ± 0.2</td>
<td>1.5 ± 0.5 *</td>
</tr>
<tr>
<td>Ventral SG Zone</td>
<td>4.4 ± 2.0</td>
<td>5.9 ± 1.0</td>
<td>6.6 ± 0.9</td>
<td>7.5 ± 0.1</td>
</tr>
<tr>
<td>Ventral GC Layer</td>
<td>2.9 ± 1.5</td>
<td>3.3 ± 0.6</td>
<td>0.8 ± 0.4</td>
<td>3.1 ± 0.6 *</td>
</tr>
<tr>
<td>Ventral Hilus</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.2</td>
<td>0.3 ± 0.2</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>Entire SG Zone</td>
<td>15.6 ± 5.4</td>
<td>14.1 ± 2.1</td>
<td>16.0 ± 4.2</td>
<td>23.8 ± 1.3</td>
</tr>
<tr>
<td>Entire GC Layer</td>
<td>9.5 ± 3.3</td>
<td>8.2 ± 1.6 *</td>
<td>4.7 ± 1.8</td>
<td>11.6 ± 3.4</td>
</tr>
<tr>
<td>Entire Hilus</td>
<td>1.3 ± 0.4</td>
<td>1.4 ± 0.4</td>
<td>1.3 ± 0.8</td>
<td>3.3 ± 1.0</td>
</tr>
<tr>
<td>Entire Hippocampus</td>
<td>28.4 ± 9.7</td>
<td>24.8 ± 3.1 *</td>
<td>23.6 ± 6.9</td>
<td>44.2 ± 5.2</td>
</tr>
</tbody>
</table>

*p < 0.05 within photoperiod
CHAPTER 5

PHOTOPERIOD AND LONG-TERM POTENTIATION

Long-term potentiation (LTP) is recognized as the strongest candidate for a mechanism by which new memories are formed and stored in the brain (reviewed in; Lynch, 2004). LTP is defined as an increase in efficiency of synaptic transmission following high-frequency stimulation (Bliss & Gardner-Medwin, 1973) and is hypothesized to be involved in memory because it is characterized by synaptic cooperativity, associativity, input specificity, and durability (Bliss & Collingridge, 1993). Regarding spatial memory, LTP is easily demonstrated in the hippocampus and occurs naturally during exploratory behaviors (Greenstein, Pavlides, & Winson, 1988; Rose & Dunwiddie, 1986). Stimulation of the perforant pathway (angular bundle) results in excitatory post-synaptic potentials (EPSPs) in the dentate gyrus and high-frequency stimulation of this pathway induces LTP. Evidence that inhibition of LTP impairs spatial learning (Morris, Anderson, Lynch, & Baudry, 1986) substantiates the hypothesis that LTP mediates hippocampal-based learning and memory. However, a complete understanding of how LTP translates into memory is unknown.
Other aspects of hippocampal plasticity are associated with induction of LTP. For example, LTP increases dendritic spine density in the CA1 region of the hippocampus (Engert & Bonhoeffer, 1999). Hormones, which vary by photoperiod (see Chapter 7), alter parameters of LTP in addition to other forms of hippocampal plasticity (Lathe, 2001). For example, melatonin dampens LTP in hippocampal slices, which is directly relevant to seasonality research (El-Sherif et al., 2003). Additionally, non-photoperiodic environmental factors also alter hippocampal LTP. Exercise (van Praag et al., 1999a) or environmental enrichment (Duffy, Craddock, Abel, & Nguyen, 2001) enhance LTP in mice and behavioral (e.g., water maze) or restraint stressors impair hippocampal LTP (Kavushansky et al., 2006; Pavlides et al., 2002). Finally, vertebrate studies suggest that a positive relationship between the strength of LTP and learning and memory performance or experience exists (Doyere & Laroche, 1992; Fagnou & Tuchek, 1995).

Based on the previously described photoperiod-provoked changes in spatial learning (Chapters 3, 4, and 6), hippocampal dendrite structure (Chapter 3), and hormonal changes (Chapter 7), I predicted that photoperiod would affect LTP. The hypothesis that short days weaken LTP in the hippocampus compared with long days was tested in anesthetized male white-footed mice (Peromyscus leucopus).

Materials and Methods

Animals. Twelve adult (>55 days of age) male white-footed mice (Peromyscus leucopus) from our breeding colony were used in these experiments. Animals were housed individually in polypropylene cages (27.8 x 7.5 x 13 cm) with a
constant temperature and humidity of 21 ± 5° C and 50 ± 10%, respectively, and *ad libitum* access to food (Harlan Teklad 8640 rodent diet, Indianapolis, IN) and filtered tap water. Mice were either housed in reversed long days (LD; 16 h light/day; lights illuminated at 2300 Eastern Standard Time [EST]; n = 6), or in short days (SD; 8 h light/day; lights illuminated at 700 h EST; n = 6) for 12-15 wks (mean = 13.5 wks).

**Surgery.** Mice were anesthetized with urethane (1.5 g/kg i.p. in 0.08 – 0.15 ml saline) and placed in the stereotaxic apparatus using ear cups. Body temperature was maintained with an isothermal pad. An anterior-posterior incision (~1 cm) was made in the skin above the skull and three holes were made in the skull with a surgical drill. Recording electrodes were made from Teflon-insulated stainless steel wire (150 µm diameter). Bipolar stimulating electrodes were made from Teflon-insulated twisted tungsten wire (125 µm diameter each). Ground electrodes were made from Teflon-insulated, stainless steel wire (250 µm diameter). Stimulating, recording, and ground electrodes were slowly lowered into the perforant pathway (approximately 0 mm from lambda, 2.8 mm lateral, 1.5 mm ventral), dentate gyrus (approximately -3.0 mm from bregma, 1.3 mm lateral, 1.5 mm ventral), and anterior cortex (approximately -1.0 mm from bregma, 2.0 mm lateral, 0.5 mm ventral), respectively (Franklin & Paxinos, 1997). Initial responses were obtained using a cathodal stimulation intensity of 400-800 µA with 0.1 ms monophasic, square-wave pulse width and 0.1 Hz stimulation frequency. Ten minutes of baseline recording was then collected at approximately half effective (EC$_{50}$) current intensity with stimulation every 10 sec. High-frequency stimulation consisted of a single train of 20 pulses over 100 ms at 200 Hz at the same current as used for the test pulse. Post-
High-frequency stimulation waveforms were elicited and recorded every 10 sec for 10 min. Waveforms were analyzed for pre- and post-high-frequency stimulation EPSP slope and population spike amplitude using Spike2 software (Cambridge Electronic Design, UK). Following recording, electrode placement lesions were made with two 10-sec 1000 µA pulses. Data from one long-day mouse was removed from analysis due to a technical problem with the recording. Mice were decapitated, brains were removed and fixed in 10% buffered formalin, and paired testes were removed and weighed to verify photoperiodic responsiveness.

**Histology.** After at least 48 h, brains were transferred from formalin to 30% sucrose in 0.1 M phosphate buffered saline at 4°C until permeated and then frozen on dry ice and stored at -70°C until being sectioned. Thirty-five µm sections were cut with a cryostat at -22°C and every other section throughout the majority of the brain (from approximately bregma -0.94 to -4.72) (Franklin & Paxinos, 1997) was dry-mounted on 3% gelatin coated slides, dried overnight, and then stored at -20°C until they were stained with 2.5% cresyl violet. Slides were coverslipped with Permount (Fisher, St. Louis, MO) and correct placement of electrodes was verified. Mice with inaccurate electrode placement were removed from the electrophysiological analysis (n = 2/photoperiod).

**Results**

Short days decreased absolute (long-day: 390.0 ± 38.5, short-day: 125.9 ± 45.4 mg) and relative (to body mass) testes mass (p < 0.05 in both cases) (Figure 5.1). Stimulation of the perforant pathway elicited potentials in the dentate gyrus.
(Figure 5.2a). Placement of the recording and stimulating electrodes was verified to be within the granule cell layer of the dentate gyrus and the perforant pathway, respectively (Figure 5.2b). Characteristic evoked responses (Figure 5.2a) contained a stimulus artifact followed by a notched population spike. Following LTP, these responses were potentiated. However, short days did not significantly decrease the percent change in EPSP slope (Figure 5.3 and 5.4a) or EPSP amplitude (Figure 5.4b) between post-high-frequency stimulation and baseline compared with long days (p > 0.05 in both cases).

Discussion

These data are suggestive that electrophysiological potentiation within the dentate gyrus is greater in long-day mice compared with short-day mice following high-frequency stimulation of the perforant pathway. Although statistically significant differences between photoperiod treatments in EPSP slope and amplitude were not observed in this experiment, further examination of potential photoperiodic differences in LTP with larger sample sizes is warranted. Because of the strict criteria used in analyzing the LTP data, less than five mice per group were acceptable. Potential impairment of short-day LTP may mediate the impairment of spatial learning and memory described in previous Chapters (3, 4, and 6).

Impaired spatial learning and memory performance of short-day mice (Chapters 3, 4, and 6) and the tendency for decreased hippocampal LTP in the present study corroborates previous studies that describe positive associations between spatial learning and memory and LTP (Lynch, 2004). However, direct evidence that LTP
mediates spatial learning and memory remains elusive. The leading hypothesis suggests that strengthened synaptic connections induced by LTP promote memory formation for spatial cues (Lynch, 2004). Supportive molecular evidence for the positive relationship between LTP and learning and memory includes the requirement of protein synthesis for the storage of long-term memories (necessary for some spatial learning and memory tasks) and for long-lasting LTP (Bliss & Collingridge, 1993). If protein synthesis is inhibited, then memory storage is impaired (Davis & Squire, 1984) and LTP is dampened (Bliss & Collingridge, 1993). Thus, indirect evidence suggests that photoperiodic modulation of LTP may result in altered spatial learning and memory performance.

This study is the first to demonstrate hippocampal LTP in this species. The induced potentiation in the dentate gyrus following high-frequency stimulation observed in this study is comparable to that described with hormonal and other environmental manipulations in previous studies (Bampton, Gray, & Large, 1999; Pavlides et al., 2002; Wang, Suthana, Chaudhury, Weaver, & Colwell, 2005). Accurate electrode placement, however, required some deviation from the coordinates described in the domestic mouse (Mus musculus) atlas as determined in a pilot study. Facilitation of accurate electrode placement in new species by using hippocampal slices (instead of in vivo) is recommended for future studies.

The influence of environmental photoperiod on LTP has not previously been reported. Short-day mammals are exposed to longer nightly durations of melatonin secretion (due to longer night lengths) than long-day mammals. Melatonin receptors are located in the hippocampus of mammals (Reppert, 1997; Reppert et al., 1995) and
the location of the hippocampus relative to the ventricles suggests that changes in circulating melatonin concentration may readily influence the hippocampus (El-Sherif et al., 2003). In rodent hippocampal slices, melatonin infusion decreases the magnitude of high-frequency stimulated LTP (El-Sherif et al., 2003; Wang et al., 2005) by a non-NMDA-dependent mechanism (Collins & Davies, 1997; El-Sherif et al., 2003; Wang et al., 2005). Exposure to short days (increased melatonin durations) similarly reduced EPSP slope and amplitude following high-frequency stimulation in the present study. Therefore, melatonin may be a signal by which photoperiod may alter hippocampal electrophysiology.

LTP increases dendritic spine density on postsynaptic dendrites in the CA1 region of the hippocampus (Engert & Bonhoeffer, 1999). A similar relationship may exist between environmental-induced changes in LTP and hippocampal dendritic structure. For example, stressors reduce LTP and decrease spine density in the CA3 region (Pavlides et al., 2002). The reduced LTP observed in short-day mice also corresponds with decreased spine density in the CA1 region of the hippocampus (Chapter 3). Structural changes to hippocampal dendrites caused by photoperiod may modulate synaptic transmission involved in LTP.

In sum, this study suggests that short days decrease high-frequency induced LTP in the dentate gyrus of the hippocampus. The negative relationship between LTP and spatial learning and memory, hippocampal spine density, or exposure to melatonin that is suggested by previous studies is in agreement with the short-day provoked, albeit nonsignificant, dampened LTP observed in this study and results from previous chapters.
(Chapters 3, 4, and 6). Further investigation is necessary to elucidate the mechanisms by which photoperiod may alter hippocampal electrophysiology that mediate changes in spatial learning and memory.
Figure 5.1. Short days decrease testes mass relative to body mass.

* p < 0.05
Figure 5.2. Representative waveform and histology.

A) EPSP waveform from individual mouse with designation for EPSP slope and population spike amplitude. Schematic brain sections are modified and reprinted from the *Mouse brain atlas in stereotaxic coordinates* (Paxinos & Franklin, 2001), with permission from Elsevier. B) Representative recording and stimulating electrode placements in the dentate gyrus and perforant pathway, respectively, with histological examples of lesions magnified 100X. Measurement bar is the same for both histological examples.
Figure 5.3. Representative slopes of EPSPs as a percent of baseline from an individual long-day and short-day mouse before and after high frequency stimulation.
Figure 5.4. Photoperiod alters EPSP parameters following high frequency stimulation.

A) Mean percent change in EPSP slope between post-high frequency stimulation and baseline in long- and short-day mice.  B) Mean percent change in EPSP amplitude between post-high frequency stimulation and baseline in long- and short-day mice.
Sex differences in rodent spatial learning and memory favors males (Galea et al., 1996; Galea et al., 1995). These sex differences are usually specific to polygynous as compared to monogamous species, and are therefore hypothesized to benefit the territorial navigation and mate searching behaviors characteristic of polygynous males (Sherry et al., 1992). Gonadal hormones may mediate sex differences in learning and memory. Testosterone injections facilitate water maze performance in male rats (Vazquez-Pereyra, Rivas-Arancibia, Loaeza-Del Castillo, & Schneider-Rivas, 1995) and testosterone implants enhance spatial memory performance in castrated male zebra finches (Poephila gattata) (Oberlander et al., 2004). However, no differences in water maze learning performance are observed in male meadow voles (Microtus pennsylvanicus) with high versus low testosterone concentrations (Galea et al., 1995). In comparison with activational effects, stronger evidence for organizational effects of testosterone on adult spatial learning and
memory exists (Dawson, Cheung, & Lau, 1975; Goto et al., 2005; Isgor & Sengelaub, 1998). One example is that individual meadow voles (both male and female) from male-biased litters (i.e., high in utero testosterone exposure) perform better at the spatial water maze task than voles from female-biased litters (Galea, Ossenkopp, & Kavaliers, 1994b).

In addition to sex differences in spatial learning and memory, seasonal differences in learning and memory performance may also exist. Gray squirrels (Sciurus carolinensis) and black-capped chickadees (Parus atricapillus) display seasonal differences in spatially-dependent food-caching behavior (Smulders et al., 1995; Thompson & Thompson, 1980). In two murine species (Peromyscus maniculatus and P. leucopus), long-day males (in reproductive condition with high testosterone concentrations) perform better at a spatial water maze task and have larger hippocampi than short-day males (in non-reproductive condition with low testosterone concentrations) (Galea et al., 1994a; Perrot-Sinal et al., 1998; Chapter 3). Enhanced spatial learning and memory in breeding male rodents compared with non-breeding males is consistent with the hypothesis that spatial learning and memory is advantageous for territorial navigation and mate searching (Jacobs, 1996a). The possibility that seasonal changes in testosterone may mediate these seasonal differences in spatial learning and memory has not been directly tested.

If testosterone mediates the photoperiodic differences in spatial learning and memory, then castration should impair spatial learning and memory performance in long-day mice and testosterone replacement should enhance performance in short-day mice. It is also possible that differences in tissue sensitivity to testosterone, that is
differential steroid hormone receptor quantity, interact with circulating testosterone concentrations to affect learning and memory. To assess this possibility, we also tested whether photoperiod alters gene expression of sex steroid receptors within the hippocampus that mediates the putative photoperiod-provoked modulation of testosterone on learning and memory.

Materials and Methods

Animals. Seventy-one adult (>55 days of age) male white-footed mice (Peromyscus leucopus) from our breeding colony were used in these experiments. Siblings were pseudo-randomly distributed among all groups. Animals were housed individually in polypropylene cages (27.8 x 7.5 x 13 cm) with a constant temperature and humidity of 21 ± 5° C and 50 ± 5%, respectively, and ad libitum access to food (Harlan Teklad 8640 rodent diet, Indianapolis, IN, USA) and filtered tap water. Mice were either housed in reversed long days (LD; 16 h light/day; lights illuminated at 2300 h Eastern Standard Time [EST]), or in short days (SD; 8 h light/day; lights illuminated at 700 h).

Experiment 1: Effects of photoperiod and testosterone manipulation on spatial learning and memory. Forty-seven mice were used in this experiment. Mice from both photoperiod treatments were divided into three surgical groups: sham-castration (SHAM; LD: n = 9; SD: n = 7), bilateral castration plus saline implant (CAST; LD: n = 8; SD: n = 8), or bilateral castration plus testosterone propionate (Sigma-Aldrich, St. Louis, MO) implant (CAST + T; LD n = 7; SD n = 8). Implants were designed to mimic long-day-typical testosterone concentrations as
previously described (Demas et al., 1998). Mice were allowed approximately one week recovery prior to onset of photoperiod treatment. All mice were exposed to their respective photoperiod conditions for a total of 14 weeks; water maze training occurred during the final two weeks. After the termination of behavioral testing, mice were rapidly decapitated and castration was verified. For SHAM mice, testes were removed and weighed.

Water maze. The water maze procedures were identical to those described in Chapter 4.

Experiment 2: Effects of photoperiod on estrogen (ERα and ERβ) and androgen (AR) receptor gene expression in the hippocampus. Twenty-four mice were used in this experiment. All mice were exposed to their respective photoperiod conditions for either 7 (n = 6/photoperiod) or 14 (n = 6/photoperiod) weeks. At 7 or 14 weeks, mice were rapidly decapitated, brains were removed, and the hippocampus was dissected and stored in RNALater solution (Qiagen, Valencia, CA, USA) at -70° C until RNA processing.

RNA extraction, gene sequencing, and quantitative PCR. Total RNA was extracted from ≤ 30 mg of hippocampus of individual mice and cDNA as previously described in Chapter 1. AR, ERα, and ERβ were sequenced as previously described (Chapter 1) and primers and a probe were synthesized with the probe labeled with 6-FAM and MGB (non-fluorescent quencher) at the 5’ and 3’ ends, respectively: ERα forward 5’-GAACAGCCCCCGCCTTG-3’, ERα reverse 5’-GCATCCAGCAAGGACTGA-3’, ERβ probe 5’-TGACAGCTGACCAGATG-3’; ERβ forward 5’-GCTGATGTGGCGCTTG-3’, ERβ reverse 5’-
CCCTCATCCCTGTCCAGAAC-3’, \(ER\beta\) probe 5’-

ACCACCCCTGGCAAGCTCATCTTT-3’; \(AR\) forward 5’-

GTGGTGTGTGCTGGACATGAC-3’, \(AR\) reverse 5’-

GGCTAGATAACAGGGCAGCAA-3’, \(AR\) probe 5’-ACAACCAACCTGACTCC-3’. The primers and probe used for 18S Ribosomal RNA and qPCR conditions were previously described in Chapter 1.

**Statistical analyses.** Repeated measures ANOVAs were used to compare water maze performance over time. Within days, pairwise comparisons were planned *a priori* in the analysis models and were conducted using two-tailed student’s *t*-tests (Keppel & Wickens, 2004). Student’s *t*-tests were also used for other behavioral and physiological comparisons. Data with unequal variances were compared using nonparametric Mann-Whitney tests to compare photoperiod differences. All comparisons were considered statistically significant when \(p < 0.05\). StatView software was used for all analyses (v. 5.0.1, Cary, NC, USA).

**Results**

**Experiment 1.** Short days decreased testes mass (both relative and absolute) compared with long days in SHAM mice (absolute: LD, 377.5 ± 27.6 mg; SD, 167.8 ± 36.5 mg; relative: LD, 17.3 ± 0.9 mg/g body mass; SD, 8.6 ± 1.9 mg/g body mass) (\(p < 0.05\) in both cases).

The latency to reach the hidden platform and swim route length decreased over blocks of trials in all groups (Figure 6.1) (\(p < 0.05\)) whereas, swim speed did not change (data not shown) (\(p > 0.05\)). Post-hoc analyses revealed that in SHAM mice,
short days increased the latency to reach the hidden platform compared with long
days on blocks 3 and 5 of hidden platform training (Figure 6.1a) \( (p < 0.05) \). In long-
day mice, the latency to reach the hidden platform did not differ among surgical
treatments in any of the blocks of trials (Figure 6.1b) \( (p > 0.05) \). In short days,
however, CAST + T mice decreased the latency to reach the platform compared with
CAST mice on block 5, compared with SHAM mice on block 3, and compared with
both CAST and SHAM mice on block 7 (Figure 6.1c) \( (p < 0.05 \text{ in all cases}) \). No
significant differences in path length were observed in SHAM mice (Figure 6.1d) \( (p
> 0.05) \). In long days, CAST + T mice reduced the path length compared with SHAM
mice on block 4 (Figure 6.1e) \( (p < 0.05) \). Similarly, in short days, CAST + T mice
reduced the path length compared with CAST mice on blocks 5-8 (Figure 6.1f) \( (p <
0.05 \text{ in all cases}) \). Swim speed did not differ among any of the groups (data not
shown) \( (p > 0.05) \). Mice from all groups persisted in spending more time in the
quadrant from which the platform had been removed from the original hidden
platform trials (Quadrant 1) compared with other quadrants (data not shown) \( (p <
0.05 \text{ in all cases}) \). No differences in the percent time spent in Quadrant 1 were
observed among groups (data not shown) \( (p > 0.05) \).

Throughout reversal training to a hidden platform, latency to reach the hidden
platform decreased over blocks of trials in all groups (Figure 6.2a-c) \( (p < 0.05) \). In
SHAM mice, short-day mice took longer to reach the hidden platform on block 1 of
reversal training (Figure 6.2a) \( (p < 0.05) \). In long-day mice, testosterone
manipulations did not affect the latency to reach the platform (Figure 6.2b) \( (p >
0.05) \). However, in short-day mice, CAST + T decreased the latency to reach the
platform compared with CAST mice on block 2, compared with SHAM mice on block 1, and compared with both CAST and SHAM mice on blocks 3 and 4 (Figure 6.2c) (p < 0.05 in both cases). The path length to reach the platform decreased over blocks of reversal trials in all mice (Figure 6.2d-f) (p < 0.05). In SHAM mice and within long-day mice, no differences in path length were observed during reversal training (Figure 6.2d-e) (p > 0.05). However, in short day mice, CAST + T mice swam a more direct route to the platform compared with CAST mice on blocks 2 and 4 and compared with both CAST and SHAM mice on block 3 (Figure 6.2f). Swim speed did not differ among any of the groups (data not shown) (p > 0.05). No differences in the amount of time spent among pool quadrants following the reversal probe trial were observed (data not shown) (p > 0.05). Also, no differences in the latency to reach the visible platform during the visible platform trial were observed among groups (data not shown) (p > 0.05).

**Experiment 2.** AR mRNA expression was greater than ERα and ERβ in the hippocampus of all groups (p < 0.05) (Figure 6.3). However, no significant photoperiodic differences in AR, ERα, or ERβ expression were observed after 7 or 14 weeks of photoperiod (p > 0.05) (Figure 6.3).

**Discussion**

This study provides evidence for photoperiodic modulation of hormonal effects on behavior. Specifically, removal of long-day typical concentrations of testosterone did not alter spatial learning and memory performance in long-day mice, but testosterone supplementation in short-day mice significantly improved spatial
learning and memory. The photoperiodic differences in behavioral responses to testosterone were not manifested in the amount of androgen or estrogen receptor gene expression in the hippocampus. Thus, the mechanism by which the hippocampal function is more sensitive to testosterone in short-day mice is likely indirect.

Short-day CAST + T mice outperformed short-day SHAM and CAST mice in both original hidden platform learning trials and reversal learning trials. The water maze performance of short-day CAST + T mice was enhanced beyond the performance of long-day mice. Facilitation of learning and memory performance by testosterone has also been demonstrated in photoperiod-nonresponsive rodents and humans (Lessov-Schlaggar et al., 2005; Vazquez-Pereyra et al., 1995; but see Gouchie & Kimura, 1991). Castration of short-day mice did not alter spatial learning and memory. This finding was predictable because, similar to castration, gonadal regression in short days results in low to undetectable testosterone concentrations (Pyter et al., 2005a). Thus, in contrast to long days, short days appear to increase the sensitivity of spatial learning and memory brain circuitry to the effects of testosterone. Similarly, spatial learning and memory in female photoperiod-responsive rodents may be dependent upon photoperiodic modulation of estrogen (Galea et al., 1995).

Our results corroborate previous studies in which intact long-day male rodents outperform intact short-day rodents in spatial learning and memory tasks (Galea et al., 1995; Perrot-Sinal et al., 1998; Chapter 3). These differences in learning and memory performance in SHAM mice may be mediated by structural changes in hippocampal spine density (Chapter 3). Although previous studies in photoperiodic rodents have
attempted to correlate testosterone concentrations with learning and memory performance (Perrot-Sinal et al., 1998), this is the first study to directly test the effects of testosterone on photoperiod-induced learning and memory differences.

In contrast to the short-day mice, our results suggest that the activational effects of long-day typical concentrations of testosterone are not necessary for long-day typical spatial learning performance. Regardless of their testosterone manipulations, all long-day mice performed comparably to SHAM long-day mice. These results concur with those reported in a previous study in which variation in testosterone concentrations of long-day male voles did not affect water maze performance (Galea et al., 1995). The unaffected learning performance in long-day mice that were castrated with testosterone replacement in the present study was predictable given that the testosterone treatment was designed to attain testosterone concentrations comparable to intact long-day mice (Demas & Nelson, 1998). In sum, long photoperiods appear to enhance spatial learning and memory performance compared with short photoperiods regardless of circulating testosterone concentrations.

I also tested whether the potential mechanism by which testosterone sensitivity may differ between photoperiods and affects spatial learning relates to the expression of androgen or estrogen receptors in the hippocampus. In addition to directly binding to androgen receptors, androgens can be aromatized to estrogens within the brain and thereby affect brain function (i.e., behavior) via estrogen receptors (Roselli & Resko, 1993), although in adult male mammals hippocampal aromatase activity is typically low (Roselli & Resko, 1993). Androgen and estrogen
receptors are located within the rodent hippocampus and entorhinal cortex (Loy, Gerlach, & McEwen, 1988; Xiao & Jordan, 2002). Testosterone antagonists administered directly into the hippocampus impair spatial learning and memory in rats (Naghdi, Nafisy, & Majlessi, 2001) and androgen insensitive rats display impaired spatial learning and memory (Jones & Watson, 2005). In the present study, however, no differences in androgen or either subtype of estrogen receptor gene expression was detected in the hippocampus. However, comparison of hippocampal samples from mice that have undergone photoperiod and testosterone manipulation are necessary to determine whether photoperiod and testosterone treatment interacted to affect hippocampal steroid receptor expression. Previous studies that have examined photoperiodic regulation of AR, ERα, or ERβ (excluding studies prior to technical differentiation between ERα and ERβ) in the brain did not examine the hippocampus (Mangels, Powers, & Blaustein, 1998; Tetel, Ungar, Hassan, & Bittman, 2004; Trainor & Nelson, 2005). However, photoperiod and testosterone treatment interact to affect steroid receptor expression in regions of the brain outside of the hippocampus (Bittman, Ehrlich, Ogdahl, & Jetton, 2003; Wood & Newman, 1993). Although I detected no differences in steroid receptor mRNA expression, photoperiod could affect local steroid production in the hippocampus (Mukai et al., 2005). Thus, it is possible that photoperiod directly affects hippocampal hormone production leading to differences in hippocampal function (i.e., learning and memory). It is also
possible that the differential interaction between testosterone and photoperiod on hippocampal function described in the present study is not mediated directly via the hippocampus.

Testosterone inhibits release of gonadotropin releasing hormone (GnRH) at the level of the hypothalamus and gonadotropins at the level of the pituitary. In photoperiod-responsive rodents, short days increase hypothalamic sensitivity to testosterone negative feedback of the hypothalamic-pituitary-gonadal (HPG) axis, whereas long days do not (Ellis & Turek, 1979; Ellis & Turek, 1980; Turek, 1977). The increased sensitivity to HPG negative feedback allows the reduced short-day testosterone concentrations to suppress GnRH and gonadotropin release. This increase in testosterone sensitivity in the HPG axis of short-day mice may indirectly mediate the observed differences in hippocampal function in the present study. For example, increased hypothalamic sensitivity to testosterone that is specific to short-day brains may affect synaptic communication to the hippocampus via the fimbria and thereby modulate hippocampal function (i.e. learning performance) described in the present study. In support of this hypothesis, lesions or electrical stimulation of the fimbrial input to the hippocampus alters learning and memory behavior in rodents (Jarrard, Okaichi, Steward, & Goldschmidt, 1984; Weiler et al., 1998).

In sum, short days appear to increase the sensitivity of hippocampal function (i.e., spatial learning and memory) to testosterone. These changes are not due to photoperiodic differences in hippocampal steroid receptor expression. In the field, winter mice may not typically be exposed to testosterone concentrations characteristic
of long-day mice (but see) (Prendergast et al., 2001). However, the increased testosterone sensitivity of the short-day hypothalamus may result in the observed enhanced spatial learning performance because of hypothalamic-hippocampal communication. Finally, our study also suggests that seasonal regulation of hormone concentrations may impact cognitive functions.
Figure 6.1. Photoperiod and testosterone affect spatial learning and memory (via water maze).

Latency to reach the original hidden platform in SHAM (A), long-day (B), and short-day (C) mice. Path length swam to reach the original hidden platform in SHAM (D), long-day (E), short-day (F) mice. Each block = 3 trials.
Figure 6.2. Photoperiod and testosterone affect reversal spatial learning and memory (via water maze).

Latency to reach the reversed hidden platform in SHAM (A), long-day (B), and short-day (C) mice. Path length swam to reach the reversed hidden platform in SHAM (D), long-day (E), short-day (F) mice. Each block = 3 trials.
Figure 6.3. Effects of 7 or 14 weeks of photoperiod on relative androgen receptor (AR), estrogen receptor α (ERα), and estrogen receptor β (ERβ) gene expression in the hippocampus.
Short days reduce brain size, impair hippocampal-based spatial learning and memory, reduce hippocampal volume, and alter hippocampal spine density in adult male white-footed mice (*Peromyscus leucopus*) (Chapter 5). Impaired spatial performance and reduced brain tissue outside of the breeding season, when home range size decreases and mate seeking behaviors diminish in rodents, may conserve energy in the winter and promote survival similar to winter suppression of breeding (Jacobs, 1996a). The mechanisms underlying seasonal plasticity of brain and behavior remain unspecified. Because spatial learning and memory performance and adult hippocampal plasticity is influenced by steroid hormones including glucocorticoids (Breedlove & Jordan, 2001; Lathe, 2001; McEwen, 1994), I hypothesized that photoperiod-evoked changes in glucocorticoid regulation and stress responses underlie seasonal plasticity in brain and learning and memory performance.

The hypothalamic-pituitary-adrenal (HPA) feedback loop involves glucocorticoids produced in the adrenal cortex which feed back on the hypothalamus...
and pituitary to inhibit corticotropin releasing hormone (CRH) and
adrenocorticotropic hormone (ACTH), respectively; glucocorticoids also bind to receptors in
the hippocampus which suppresses the HPA axis (Jacobson & Sapolsky, 1991). The
HPA axis regulates energy availability within the body by continuously readjusting
mobilized energy levels following disruptions to homeostasis (i.e., allostasis) and is
often associated with responses to stressors (McEwen, 2000). This fragile balance is
challenged vigorously by seasonally-changing environmental conditions (i.e.,
seasonal fluctuations in temperature, food availability, agnostic interactions, etc.)
(Prendergast et al., 2001). However, seasonal regulation of the HPA axis and
response to stressors are poorly understood. I report here that short days increase
corticosterone responses to various stressors, alter negative feedback regulation of the
HPA axis via differential glucocorticoid receptor expression in the brain and
dexamethasone suppression of corticosterone, and modify the effects of
corticosterone and stressors on spatial learning and memory.

Materials and Methods

Animals. One hundred twenty-nine (>55 days of age) male white-footed
mice (Peromyscus leucopus) from our breeding colony were used in these
experiments. Animals were housed individually in polypropylene cages (27.8 x 7.5 x
13 cm) with a constant temperature and humidity of 21 ± 5° C and 50 ± 10%,
respectively, and ad libitum access to food (Harlan Teklad 8640 rodent diet,
Indianapolis, IN) and filtered tap water. Mice were either housed in reversed long
days (16 h light/day; lights illuminated at 2300 Eastern Standard Time [EST]), or in
short days (8 h light/day; lights illuminated at 700 h EST). Siblings were pseudo-randomly distributed among all groups. The reproductive system of one mouse from Experiment 3 did not respond to short days and was removed from the study.

Experiment 1: Effects of photoperiod on corticosterone responses to stressors. **Restraint stressor.** Thirty-eight mice were used in this experiment. All mice were exposed to their respective photoperiod conditions for 12 weeks before testing. I operationally define the acute restraint procedures used in this experiment as a physical and psychological stressor based on the subsequent elevation of corticosterone concentrations (Glavin, Pare, Sandbak, Bakke, & Murison, 1994). Mice from each photoperiod were further divided into 3 subgroups: 1) 1 h of restraint followed by an immediate blood collection (long-day: n = 6, short-day: n = 7), 2) 1 h of restraint with a delayed blood collection (long-day: n = 6, short-day: n = 7), or 3) no restraint (long-day: n = 6, short-day: n = 6). Restrained mice were placed in well-ventilated, Plexiglas tubes without compression for 1 h (0900 – 1000 h EST). Immediately before these treatments, a 50 µl retro-orbital baseline blood sample was collected from all mice (0800 – 0900 h EST). Post-treatment trunk blood was collected after rapid decapitation. Mice with delayed blood collections were returned to their home cage for 1 h following restraint before trunk blood was collected. Unrestrained mice remained undisturbed in their home cage while the other mice were restrained and trunk blood was collected at the same time as the immediate group. Body mass was recorded and paired testes were removed and weighed for all
mice to determine reproductive responsiveness to photoperiod treatment. All blood samples were stored on ice until centrifuged at 3,000 rpm for 30 min at 4°C. Plasma was removed from the samples and stored at -70°C until hormone analyses.

*Water maze stressor.* Thirty-seven mice were used in this experiment. All mice were exposed to their respective photoperiod conditions for a total of 14 weeks; water maze testing occurred during the final two weeks. Body mass was recorded bi-weekly. Mice from each photoperiod were further divided into two treatments: 1) 200 mg/kg metyrapone (long-day: n = 9, short-day: n = 11) or 2) saline injections (long-day: n = 8, short-day: n = 9) throughout water maze testing. Metyrapone (2-methyl-1,2-di(3-pyridyl)-1-propanone; Sigma-Aldrich, St. Louis, MO) is a corticosterone synthesis inhibitor. Injections were administered i.p. once daily 20 min prior to the onset of water maze testing (in 0.1 - 0.2 ml saline) beginning on the first day of training. Three days prior to water maze, a 50 µl retro-orbital blood sample was collected (between 1400 – 1430 h EST) from each mouse to compare basal corticosterone concentrations. Additional 50µl retro-orbital blood samples were collected (between 1400 – 1430 h EST) after the 60-sec free swim and both 60-sec probe trials (see description of water maze below). Samples were collected after these trials because all mice swam in the water maze for the same duration regardless of treatment (60 sec). Testing and treatments were divided into two groups of mice representing all experimental treatments and were staggered by 1 week. Blood samples were collected and stored as previously described.

*Injection stressor.* Mice used in this experiment were described previously for the water maze stressor experiment. To determine corticosterone responses to an
injection alone, mice received a single i.p. injection of 0.1ml saline and blood samples were taken 1 and 2 h post-injection. Half of the mice representing all experimental treatments received the saline injection 2 d prior to all water maze testing and drug injections to measure the “naïve” corticosterone response to an injection. The remaining mice received the saline injection 1 d following the completion of water maze testing and injection treatment (“experienced”) to measure the potential habituation of corticosterone responses to injection. Blood samples were collected and stored as previously described.

**Experiment 2: Effects of photoperiod on corticosterone negative feedback.** Corticosterone receptor (glucocorticoid [GR] and mineralocorticoid [MR]) mRNA expression in the brain. Twenty-four mice were used for this experiment. All mice were exposed to their respective photoperiod conditions for either 7 (n = 6/photoperiod) or 14 (n = 6/photoperiod) weeks. Body mass was recorded bi-weekly. At 7 or 14 weeks, mice were rapidly decapitated, brains were removed, and the hippocampus and hypothalamus were dissected out and stored in RNALater solution (Qiagen, Valencia, CA) at -70°C until RNA processing. These regions of the brain are involved in the feedback loop that regulates corticosterone release (McEwen, 2000).

**RNA extraction, gene sequencing, and qPCR.** Total RNA was extracted from ≤30 mg of hypothalami and hippocampi of individual mice and cDNA as previously described in Chapter 1. MR and GR were sequenced as previously described (Chapter 1) and primers and a probe were synthesized with the probe labeled with 6-FAM and MGB (non-fluorescent quencher) at the 5’ and 3’ ends, respectively: GR
forward 5’-CCCAAGTGAAAACAGAAAAAGATGA-3’, \textit{GR} reverse 5’-
GCCCAGTTTCTCTTGCTTAATTACC-3’, \textit{GR} probe 5’-
TTCATTGAACTTTGCACCC-3’; \textit{MR} forward 5’-GGCTGTGTTTCCGGTGCTA-
3’, \textit{MR} reverse 5’-TTCGTGTTTTTCTTGTGCTTT-3’, \textit{MR} probe 5’-
TCCAAACAAACAGTACTCAAG-3’. The primers and probe used for 18S
Ribosomal RNA and qPCR conditions were previously described in Chapter 1.

\textit{Dexamethasone suppression of corticosterone.} Thirty mice were used in this
experiment to characterize corticosterone negative feedback with the dexamethasone
suppression test (Oxenkrug, McIntyre, Stanley, & Gershon, 1984). A stock solution
(5 mg/ml) of dexamethasone crystalline (Sigma-Aldrich, St. Louis, MO) was created
by mixing 25 mg/ml in 100% EtOH followed by dilution in saline. Based on a pilot
study, a 1.5 ug/kg body mass injection (in 0.08 - 0.13 ml volumes) of dexamethasone
(long-day: \(n = 10\), short-day: \(n = 5\)) or saline (long-day: \(n = 10\), short-day: \(n = 5\)) was
administered i.p. 6 h prior to retro-orbital blood sampling (1415 – 1445 h EST).
Blood samples were collected and stored as previously described.

\textbf{Experiment 3: Effects of corticosterone and photoperiod on spatial
learning and memory performance.} Mice used in this experiment were described
previously for the water maze stressor experiment. After the termination of water
maze, mice were rapidly decapitated, trunk blood was collected on ice, and testes and
epididymal fat pads were removed and weighed.

\textit{Water maze.} The water maze procedures used in this study are identical to
those described in Chapter 6.
Radioimmunoassays. Plasma corticosterone concentrations were determined using a single $^{125}$I kit purchased from ICN Biomedicals (Costa Mesa, CA) for each experiment. Each sample was assessed in duplicate according to the manufacturer’s protocol with two exceptions. Because corticosterone concentrations in *Peromyscus* are elevated relative to *Mus musculus* and *Rattus rattus*, serum was diluted 5.2-fold more than recommended for domestic rodents and two additional standard dilutions were added to the low end of the standard curve (5 and 10 ng/ml). Cross-reactivity with other steroid hormones was < 0.5%. Intraassay and interassay variances were < 10% with a minimum detection level of 5 ng/ml.

Statistical analyses. Repeated measures ANOVAs were used to compare water maze performance over time. Within days, pairwise comparisons were planned *a priori* in the analysis models and were conducted using two-tailed student’s $t$-tests (Keppel & Wickens, 2004). ANOVAs or student’s $t$-tests were used for physiological comparisons depending on the number of groups. Data with unequal variances were compared using nonparametric Mann-Whitney tests to compare photoperiod differences. All comparisons were considered statistically significant when $p < 0.05$. StatView software was used for all analyses (v. 5.0.1, Cary, NC).

Results

Experiment 1. Restraint stressor. Twelve weeks of short days reduced mean ($±$ SEM) relative and absolute testes masses (absolute: long-day, 336.1 ± 23.8 mg;
short-day, 180.4 ± 24.6 mg; relative: long-day, 16.7 ± 1.7 mg/g body mass; short-day, 9.1 ± 1.2 mg/g body mass) (p < 0.001 in both cases). Body mass did not differ between photoperiods after 12 weeks (p > 0.05).

Corticosterone responses in unrestrained mice did not differ between photoperiods (p > 0.05; Figure 7.1a). Immediately following 1 h restraint, corticosterone concentrations increased in both short- and long-day mice (p < 0.001; Figure 7.1a), although the increase in short-day mice relative to unrestrained controls was greater than in long-day mice (p < 0.05; Figure 7.1a). Corticosterone concentrations remained elevated in long-day mice 1 h after restraint, but decreased significantly in short-day mice (p < 0.005) to concentrations comparable to those of unrestrained mice. The same statistical differences were achieved following normalization of corticosterone responses to those of unrestrained controls within the same photoperiod.

Water maze stressor. Fourteen weeks of short days reduced relative and absolute testes (absolute: long-day, 383.6 ± 29.0 mg; short-day, 112.7 ± 8.7 mg; relative: long-day, 17.8 ± 1.1 mg/g body mass; short-day, 6.2 ± 0.4 mg/g body mass) and epididymal fat pad (absolute: long-day, 357.3 ± 39.3 mg; short-day, 139.2 ± 16.5 mg; relative: long-day, 16.3 ± 1.5 mg/g body mass; short-day, 7.6 ± 0.8 mg/g body mass) masses relative to long days (p < 0.001 in all cases). Twelve and 14 weeks of short days also significantly reduced body mass compared with long days (12 weeks: long-day, 20.3 ± 0.6 g; short-day, 18.2 ± 0.5 g; 14 weeks: long-day, 21.3 ± 0.6; short-day, 18.1 ± 0.4) (p < 0.05 in both cases).
Short days slightly increased baseline corticosterone concentrations relative to long days 30 min before lights-off (data not shown) (p < 0.02). Following the initial exposure to water maze (60-sec free swim) corticosterone concentrations were increased in short-day mice (p < 0.002), but not long-day mice (Figure 7.1b) (p > 0.05). The elevation in short-day corticosterone concentrations in response to a 60-sec probe trial mid-water maze was attenuated to concentrations comparable to baseline and long-day mice (Figure 7.1b). Similarly, no differences in corticosterone responses to a 60-sec probe trial at the end of water maze training were observed between photoperiods or compared with baseline concentrations (Figure 7.1b) (p > 0.05). Metyrapone significantly decreased corticosterone concentrations in all mice in the beginning (free swim), middle (probe 1), and end (probe 2) of water maze training compared with baseline and saline-treated control concentrations (Figure 7.1b) (p < 0.001). The differences in corticosterone concentrations observed during the water maze testing remained significant following normalization of all values to baseline concentrations.

Injection stressor. One h after a saline injection, short-day mice displayed an elevated corticosterone response compared with long-day mice before exposure to water maze and drug injection procedures (Figure 7.1c) (p < 0.03). Saline injection did not elicit a prolonged elevation in corticosterone response 1 h later in mice from either photoperiod that had completed the water maze training and daily drug injections (Figure 7.1c) (p > 0.05). Corticosterone concentrations increased 2 h after a saline injection compared with 1 h (p < 0.05); post-hoc analyses revealed that this increase was largely due to the pre-water maze groups (Figure 7.1c) (p < 0.03).
However, the short-day increase in corticosterone concentrations 2 h-post saline injection was not significantly greater than the long-day increase (p > 0.05). Short-day mice that had completed the water maze training displayed a greater corticosterone elevation compared with long-day mice 2 h post-saline injections (Figure 7.1c) (p < 0.02).

**Experiment 2. Glucocorticoid receptor gene expression.** The mice used for this experiment were a subset of mice used in a previous study that reported significant decreases in reproductive tissue masses following 7 and 14 weeks of short days and a decrease in body mass following 14 weeks of short days (Chapter 1).

*GR* or *MR* gene expression in the hippocampus did not differ between photoperiod after 7 or 14 weeks (Figure 7.2a,b) (p > 0.05 in all cases). However, both *MR* and *GR* expression increased between 7 and 14 weeks in short-day hippocampi (Figure 7.2a,b) (p < 0.003 in both cases). In the hypothalamus, time spent in either photoperiod treatment reduced *GR* gene expression (Figure 7.2a) (p < 0.003 in both cases), although photoperiodic differences were not significant within individual weeks (p > 0.05). Short days significantly reduced hypothalamic *MR* gene expression relative to long days after 14 weeks (p < 0.05) and time spent in photoperiod treatment increased hypothalamic *MR* expression (Figure 7.2b) (p < 0.001).

*Dexamethasone suppression test.* Dexamethasone significantly reduced corticosterone concentrations in short-day (Figure 7.2c) (p < 0.03), but not long-day mice (p > 0.05). Saline-treated long- and short-day mice did not differ in corticosterone concentrations (Figure 7.2c) (p > 0.05).
Experiment 3. The latency to reach the hidden platform decreased over blocks of trials in all groups (Figure 7.3a) (p < 0.001). Corticosterone synthesis inhibition increased the latency to reach the hidden platform compared to saline-injected controls (Figure 7.3a) (p < 0.04). Post-hoc analysis revealed that short days reduced the latency to reach the hidden platform compared with long days in metyrapone-treated mice on block 7 (Figure 7.3a) (p < 0.05). The path length to reach the hidden platform decreased over blocks of trials in all groups (data not shown) (p < 0.001). Short days also reduced the path length over blocks of trials (p < 0.03), whereas metyrapone increased the path length compared with saline treatment (data not shown) (p < 0.03). Swim speed increased over blocks of trials (p < 0.001), but metyrapone decreased swim speed relative to saline-treatment (data not shown) (p < 0.03). Mice from all groups spent more time in the quadrant of the pool from which the platform had been removed (Quadrant 1) during the first memory probe trial compared with all other quadrants (data not shown) (p < 0.01). Although metyrapone decreased the time spent in quadrant one, this difference was not statistically significant (p = 0.2).

Throughout reversal training to a hidden platform, latency to reach the hidden platform decreased over blocks of trials in all groups (Figure 7.3b) (p < 0.002). Metyrapone significantly increased the latency to reach the platform compared to saline treatment (Figure 7.3b) (p < 0.05). Similarly, the path length to reach the platform decreased over blocks of trials and metyrapone increased the path length (data not shown) (p < 0.05 in both cases). Swim speed was not significantly affected by metyrapone (p = 0.07), however, post-hoc analyses revealed that metyrapone
decreased swim speed on blocks 1 and 3 of reversal training (data not shown) (p < 0.05 in both cases). Mice persisted in spending more time in the quadrant from which the platform had been removed from the original hidden platform trials (Quadrant 1) (data not shown) (p < 0.05), and did not spend more time in the quadrant from which the platform had been removed during the more recent reversal training (Quadrant 3) (data not shown) (p > 0.05). However, saline-treated mice in long days spent less time in Quadrant 1 and more time in Quadrant 3 than metyrapone-treated mice in long days, although the interaction between time spent in these quadrants and drug treatment was not statistically significant (data not shown) (p = 0.1). No differences in latency or path length to reach the visible platform were observed among groups (data not shown) (p > 0.05 in all cases), although metyrapone reduced swim speed (p < 0.05).

Discussion

These experiments demonstrate a link between photoperiod, stress responsivity, brain plasticity, and behavior. These results have physiological, behavioral, ecological, and clinical relevance. Specifically, short days increased HPA responsivity to various stressors (restraint, water maze, injection), as well as increased sensitivity to HPA negative feedback illustrated by increased GR and MR expression in the hippocampus, faster return to basal corticosterone concentrations following restraint, and increased sensitivity to dexamethasone suppression of corticosterone. These short-day alterations in HPA regulation had functional consequences with respect to spatial learning and memory performance. Short days alone impair spatial
learning and memory (Chapters 3 and 6) possibly due to the increased glucocorticoid response to water maze training in short-day mice observed in the present study. However, additional stressors, such as repeated injections complicated the effects of glucocorticoids on water maze performance such that daily saline injection stressors improved learning in short-day mice. In contrast, inhibition of corticosterone synthesis impaired spatial learning in all mice. Overall, these results suggest that photoperiod-evoked modification of the HPA axis and brain receptivity and their behavioral consequences may be adaptive for winter survival.

Physiologically, short days promoted a rapid, more efficient HPA feedback system by increased sensitivity of the HPA feedback loop demonstrated by increased glucocorticoid receptor expression in the brain, increased dexamethasone suppression, greater corticosterone responses to stressors, and a faster return to baseline corticosterone concentrations following a stressor. Baseline glucocorticoid concentrations display a seasonal pattern in many vertebrates, although mammals are the least studied taxa in this regard (Romero, 2002). Seasonal regulation of baseline glucocorticoids suggests that seasonal regulation of the HPA axis in response to stressors may also exist. Indeed, the speed of return to basal cortisol concentrations following restraint stress in this study corroborates previous findings in short-day Syrian hamsters (*Mesocricetus auratus*; another photoperiod-responsive species) following an anesthetic stressor (Ronchi, Spencer, Krey, & McEwen, 1998). Short-day Siberian hamsters (*Phodopus sungorus*) display greater cortisol responses to restraint compared with long-day hamsters (Bilbo et al., 2002a), although these differences were not normalized to elevated basal cortisol concentrations. Rodents
living in arctic conditions year-round display greater sensitivity and efficiency of HPA negative feedback even during the breeding season compared with non-arctic confamilials (Boonstra & McColl, 2000).

Because exogenous glucocorticoids trigger the HPA negative feedback loop and dampen endogenous glucocorticoid production, the dexamethasone suppression test indicates relative sensitivity of the HPA negative feedback loop (Oxenkrug et al., 1984). In this study, corticosterone suppression in short-, but not in long-day mice, was observed following a low dose of dexamethasone corrected for body mass. These results suggest that the negative feedback loop upstream of adrenal corticosterone production is enhanced in short-day mice. Seasonal changes in dexamethasone suppression have also been reported in horses (Equus sp.) (Donaldson et al., 2005). Based on the increase in short-day hippocampal glucocorticoid receptor gene expression, it is likely that the more sensitive negative feedback loop observed in short-day mice is due to increased glucocorticoid receptors in the brain regions that regulate the HPA axis.

Photoperiod-induced changes in glucocorticoid receptor gene expression in the brain provide further evidence of seasonal plasticity in adult mammalian brains. The present findings support a previous study in which short days increased hippocampal MR mRNA expression in Syrian hamsters (Lance, Miller, Holtsclaw, & Turner, 1998) that was associated with increased cortisol binding to MR in the hippocampus and hypothalamus and rapid cortisol recovery following an anesthetic stressor (Ronchi et al., 1998). The functional or adaptive significance of these data was not proposed or tested. In the present study, altered HPA feedback is associated
with changes in hippocampal-based learning. Exposure to 14 weeks of short days (time frame necessary to induce gonadal regression in this species) increased hippocampal GR and hippocampal and hypothalamic MR expression and may be the mechanism by which short-day mice display more sensitive HPA negative feedback than long-day mice (Jacobson & Sapolsky, 1991). MR has a higher affinity for corticosterone, however, GR is thought to mediate stress effects on the brain (de Kloet, 1991). Therefore, the short-day increase in hippocampal GR expression specifically may mediate the proposed stress-induced changes in spatial learning. Membrane and intracellular corticosteroid receptors in the brains of white-crowned sparrows also vary by season (Breuner & Orchinik, 2001), although the specific regions within the brain in which these changes occur is unknown. The changes in glucocorticoid gene expression in long-day hypothalami from 7 to 14 weeks were unexpected because of the absence of other phenotypic changes. It is possible that these slight changes were due to circadian differences in gene expression based on the time of day of tissue collection (Herman, Watson, Chao, Coirini, & McEwen, 1993) or an affect of aging. The former is unlikely because all tissue samples were collected within 4 h of each other. In general, the increased HPA feedback sensitivity described in short-day mice in the present study is comparable to the increased HPG feedback sensitivity described in short-day rodents (Ellis & Turek, 1979; Ellis & Turek, 1980; Turek, 1977). Also, similar to the influence of melatonin on the photoperiodic regulation of the HPG axis (Sisk & Turek, 1982), the described photoperiodic changes in the HPA axis is likely via melatonin.
On a behavioral level of analysis, previous studies demonstrated that short-
day rodents display impaired spatial learning and memory compared with long-day
rodents (Perrot-Sinal et al., 1998; Chapter 5). The present study suggests that
regulation of the stress response (via the HPA axis) may be the means by which
photoperiod affects behavior. The water maze task itself is considered to be a
relatively stressful behavioral test (Crawley, 2000). In the present study, all mice
were subjected to daily injections (either metyrapone or saline) prior to daily water
maze testing. Therefore, it is likely that the effects of the injection stressor (and the
photoperiodic differences in corticosterone response to injections) before each block
of behavioral training masked the photoperiodic differences in water maze
performance in the saline-treated mice. In contrast, widespread corticosterone
synthesis inhibition impaired both original and reversal learning performance in all
mice. A similar dichotomy of the effects of stressors or glucocorticoids on learning
and memory has been described (Wolf, 2003). Repeated stressors (e.g., repeated
injections) impair whereas, acute stressors enhance learning and memory
performance (Shors, 2004). In this study it is difficult to determine whether 10 days
of water maze training with or without daily injection exposure is more comparable to
an acute or chronic stressor paradigm. Decreased swim speed in metyrapone-treated
mice during some of the blocks of training may have increased the latency to reach
the platform on some of the blocks of trials, however, should not have affected the
comparable differences observed via path length. Based on the corticosterone
measurements throughout water maze training, it appears that unmanipulated short-
day corticosterone responses to water maze training are responsible for impaired
short-day learning, whereas the unmanipulated long-day corticosterone responses to water maze promote spatial learning (Chapters 3 and 6). The greatest spatial learning performances are displayed in either unmanipulated long-day mice or saline-injected short-day mice. Thus, optimal corticosterone exposure for enhanced spatial performance differs between long- and short-day mice. This is not unexpected however, given the photoperiodic differences in HPA regulation and receptivity observed in the other experiments.

Ecologically, the HPA axis is an important physiological system that mediates both adaptive and maladaptive responses in nature (McEwen, 2000). For example, acutely elevated glucocorticoids mobilize emergency energy necessary for survival from stressors (e.g., predators), whereas continuously elevated glucocorticoids impair brain and immune function and consume extensive energy reserves that are crucial for winter survival (Korte, Koolhaas, Wingfield, & McEwen, 2005; McEwen, 2000). Therefore, photoperiodic regulation of the HPA axis may help to modulate the balance between beneficial and detrimental effects of Glucocorticoids depending on the season. The numerous differences between long- and short-day HPA responses to stressors, the photoperiod-induced brain plasticity, and the effects of corticosterone on spatial learning reported in this study suggest that an altered HPA feedback axis is important for seasonal adaptation (Romero, 2002). Because the HPA axis modifies numerous other behaviors and physiological processes (McEwen, 2005; Nelson et al., 2002; Proulx & Seeley, 2005; Roozendaal, 2000), photoperiodic regulation of the HPA axis may underlie many other physiological and behavioral adjustments reported in seasonality research (Romero, 2002). Studies in wild populations are
necessary to test the functional consequences (including, but not limited to, spatial learning and memory) of a seasonally-changing HPA axis. It is possible that a more efficient HPA response represented by the initial increased corticosterone response to stressors in short-day mice followed by a faster return to baseline concentrations represents a coping strategy evolved specifically for winter stressors (Korte et al., 2005). Additionally, seasonal changes in cortisol concentrations, tissue sensitivity to glucocorticoids (Walker, Best, Noon, Watt, & Webb, 1997), and dexamethasone suppression have been reported in normal and seasonally depressed humans (Levitan, Vaccarino, Brown, & Kennedy, 2002) suggesting that mechanisms underlying seasonal regulation of the HPA may be applicable to treatment of seasonal cognitive and affective disorders.
Figure 7.1. Photoperiod influences plasma corticosterone responses to stressors.

(a) Corticosterone concentrations immediately and 1 h after restraint or no restraint. Differences in letters represent differences of p < 0.05. (b) Water maze-induced corticosterone concentrations at beginning (free swim), middle (probe 1), and end (probe 2) of training in mice injected with metyrapone (Met) or saline prior to testing. * p < 0.05 between injection treatments within photoperiod; # p < 0.05 between photoperiods of saline-treated mice. (c) Saline injection-induced corticosterone concentrations 1 and 2 h post-injection in mice naïve (Pre-MWM) and experienced (Post-MWM) to water maze training. * p < 0.05
Figure 7.2. Photoperiod affects HPA axis negative feedback loop.

(A) Relative glucocorticoid receptor (GR) gene expression in the hippocampus and hypothalamus after 7 and 14 weeks of photoperiod. (B) Relative mineralocorticoid receptor (MR) gene expression in the hippocampus and hypothalamus after 7 and 14 weeks of photoperiod. (C) Corticosterone concentrations 6 h following dexamethasone (1.5 µg/kg) or saline injection. * p < 0.05.
Figure 7.3. Photoperiod and corticosterone affect spatial learning and memory (via water maze).

(A) Latency to reach the original hidden platform in metyrapone (Met) or saline-injected mice. (B) Latency to reach the reversal hidden platform. Each block = 3 trials.
CHAPTER 8

PHOTOPERIOD, OLFACTION, AND OLFACTORY BULB PLASTICITY

Non-pathological adult brain plasticity is primarily limited to the hippocampus and olfactory bulbs. In adult mammals, changes in olfactory bulb cell proliferation and neurogenesis and spine density in the piriform (olfactory) cortex have been reported (Knafo, Grossman, Barkai, & Benshalom, 2001; Lemasson, Saghatelyan, Olivo-Marín, & Lledo, 2005; Winner, Cooper-Kuhn, Aigner, Winkler, & Kuhn, 2002). This plasticity is modulated by environmental influences such as social cues (e.g., sexually receptive conspecifics) (Fowler et al., 2002; Huang & Bittman, 2002), olfactory enrichment (Doving & Pinching, 1973; Rehn, Breipohl, Mendoza, & Apfelbach, 1986), and olfactory learning (Knafo et al., 2001), but not physical environmental enrichment (Brown et al., 2003). In general, olfactory stimulation or learning is associated with increased neurogenesis and spine density (Fowler et al., 2002; Knafo et al., 2001), whereas olfactory deprivation is associated with cell death (Mandairon, Jourdan, & Didier, 2003).

Social information is often communicated among individual mammals via pheromones that are perceived via the vomeronasal organ within the olfactory system.
(Buck, 2004) and therefore, olfaction is tightly linked to reproduction and other social behaviors (Rekwot, Ogwu, Oyedipe, & Sekoni, 2001). Additionally, intact olfactory bulbs appear to be important for appropriate physiological and morphological modifications in response to changes in season or photoperiod (Clancy, Goldman, Bartke, & Macrides, 1986; Pieper, Tang, Lipski, Subramanian, & Newman, 1984; Seguy & Perret, 2005). Thus, I hypothesized that photoperiodic control of reproduction displayed by white-footed mice (*Peromyscus leucopus*) (Whitaker, 1940) is associated with photoperiodic regulation of olfaction and olfactory bulb plasticity. In support of this hypothesis, European starlings (*Sturnus vulgaris*) display greater olfactory sensitivity during the breeding season compared with the non-breeding season (Clark & Smeraski, 1990). In meadow voles (*Microtus pennsylvaniaicus*) and mole rats (*Spalax ehrenbergi*), photoperiod influences responses to predator odor and olfactory preferences (Ferkin & Gorman, 1992; Heth, Nevo, & Todrank, 1996; Perrot-Sinal, Ossenkopp, & Kavaliers, 2000). Finally, olfactory discrimination in humans varies seasonally (Goel & Grasso, 2004) and humans afflicted with seasonal affective disorder display lower odor detection thresholds than control subjects (Postolache et al., 2002). Previous chapters have described significant effects of photoperiod on adult hippocampal plasticity and learning and memory (Chapters 2-7), and the present experiments were designed to test the influence of photoperiod on olfaction and similar types of adult brain plasticity in the olfactory bulbs.
Materials and Methods

**Animals.** One-hundred sixteen adult (>55 days of age) male white-footed mice (*Peromyscus leucopus*) from our breeding colony were used in these experiments. Animals were housed individually in polypropylene cages (27.8 x 7.5 x 13 cm) with a constant temperature and humidity of 21 ± 5° C and 50 ± 10%, respectively, and *ad libitum* access to food (Harlan Teklad 8640 rodent diet, Indianapolis, IN) and filtered tap water. Mice were either housed in reversed long days (LD; 16 h light/day; lights illuminated at 2300 Eastern Standard Time [EST]), or in short days (SD; 8 h light/day; lights illuminated at 700 h EST) for 12-15 weeks.

**Experiment 1: Effects of photoperiod on olfaction and olfactory bulb dendritic plasticity.** Behavioral testing. Nineteen mice were used for this study (n = 9-10/photoperiod). Olfactory testing occurred at the beginning of the dark phase (1500-1800 h EST). Modified “find the hidden cookie” paradigms (Crawley, 2000) were used on separate days of testing for the first 3 olfactory tests. For the first test, individual mice were placed in a clean cage for 3 min while a 1 cm piece of fruit (orange) was buried in the corner of the home cage. Mice were then returned to their home cages and latency to explore the fruit was recorded for up to 300 sec. Exploring was operationally defined as touching the food item with the mouth or manipulating with one or both paws. For the second and third tests (cookie and bacon), all food was removed from the animals’ cage 12 h before testing. A small piece (approximately 0.5 g) of peanut butter cookie (Nutter Butters, Nabisco) or bacon (Beggin’ Strips, Purina Brand) was buried under the bedding in one of the corners of a fresh cage. The latency to explore the food items was recorded. For the
fourth olfactory test, female urine was used as the olfactory stimulus. To obtain urine devoid of influence by estrous stage, eight adult female *P. leucopus* were bilaterally ovariectomized. Females were allowed 1 week recover and then urine was collected, pooled, and frozen at \(-70^\circ\text{C}\) until behavioral testing. Test sessions consisted of 4 consecutive presentations of increasing concentrations (0, 10\(^{-4}\), 10\(^{-3}\), 10\(^{-2}\), and 10\(^{-1}\) by volume) of female urine diluted in distilled water. Forty µl of each solution was placed onto a piece (1 sq. inch) of filter paper taped to a plastic weigh boat. The weigh boat was placed in one corner of the home cage. The test sessions were videotaped and scored using The Observer software (version 5, Exeter Software, Setauket, NY) for the latency to explore and the amount of time spent exploring the weigh boat.

*Tissue collection and histology.* Twenty-four h following the completion of all behavioral testing, mice were rapidly decapitated. Paired testes were removed and weighed to determine reproductive responsiveness to photoperiod treatment. Brains were removed and processed for Golgi staining as described in Chapter 3. Granule cells in the olfactory bulbs (n = 5/mouse) were traced using a camera lucida at 400X magnification (Neurolucida, MicroBrightField, Williston, VT). Dendritic spines were traced on five 10 µm distal segments of each neuron at 1000X magnification on the terminal tips of randomly chosen granule cell dendrites that had at least one branch point. Using the accompanying software (NeuroExplorer, MicroBrightField) dendritic complexity (via Sholl analysis), dendritic length, and spine density were calculated. All samples were number-coded and the experimenter was unaware of the treatments.
Experiment 2: Effects of photoperiod on olfactory bulb neurogenesis.

Injections. To estimate hippocampal cell proliferation and neurogenesis, 33 mice were injected with the cell division marker, bromodeoxyuridine (BrdU; Sigma-Aldrich, St. Louis, MO). Subsets of each of the photoperiod groups described in Chapter 4 were used to examine cell proliferation or neurogenesis (n = 6-11/photoperiod). To estimate cell proliferation, a single i.p. BrdU injection (50 mg/kg in 0.1 ml saline) was administered to each mouse 1.5-2.5 h prior to perfusion at week 14. To estimate neurogenesis, daily i.p. BrdU injections (50 mg/kg in 0.1 ml saline) were administered to the mice over 6 consecutive days at week 10. The time of injections was randomized to control for circadian differences in cell division among the treatment groups.

Tissue collection and histology. At week 14, mice were deeply anesthetized with sodium pentobarbital (Nembutal®; Abbott Laboratories, North Chicago, IL) and transcardially perfused with 50 ml saline followed by 75 ml 4% paraformaldehyde. Paired testes were removed and weighed to determine reproductive responsiveness to photoperiod treatment. Brains were removed and fixed as described in Chapter 4. Four series of every eighth section throughout the olfactory bulbs was dry-mounted on plus-slides, dried overnight, and then stored at -20°C until immunohistochemistry processing.

Cell proliferation and neurogenesis immunohistochemistry. One series of every eighth section from all mice were triple labeled fluorescently for BrdU, a neuronal marker, and a glial marker to determine which types of cells were newly born. Immunohistochemistry was performed as described in Chapter 4. A mean (±
SEM) of $13.9 \pm 0.4$ sections were analyzed per mouse. BrdU+ cells were manually counted in each section within the periglomerular and plexiform regions of the olfactory bulbs at 400X magnification with a fluorescent microscope. Sections were designated as being rostral (~300 µm), central (~500 µm), or caudal (~750 µm) from the tip of the olfactory bulbs. The number of BrdU+ cells within the granule cell layer was estimated by manually counting the number of cells within 3 (dorsal, medial, ventral) 300 µm x 225 µm grid boxes of the granule cell layer at 400X magnification. To determine the cellular phenotypes of BrdU+ cells in the granule cell layer of the neurogenesis brains (i.e., 6 injections of BrdU + 4 weeks), between 35 – 60 cells (mean 46.7 ± 9.3) from 8 -15 sections (mean 12 ± 2.7) per animal were analyzed at 400X magnification using a confocal laser scanning microscope (Zeiss 510 META, Thornwood, NY) with excitation wavelengths of 488, 543, and 633 nm at the Microscope and Imaging Facility at Ohio State University.

**Experiment 3: Brain-derived neurotrophic factor (BDNF) gene expression in the olfactory bulbs.** The 64 mice used in this experiment were previously described in Experiment 2 in Chapter 2 (n = 4-10/group). Briefly, all mice were exposed to long or short photoperiod conditions for 14 weeks (to induce long- and short-day phenotypes determined by maximum testicular regression in short-day mice; Chapter 1) and then transferred to the opposite photoperiod for 0, 2, 7, 21, or 49 days.

**RNA extraction, gene sequencing, and qPCR.** Total RNA was extracted from ≤30 mg of olfactory bulbs and cDNA was transcribed as described in Chapter 1.
Bdnf was sequenced as previously described (Chapter 1) and primers and a probe used for Bdnf and 18S Ribosomal RNA and qPCR conditions were previously described in Chapters 1 and 4.

Statistical analyses. Two-tailed student’s t-tests were used for most behavioral and physiological comparisons between photoperiods. ANOVAs were used to compare photoperiod by neurogenesis treatments. Data with unequal variances were compared using nonparametric Mann-Whitney tests to compare photoperiod differences (e.g., urine olfactory behavior and BDNF data). All comparisons were considered statistically significant when p < 0.05. StatView software was used for all analyses (v. 5.0.1, Cary, NC, USA).

Results

Experiment 1. Short days decreased testes mass (both absolute [LD: 322.2 ± 32.5, SD: 145.5 ± 21.9 mg] and relative to body mass [LD: 15.9 ± 1.7, SD: 7.0 ± 1.0 mg/g]) compared with long days (p < 0.05). No photoperiodic differences in the latency to explore the orange, cookie, bacon, or urine were observed (p > 0.05 in all cases) (Figure 8.1). The latency to explore urine decreased over trials for all mice (p > 0.05) (Figure 8.1c). No differences in the time spent exploring the urine were observed (p > 0.05) (Figure 8.1d). Photoperiod did not alter granule cell dendritic complexity (i.e., Sholl analysis) in the olfactory bulbs, or the number of dendrites, dendritic length, or spine density (p > 0.05 in all cases) (Figure 8.2).

Experiment 2. Mice treated to examine neurogenesis (i.e., 6 BrdU injections + 4 weeks) displayed more BrdU+ cells in the periglomerular, plexiform, and all
regions of the granule cell layer measured compared with mice treated to examine cell proliferation (i.e., 1 BrdU injection + 2 h) (p < 0.05 in all cases) (some of data in Figure 8.3b). However, photoperiodic differences in BrdU+ cell numbers only significantly differed in the caudal olfactory bulbs. Short days increased the number of BrdU+ cells in the plexiform region and the dorsal, medial, and ventral portions of the granule cell layer (p < 0.05 in all cases) (Figure 8.3b). Short days also increased the overall total number of BrdU+ cells in the entire granule cell layer (p < 0.05) (Figure 8.3c, e, f). The interaction between photoperiod and BrdU treatment was also significant for all of these regions (p < 0.05 in all cases). Within the granule layer of the olfactory bulbs, most BrdU+ cells were co-labeled with the neuronal marker NeuN (p < 0.05) (Figure 8.3d). No differences in the percentages of different BrdU+ cell phenotypes were observed between photoperiods (p > 0.05) (Figure 8.3d).

**Experiment 3.** No photoperiodic differences in hippocampal Bdnf gene expression were observed among groups (p > 0.05) (Figure 8.4).

**Discussion**

Although olfaction is important for rodent communication and survival, the potential for seasonal regulation of the olfactory system is understudied. Results from this study suggest that photoperiod may alter olfactory bulb neurogenesis, although these differences were not observed to functionally affect olfactory behaviors. Dendritic arborization and spine density of neurons in the granule cell layer of the olfactory bulbs did not vary by photoperiod. The short-day increases in
olfactory bulb neurogenesis did not appear to be mediated by changes in BDNF gene expression. Overall, further studies are necessary to elucidate the potential functional effects of photoperiod-evoked changes in olfactory bulb neurogenesis.

The caudal region of the olfactory bulbs is closest in proximity to the origin of newly born cells (i.e., the subventricular zone of the lateral ventricles). Because photoperiodic differences in neurogenesis were only observed in the caudal region (as opposed to the more rostral regions) of the olfactory bulbs, it is possible that more cells are dividing in the subventricular zone of short-day brains, but not surviving migration through the rostral migratory stream to the rostral olfactory bulbs (Peretto, Merighi, Fasolo, & Bonfanti, 1999). Thus, the possibility remains that the photoperiodic differences in caudal olfactory bulb neurogenesis are not functionally reflected in the behavioral paradigms used in this study because these newly born cells are not surviving migration, and are therefore, not making functional connections. A study in Syrian hamsters (Mesocricetus auratus) also reported increased BrdU+ cell numbers in the subventricular zone of short-day brains compared with long-day brains (Huang et al., 1998). Cell proliferation in the chemosensory epithelium of red-backed salamanders (Plethodon cinereus) increases prior to the breeding season (Dawley, Fingerlin, Hwang, John, & Stankiewicz, 2000) and increases in the central olfactory regions in the spring/summer of shore crabs (Carcinus maenas) (Hansen & Schmidt, 2004). Other studies that have examined seasonal or photoperiodic differences in cell proliferation or neurogenesis have not investigated the olfactory bulbs (Galea & McEwen, 1999; Lavenex et al., 2000a; Ormerod & Galea, 2003).
In contrast to the short-day impairment of hippocampal-based performance and inhibition of hippocampal size and structure (Chapter 3), short days increased neurogenesis in the olfactory bulbs. However, these results are not unexpected. An anecdotal report in shrews and voles describes an increase in olfactory bulb mass in the winter compared to summer; this pattern of brain structure size is opposite to that of the hippocampus (Yaskin, 1984). The function of enlarged olfactory bulbs or increased cell birth remains to be determined because the few studies examining olfactory bulb function with respect to photoperiod suggest that olfactory sensitivity may actually decrease during the non-breeding season (i.e., short days for rodents) (Clark & Smeraski, 1990; Ferkin & Gorman, 1992; Heth et al., 1996; Perrot-Sinal et al., 2000).

The finding in the present study that the overwhelming majority of BrdU+ cells in the granule cell layer were co-labeled with NeuN was predictable. Previous studies have demonstrated a similar bias towards neurogenesis as opposed to gliogenesis in the olfactory bulbs (Liu & Martin, 2003). Given that the differences in BrdU+ cell counts were restricted to the granule zone, cell phenotyping of BrdU+ cells in other olfactory regions were not determined. Anecdotally, however, some BrdU+ cells within the periglomerular layer were not co-labeled with NeuN or GFAP (data not shown). This suggests that some newly-born cells that migrate to the periglomerular layer may be neither neurons nor glia.

**BDNF** expression is associated with neurogenesis in the olfactory bulbs (Mackay-Sim & Chuah, 2000). Although no photoperiodic differences in **Bdnf** expression were observed in the present study, it is possible that discrimination of
**BDNF** gene expression between caudal and rostral olfactory bulb regions may correlate with the described differences in neurogenesis. Additionally, further investigation of other growth factors (Mackay-Sim & Chuah, 2000) involved in neurogenesis may elucidate potential mechanisms by which photoperiod alters olfactory bulb neurogenesis.

In contrast to my predictions, significant differences in olfactory behaviors were lacking. However, a statistically nonsignificant trend towards shorter latencies to explore female urine in short-day mice compared with long-day mice warrants further investigation. It is important to note that studies describing photoperiodic or seasonal differences in olfactory acuity use socially-relevant olfactory cues such as female or predator odors and not food cues (Clark & Smeraski, 1990; Ferkin & Gorman, 1992; Heth et al., 1996; Perrot-Sinal et al., 2000). It is possible that the skittishness of this species masked potential olfactory differences that may be elucidated by using a different photoperiod-responsive rodent model or more elaborate testing paradigms.

The lack of changes in dendritic arborization or spine density in the olfactory bulbs was predictable. Few studies have described structural plasticity in this region of the brain (Knafo, Ariav, Barkai, & Libersat, 2004; Knafo et al., 2001; Rehn et al., 1986). Although adult plasticity in the olfactory bulbs is understudied compared with the hippocampus, it is possible that changes in dendritic structure are less likely in this region relative to the hippocampus.

In sum, this study suggests that short days increase neurogenesis in the olfactory bulbs compared with long days. The functional (i.e., behavioral)
significance of this finding requires further investigation, possibly in a rodent model more tolerable of olfactory behavior testing. The molecular mechanisms mediating the described photoperiodic differences in olfactory bulb neurogenesis do not appear to involve $Bdnf$ gene expression. Finally, photoperiod does not affect granule cell dendritic morphology. Based on the photoperiod-evoked differences in olfactory bulb neurogenesis and the relationship between olfaction and social communication, it remains likely that seasonal modifications of the olfactory system are adaptive.
Figure 8.1. Effects of photoperiod on olfactory behaviors.

A) Latency to explore hidden orange, cookie, and bacon over a single 300-sec trial.  B) Latency to explore female urine (or water) over 4 consecutive 120-sec trials with increasing urine concentrations.  C) Duration of time spent exploring female urine (or water) for each trial.
Figure 8.2. Effects of photoperiod on olfactory bulb dendritic morphology.

Figure 8.3. Photoperiod alters olfactory bulb cell proliferation.

A) Schematic diagrams of 3 portions of the olfactory bulbs and 3 cell regions within the olfactory bulbs examined. Templates are modified from *Mouse brain atlas in stereotaxic coordinates* (Paxinos & Franklin, 2001) with permission from Elsevier. B) Number of BrdU+ cells in four regions of the caudal olfactory bulbs in long- or short-day brains following BrdU procedures designed to examine neurogenesis or cell proliferation. GC = granule cell layer. C) Total number of BrdU+ cells in the entire caudal granule cell layer of the olfactory bulbs. D) Percent BrdU+ cells that co-label with NeuN (neuronal marker), GFAP (glial marker) or neither in the granule cell layer of the olfactory bulbs. E) Photomicrograph of long-day, triple-labeled caudal granule cell layer section. F) Photomicrograph of short-day, triple-labeled caudal granule cell layer section. Arrow heads point to BrdU+ cells.
Figure 8.4. Effects of acute transfer (0, 2, 7, 21, or 49 d) to the opposite photoperiod following 14 weeks of original photoperiod treatment on relative *Bdnf* gene expression olfactory bulbs.

LD = long-day; SD = short-day.
TESTOSTERONE, PHOTOPERIOD, AND AFFECTIVE BEHAVIORS

Reports from various non-tropical rodent species suggests that photoperiod affects a broad range of behaviors including: aggression (Jasnow et al., 2000), learning (Chapter 3), analgesia (Kavaliers & Galea, 1995), and feeding (Dark & Zucker, 1984). Similar to the physiological and morphological changes induced by photoperiod, these behavioral changes are thought to be adaptive for specific seasons. For example, during the breeding season, many male rodents defend breeding territories. As winter approaches and breeding ceases, territorial aggression is reduced in these males (Prendergast et al., 2002). Tolerance among males is displayed and group huddling within communal nests has been reported during the winter months (West & Dublin, 1984). These seasonal changes in aggression can be reproduced in the laboratory by manipulating one seasonal cue: photoperiod (day length) (Jasnow et al., 2002). Seasonal changes in aggression presumably promote reproductive activities during the breeding season and enhance thermoregulation during the non-breeding season.
Although traditionally considered to be maladaptive in humans, behaviors similar to symptoms of human depression and anxiety disorders persist in other species and may be advantageous under certain conditions (i.e., seasons). For example, symptoms of affective disorders, such as lethargy, altered food intake, loss of sexual motivation, and fearfulness may function to conserve energy during the winter (Nesse, 2000; Nesse & Williams, 1996; Wehr et al., 2001). Numerous behavioral tests have been designed to measure “affective-like” behaviors based on the similarity to symptoms in humans with affective disorders and the effectiveness of pharmacological treatments in reversing the affective-like responses. Thus, affective behaviors and the brain regions responsible for these behaviors may be modified by seasonal information. Studies examining the effects of photoperiod treatment on affective behavior in rodents are limited. One study reports that short photoperiod treatment increased depressive-like behavior in rats (Rattus rattus), a species traditionally considered reproductively non-photoperiodic (Molina-Hernandez & Tellez-Alcantara, 2000). Short days also decreased neophobic responses in two strains of Mus musculus, another non-photoperiodic species (Kopp et al., 1999). In peripubertal Siberian hamsters (Phodopus sungorus), elevated depressive- and anxiety-like behaviors were induced by 2 weeks of short day exposure (Prendergast & Nelson, 2005). Short-day exposed male hamsters spent less time in open, exposed areas of an elevated plus maze and exhibited behavioral despair more frequently in the Porsolt forced swim test relative to long-day males. Results from a follow-up study in this species suggest that perinatal exposure to short days also has enduring
effects on affective behaviors in adult hamsters (Pyter and Nelson, *in press*). Considered together, these results are consistent with the hypothesis that depressive- and anxiety-like behaviors may be part of a constellation of adaptations to short days.

In humans, one controversial hypothesis suggests that symptoms of seasonal affective disorder (SAD) represent a vestigial evolutionary benefit (Eagles, 2004). SAD is characterized by recurrent depression during the winter months. The symptoms of SAD (e.g., lethargy, anhedonia, etc.), that are similar to symptoms of general depression and anxiety disorders, may be seasonally adaptive because of the energetic savings conferred during the winter (Nesse, 2000; Nesse & Williams, 1996; Wehr et al., 2001). Additional hypotheses on the adaptive value of winter depression have been proposed (Price, 1967; Wehr, Rosenthal, & Sack, 1988). Of course, to demonstrate the adaptive value of a trait, individuals with the trait would have to display increased reproductive fitness or survival compared with individuals lacking the trait. These sorts of studies are difficult to conduct in nonhumans, and virtually impossible to conduct in humans. However, it is possible that people afflicted with SAD represent a population that has retained some degree of dependency on environmental cues from ancestors. Co-morbidity of depression and anxiety in humans is high (Kaufman & Charney, 2000); together these affective states may be manifested in a collection of behaviors that are adaptive in the winter. Effective treatment of many SAD and non-seasonally depressed patients includes extended exposure to bright light (Rosenthal et al., 1984; Terman & Terman, 2005). This light exposure shuts off melatonin production and therefore shortens the duration of melatonin similar to that of a long day.
In addition to the potential role of melatonin in affective regulation, gonadal hormones have been also linked with mood and affective-like responses. Male rodents that are reproductively responsive to photoperiod have low circulating testosterone concentrations in short days as a result of dampened gonadotropin release compared with long-day individuals (Berndtson & Desjardins, 1974). Testosterone may reduce depressive- and anxiety-like responses in male rodents or low testosterone concentrations may enhance depressive- and anxiety-like responses. Castration of mice increases depressive-like behaviors, whereas testosterone replacement abolishes this effect (Bernardi, Genedani, Tagliavini, & Bertolini, 1989). Both negative and positive effects of testosterone on anxiety-like behavior have been reported in male rodents (Aikey, Nyby, Anmuth, & James, 2002; Blizard, Lippman, & Chen, 1975; Lucion, Charchat, Pereira, & Rasia-Filho, 1996). Testosterone may also alleviate symptoms of depression in men and women (Orengo, Fullerton, & Tan, 2004; Studd & Panay, 2004). Overall, these results suggest that testosterone modulates behaviors used to assess affect, although the mechanisms remain unspecified.

I hypothesized that short-day *Peromyscus* (another photoperiodic rodent species) display more anxiety-like and depressive-like responses than long-day rodents which is mediated by low circulating testosterone concentrations. To test this hypothesis male white-footed mice (*Peromyscus leucopus*) and closely related deer mice (*Peromyscus maniculatus*) were used, in contrast to the previous study using Siberian hamsters, to more closely follow traditional behavioral paradigms established for mice and rats (Crawley, 2000). For example, one commonly used test for depressive-like behavior, the tail suspension test, requires the rodents to be hung by their tails which would be
impractical in hamsters with short tails. For the same reason, hamsters are less efficient swimmers in tests such as the Porsolt forced swim. Additionally, hamsters have poor perception of visual cliffs making tests such as the elevated plus maze difficult to use. Finally, these species were chosen for comparative purposes between latitude. In the first experiment, to assess the role of testosterone in mediating affective behavior, male white-footed mice in long or short days were castrated and supplemented with testosterone exogenously. In the second experiment, *Peromyscus* from high latitudes were tested for affective behavioral differences between photoperiod to compare with potential differences observed in the SHAM mice from low latitude in Experiment 1.

**Material and Methods**

**Animals.** Seventy-three adult (>55 days of age) male white-footed mice (*Peromyscus leucopus*) and 16 adult male deer mice (*Peromyscus maniculatus*) from our breeding colonies were used in these experiments. The *P. leucopus* breeding colony was derived from breeders that were caught in Linville, North Carolina, USA (~36° N) and the *P. maniculatus* colony was derived from breeders caught in Kananaskas Valley, Ontario, Canada (43° N). Animals were housed individually in polypropylene cages (27.8 x 7.5 x 13 cm) with a constant temperature and humidity of 21 ± 5° C and 50 ± 5%, respectively, and *ad libitum* access to food (Harlan Teklad 8640 rodent diet, Indianapolis, IN) and filtered tap water. Mice were either housed in reversed long days (LD; 16 h/light/day; lights illuminated at 2300 h Eastern Standard Time [EST]) photoperiod, or in short days (SD; 8 h/light/day; lights illuminated at 700 h EST) photoperiod.
**Experiment 1.** This experiment was designed to determine the potential role of testosterone on photoperiod-induced affective behaviors in white-footed mice. Mice from both photoperiod treatments were divided into three surgical groups: sham (SHAM; LD: n = 10; SD: n = 14), bilateral castration plus saline implant (CAST; LD: n = 11; SD: n = 14), or bilateral castration plus testosterone propionate (Sigma-Aldrich, St. Louis, MO) implant (CAST + T; LD n = 10; SD n = 14). Silastic implants were designed as previously described (Demas & Nelson, 1998). Mice were allowed one week recovery prior to onset of photoperiod treatment. Siblings were pseudo-randomly distributed among all groups. All mice were exposed to their respective photoperiod conditions for a total of 13 weeks with the battery of behavioral testing occurring during the final two weeks. Body mass was recorded bi-weekly as a measure of photoperiod responsiveness. At week 7, all mice were lightly anesthetized under isoflurane vapors and subcutaneous implants were replaced with fresh implants in order to maintain physiological hormone concentrations. All studies were conducted with the approval of the Ohio State University Institutional Animal Care and Use Committee and were in compliance with all US federal animal welfare requirements. Mice from each experiment were tested simultaneously. All behavioral testing was performed during the first 3 h of the dark phase unless specified otherwise.

*Depressive-like behavior tests: Porsolt forced swim.* Mice were tested for early cessation of attempts to escape by placing them in 14 cm of room temperature water in a cylindrical tank (24 cm diameter) with opaque walls. Swimming behavior was
videotaped for 8-min and scored by a blind observer using The Observer program to determine 1) latency to first floating bout, 2) the total number of floating bouts, and 3) the total time spend floating.

*Sucrose intake.* Consumption of a 3% sucrose solution was measured in all mice for 6 h during the dark phase (1500 – 2100 h EST) for four consecutive days. Water bottles were replaced with modified novel bottles filled with a 3% sucrose solution in tap water at room temperature at the onset of darkness (0300 h EST). Six h later, sucrose bottles were removed, water bottles were replaced, and consumption of sucrose solution was assessed by subtracting the remaining mass of the bottles from the original mass. The tail suspension test for depressive-like behavior was excluded from the study because all suspended mice were able to right themselves by climbing up their own tail.

*Novelty behavior tests: Novel drinking apparatus versus drinking solution.* Following sucrose intake assessment, a subset of mice (LD: n = 13, SD: n = 14) were tested for neophobia to a drinking apparatus (in contrast to a drinking solution). Water bottles were replaced by ceramic drinking cups filled with tap water for 6 h during the dark phase, commencing at the onset of darkness (1500 – 2100 h EST) for one evening. After 6 h, drinking cups were removed, water bottles were replaced, and consumption of water in a novel drinking apparatus was assessed by subtracting the remaining mass of the cups from the original mass. A second subset of mice (LD: n = 23, SD: n = 34) were tested to determine if neophobia was extinguished by using a familiar drinking apparatus and/or drinking solution. Water bottles were replaced with modified bottles (no longer novel to the mice due to previous sucrose intake assessment) filled with either tap water or 3% sucrose solution (as described previously) for 6 h during the dark phase,
commencing at the onset of darkness (1500 – 2100 h EST) for one evening. After 6 h, modified bottles were removed, water bottles were replaced, and consumption of water or sucrose solution in drinking apparatus was assessed by subtracting the remaining mass of the bottles from the original mass.

**Novel cage.** To determine whether neophobia was confined to appetitive behavior, a subset of mice were assessed for neophobia to a novel environment. Mice (CAST: 6, CAST + T: 8, SHAM: 7) and their nestlets were transferred to a clean cage (home cage) that was attached to another clean cage (novel cage) via a plexiglass tunnel at the onset of darkness (0300 h EST). The tunnel was blocked to prevent access to novel cage. Mice were maintained in the home cage for 24 h. After 24 h, blockage to the tunnel was removed for 10 min during which locomotor behavior between the two cages was videotaped. Food and water was removed from cages during the 10 min testing session to minimize distractions. Videotapes were scored for 1) number of entries into the novel cage, 2) total time spent in novel cage, 3) number of grooming bouts, and 4) total time spent grooming using The Observer software (version 5, Exeter Software, Setauket, NY) by an observer unaware of treatments. An entry was considered when all four paws of mice crossed into a specific region of the apparatus (i.e., tunnel, novel cage, home cage). The tunnel was considered part of the novel cage.

**Anxiety-like behavior tests: Acoustic startle/pre-pulse inhibition.** Startle reactivity and pre-pulse inhibition were measured using a single ventilated startle chamber (SR-Lab, San Diego Instruments, San Diego, CA). Mice were placed inside a clear, nonrestrictive Plexiglas cylinder inside the chamber that was hooked up to an electrostatic sensor to measure the amplitude of the animal’s whole body movement.
Over a period of 30 min, the mice were subjected to a series of acoustic stimuli through a high-frequency loudspeaker inside the chamber over a continuous background noise of 65dB. All test sessions will consist of 60 trials including: startle trials (loud acoustic 120 dB pulse alone for 40 msec), prepulse trials (mild acoustic pre-pulse for 20 msec (73 or 81 dB) followed by 40 msec 120 dB pulse after 100 msec delay), and no-stimulus trials (no pulses). The test session began and ended with 5 presentations of the pulse alone trial; in between, each prepulse and no pulse trial type was presented 10 times in pseudo-random order. There was an average of 15 sec (range = 12 -30 sec) between trials. Sixty-five readings were taken at 1 msec intervals, starting at stimulus onset, and the average amplitude is used to determine the acoustic startle response. Before onset of the testing session mice were allowed to acclimatize to the apparatus for 10 min.

*Central tendency in an open field.* Mice were placed in a clear 36 x 36 cm acrylic box lined with bedding, inside a ventilated cabinet. A frame at the base of the box consisting of 32 photobeams in a 16 x 16 arrangement in addition to 2 rows of vertical detected the location of horizontal and rearing movements (Open Field Photobeam Activity System, San Diego Instruments, Inc., San Diego, CA). The center of the open field was designated as the central 13 x 13 cm. Total movement was tracked for 60 min and analyzed for 1) percent of time spent in the center versus periphery, and 2) number of rears.

*Elevated plus maze.* The elevated-plus maze consisted of two open and two closed 6 cm wide arms in a plus-sign configuration 1 m off the ground. The closed arms were enclosed by 15 cm high black plexiglass. All arms were covered with contact paper to prevent the mice from sliding off and all surfaces were wiped with 70% alcohol
between animals. Each mouse was removed from their cage using a small fish net, released at the center of the maze, and allowed to move freely on the maze for a 5-min. testing period that was videotaped under dim red light. The human observer was hidden behind a screen during the testing period to limit the influence of their presence on behavior. Mice that jumped off the maze into compartments below were placed back on the maze for the remainder of the testing period. An observer uninformed about the animals’ experimental treatment scored the videotapes using The Observer program for 1) total entries into all arms, 2) number entries into open arms, 3) percent entries into open arms, 4) latency to enter open arm, 5) number of rears, 6) number of grooming bouts, 7) number of freezing bouts.

Tissue collection. Following the final behavioral test, mice were rapidly decapitated. For all sham animals, paired testes were collected from mice under aseptic conditions and weighed. Complete castration was confirmed in all castrated mice. Incomplete removal of testicular tissue was found in one mouse that had been given testosterone in LD and data from this mouse were removed from the study.

Experiment 2. Based on the results from Experiment 1, this experiment was designed to determine whether *Peromyscus* derived from high latitudes would display more pronounced photoperiod-induced affective behaviors than *Peromyscus* from low latitudes. This hypothesis was based on evidence that reproductive responses to photoperiod fall along a latitudinal gradient of origin in this genus (Dark, Johnston, Healy, & Zucker, 1983). Deer mice were not exposed to any surgical manipulation and were divided into long (n = 7) or short (n =9) photoperiod treatments. The same
behavioral tests and timing of the tests for affective behaviors were used as described in Experiment 1. Tissue was collected after the behavioral testing as described in Experiment 1.

**Statistical analysis.** StatView statistical software was used for all analyses (v. 5.0.1, Cary, NC). ANOVA tests were used to compare surgery and treatment groups. Planned comparisons between photoperiods within surgery groups and among surgery groups within photoperiod were tested using t-tests and ANOVA tests, respectively. All comparisons were considered statistically significant when p < 0.05.

**Results**

**Experiment 1. Photoperiod responsiveness.** SD SHAM mice weighed significantly less than LD SHAM mice by week 12 (p < 0.05; Figure 9.1a). LD CAST + T mice weighed less than SD CAST + T mice on week 4 (p < 0.05; Figure 9.1c). There were no photoperiodic differences in body mass of CAST mice (p > 0.05 for all weeks: Figure 9.1b). Within the SD-treated group, CAST + T mice weighed more than CAST or SHAM mice on week 4 and 8 and more than SHAM mice on week 6 (p < 0.05 in all cases; Figure 9.1e). No differences in body mass were found among surgical cohorts within the LD group (p > 0.05 for all weeks; Figure 9.1d). SD SHAM testes mass (relative to body mass) was smaller than that of LD SHAM (p < 0.01; Figure 9.1f).

**Depressive-like behaviors: Porsolt forced swim test.** Photoperiod treatment did not affect the latency to float, number of floating bouts, or total float time within surgical groups (p > 0.05 for all measures; data not shown). Surgical treatment also did not affect these measures within photoperiod groups (p > 0.05 for all measures; data not shown).
Sucrose anhedonia. SD CAST mice drank less sucrose than LD CAST mice on the first day of sucrose presentation (p < 0.05; Figure 9.2). Photoperiod treatment did not affect sucrose intake in the CAST + T or SHAM mice (p > 0.05 on each day; Figure 9.2). Surgical treatment did not have an overall effect on sucrose intake in either LD or SD mice (p > 0.05 on each day; data not shown).

Novelty behaviors: Novel drinking apparatus versus drinking solution. The novel drinking device for sucrose intake did not account for the difference in sucrose consumption for CAST mice. Water and sucrose intake did not differ among surgical cohorts or photoperiodic groups in either the novel cup or familiar water bottle tests (p > 0.05 in all cases; data not shown).

Novel cage. Photoperiod did not affect the time spent in the novel cage among surgical cohorts (p > 0.05; data not shown). CAST mice spent more time in the novel cage than CAST + T mice regardless of photoperiod treatment (p > 0.05; Figure 9.3). Within photoperiodic groups, surgical treatments did not affect the time spent in the novel cage (p > 0.05; data not shown).

Anxiety-like behaviors: Acoustic startle/pre-pulse inhibition. SD SHAM mice displayed larger percents of pre-pulse inhibition compared to LD SHAM mice following 73 db and 81 db pre-pulses (p < 0.05 in both cases; Figure 9.4a). However, LD SHAM mice displayed greater activity within the startle apparatus than SD SHAM without any pulse stimulation (p < 0.05; Figure 9.4b). No differences in pre-pulse inhibition were recorded between photoperiod-treated CAST + T and CAST mice or among surgical cohorts within the SD group (p > 0.05 in all cases; data not shown). LD SHAM mice
displayed greater activity within the startle apparatus than LD CAST mice (p < 0.05; Figure 9.4b). Testosterone replacement in SD CAST + T mice did not restore activity levels to that of LD SHAM mice during the no-pulse trials (p < 0.05).

Central tendency. Total activity and central tendency were not affected by surgical or photoperiodic treatments (p > 0.5 in all cases; data not shown). LD CAST mice reared fewer times than LD SHAM mice (p < 0.05; data not shown). Rearing did not differ among surgeries within the SD group (p > 0.05; data not shown).

Elevated plus maze. SD CAST + T mice entered the open arms fewer times than LD CAST + T mice (p < 0.05; Figure 9.5a). However, SD CAST + T also entered fewer total arms than LD CAST + T (p = 0.07; Figure 9.5b) and therefore, the percent of open arm entries did not differ between photoperiod (p > 0.05; Figure 9.5c). Within both LD and SD groups, CAST mice reared more than SHAM mice (p < 0.05 in both cases; Figure 9.5d).

Experiment 2. Photoperiod responsiveness. SD deer mice weighed significantly less than LD mice by week 14 of photoperiod treatment (p < 0.05; Figure 9.6a). Relative testes mass was also decreased in SD mice compared with LD mice (p < 0.05; Figure 9.6b).

Depressive-like behaviors. No photoperiodic differences were observed between LD and SD deer mice for the Porsolt forced swim test nor the sucrose anhedonia test (p > 0.05 in all cases).

Novelty behaviors. Photoperiod also did not affect liquid intake from novel and familiar drinking apparatuses or responses to novel cage exposure (p > 0.05 in all cases).
Anxiety-like behaviors. Pre-pulse inhibition, elevated plus maze, and central tendency behaviors did not vary by photoperiodic treatment in deer mice (p > 0.05).

Latitudinal comparison within genus. High latitude deer mice did not display greater photoperiodic differences in relative testes mass or body mass compared with low latitude white-footed mice. Indeed, the difference between relative testes mass of SD and LD white-footed mice was significantly greater than the difference between relative testes mass of LD and SD deer mice (p < 0.05; (mean ± SEM) P. leucopus = 104.1 ± 14.2, P. maniculatus = 41.5 ± 11.0). Photoperiodic differences in body mass were similar in both species (mean ± SEM: P. leucopus = 13.4 ± 6.0, P. maniculatus = 15.7 ± 6.5). The lack of photoperiodic differences in affective behavior prevents the comparison of severity of photoperiod-induced affective behaviors between high and low latitude Peromyscus.

Discussion

Based on previous studies in seasonal rodents, I hypothesized that depressive- and anxiety-like responses are adaptive in short days and predicted that seasonal changes in testosterone concentration may elevate these affective responses in short-day Peromyscus. I also predicted that the differences in affective responses to photoperiod would be greater in individuals derived from higher latitudes compared with those from lower latitudes. This prediction was based on the hypothesis that because seasonal changes in the environment (e.g., temperature, day length, etc.) are more extreme at high latitudes compared with low latitudes, seasonal adaptations that have evolved in individuals from high latitudes may be more extreme to cope with these harsh
environmental conditions. In contrast to my predictions, even though white-footed mice displayed reproductive and body mass responses to photoperiod, virtually no consistent photoperiod-induced changes in depressive- and anxiety-like behaviors were observed among testing paradigms. CAST + T mice housed in short days did display greater anxiety-like behavior compared with those housed in long days in the elevated plus maze and greater neophobia in the novel cage test, but not in the open field. CAST + T mice in short days also gained significantly more body mass than other SD groups and LD groups alike. CAST mice in SD displayed a transient decrease in sucrose anhedonia compared with LD CAST mice, but no other evidence of increased depressive-like behaviors was observed. In contrast, LD SHAM mice failed to display pre-pulse inhibition, a significant difference from SD SHAM mice. LD SHAM mice also displayed increased activity within the startle chamber compared with SD SHAM mice and LD CAST mice. In sum, photoperiod alone had minimal effects on affective behaviors and testosterone manipulation interacted with photoperiod in a few affective tests. Additionally, deer mice from high latitude also did not display affective differences in response to photoperiod treatment, although photoperiodic differences in testes mass were observed.

The lack of long- versus short-day differences in depressive- and anxiety-like behaviors was unexpected given the previous studies in Siberian hamsters (Prendergast & Nelson, 2005; Pyter & Nelson, in press). Physiological responsiveness to photoperiod treatment, as measured by testicular mass and body mass, is more dramatic in Siberian hamsters as compared with Peromyscus. For example, testes mass relative to body mass of SD hamsters is >90% lower than that of LD hamsters (Prendergast, Hotchkiss, Bilbo, Kinsey, & Nelson, 2003), whereas based on the present study, this difference is only
approximately 40% - 60% in *Peromyscus*. SD hamsters also weigh >20% less than LD hamsters (Bilbo, Quan, Prendergast, Bowers, & Nelson, 2003), whereas SD *Peromyscus* mice weigh approximately 12% - 16% less than LD mice. Smaller photoperiod-induced physiological differences in *Peromyscus* compared with hamsters may be predictive of the dampened behavioral differences presently observed.

In Experiment 1, testosterone replacement increased body mass in SD mice but not LD mice. A similar divergent effect of testosterone replacement on body mass was observed in male Siberian hamsters (Bartness, 1996). This difference in body mass was correlated with increased food intake in the SD CAST + T hamsters (Bartness, 1996). Because the testosterone treatments used in the hamster study and the present study were designed to mimic physiological concentrations, it is possible that the exaggerated increase in body mass of SD CAST + T mice is due to a difference in testosterone feedback or receptivity between SD and LD animals. Indeed, the sensitivity of the hypothalamic-pituitary-gonadal feedback loop is enhanced in short-day rodents (Bittman, Jetton, Villalba, & Devries, 1996; Bittman & Krey, 1988; Korytko, Dluzen, & Blank, 1997).

The SD CAST + T mice in this study also displayed increased anxiety-like behavior in the elevated plus maze and neophobia in the novel cage test. These data in SD mice conflict with previous studies that reported that testosterone administered neonatally (but not in adulthood) to male rats reduces defecation (an anxiety-like behavior) in the open field test for anxiety (Blizard et al., 1975; Lucion et al., 1996) and testosterone administered to adult male mice reduced anxiety-like behaviors in the elevated plus maze (Aikey et al., 2002). However, the present study is the first to
examine photoperiod and testosterone interactions on affective behavior, and therefore, increased anxiety in testosterone-treated SD mice may be a novel result of this interaction. The hypothesis that photoperiod alters testosterone regulation of anxiety-like behavior is supported by the lack of anxiety-like behavior in the LD CAST + T mice. Other behaviors, such as locomotor activity in Syrian hamsters (Mesocricetus aurarus), are also differentially modulated by testosterone depending on photoperiod exposure (Ellis & Turek, 1983). Further testing of this hypothesis should use the Siberian hamster model of photoperiod-induced affective behaviors. The lack of similar anxiety-like behaviors among the 3 tests for anxiety suggests that anxiety-like behaviors are context dependent (Moore, 1986).

CAST + T mice also displayed neophobia to a novel environment in the present study. Very little research has examined the effects of testosterone on neophobic behavior. In contrast to the present data, one study reports that testosterone decreased olfactory neophobia in male Syrian hamsters (Cornwell-Jones & Kovanic, 1981). However, significant differences may exist between the effects of testosterone on olfactory neophobia versus environmental neophobia. In support of this hypothesis, the CAST + T mice did not display differences in the neophobia tests for drinking solutions and drinking apparatuses. Future research investigating different types of neophobia should directly address these discrepancies. The implications of potential photoperiodic differences in neophobia may be great with respect to numerous behavioral testing paradigms.

The single significant effect of photoperiod and surgery on depressive-like behaviors was the transient reduction of sucrose intake in the SD CAST mice relative to
LD CAST mice. This depressive-like behavior in SD mice corroborates the short-day induced depressive-like behaviors found in Siberian hamsters in the Porsolt test from a previous study (Prendergast & Nelson, 2005). The transient timing of this depressive-like behavior is not confounded by neophobic responses to the testing paradigm because the SD CAST mice did not differ from LD CAST mice in water or sucrose consumption from other novel apparatuses. In contrast to stress-induced models of depression, for which the sucrose anhedonia test is most often used and is characterized by chronic anhedonia (Monleon et al., 1995), sucrose intake in the SD CAST mice was reduced temporarily. It is possible that the concentration of sucrose administered in the present study is not hedonic to Peromyscus compared with previously tested Mus musculus. However, testosterone replacement in castrated SD mice resulted in sucrose consumption that was not significantly different from SD CAST or SD SHAM. Therefore, testosterone replacement is not sufficient to rescue the reduced sucrose intake in SD CAST mice.

The startle response of SD SHAM mice was inhibited by pre-pulses, whereas the startle response of LD mice was potentiated by pre-pulses. Potentiation as opposed to inhibition is often due to a shortened interval (< 10 msec) between the pre-pulse and the pulse. Inhibition usually requires an interval of 10-500 msec (Hoffman, 1968). Our protocol included a 100 msec interval, which has been found to produce the strongest inhibition in rats and several strains of mice (Hoffman, 1968; Plappert, 2004). It is possible, however, that LD white-footed mice are deficient in the processing or prioritizing of sensory information and require a longer interval for pre-pulse inhibition. These data suggest that photoperiod affects the neural circuitry involved in sensory-motor
processing in this species. In contrast, no differences were observed between photoperiods in deer mice or previously tested Siberian hamsters (Prendergast & Nelson, 2005). Therefore, this study is the first to find photoperiodic differences in pre-pulse response and requires further examination to elucidate the potential adaptive significance. Impaired pre-pulse inhibition is also observed in humans with schizophrenia (reviewed in Geyer, Swerdlow, Mansbach, & Braff, 1990).

These data do not rule out the hypothesis that the effects of photoperiod on affective behavior correlate positively with the regional latitude from which the species reside. Hamsters originating from Siberia that display photoperiod-induced affective behaviors (Prendergast & Nelson, 2005) are from higher latitudes than either the white-footed mice or deer mice tested in the present study. If rodents from latitudes lower than Siberian hamsters display photoperiod-induced changes in affective behavior, then it is possible that the sensitivity of current behavioral testing paradigms is insufficient to observe the changes.

These experiments suggest that affective behaviors may not necessarily be seasonally adaptive in all rodents. Based on the present data, testosterone does appear to affect anxiety-like and neophobic behaviors and may interact with photoperiod treatment. Further investigation using multiple populations of a single genus (e.g., Phodopus) among latitudes is required to determine whether a photoperiodic gradient in seasonal affective behavior exists.
Figure 9.1  Effect of photoperiod and surgery on body mass and testes mass (mean ± SEM).

Effect of photoperiod on A) SHAM mice, B) CAST mice, and C) CAST + T mice. Effect of surgery on D) long-day and E) short-day mice. F) Effect of photoperiod on relative testes mass in SHAM mice. * p < 0.05 between photoperiods; + p < 0.05 between CAST + T and other surgery groups; # p < 0.05 between CAST + T and SHAM mice.
**Figure 9.2.** Effect of photoperiod and surgery on (mean ± SEM) 6-h sucrose intake over 4 consecutive days.

Effect of 13 weeks of photoperiod treatment on sucrose intake of A) SHAM, B) CAST, and C) CAST + T mice. *p < 0.05 between photoperiods.
Figure 9.3. Effects of surgery on (mean ± SEM) time spent in a novel environment following 13 weeks of photoperiod treatment. * p < 0.05 among surgery groups
Figure 9.4. Effects of photoperiod and surgery on pre-pulse inhibition measures.

A) Effects of 13 weeks of photoperiod treatment on percent pre-pulse inhibition in SHAM mice. B) Effects of photoperiod and surgery on no-pulse activity amplitude within the acoustic startle apparatus. * p < 0.05 between photoperiods.
**Figure 9.5.** Effects of photoperiod and surgery on elevated plus maze behaviors.

A) Effects of photoperiod on number of entries into the open arms, B) total number of arm entries, and C) percent time spent in the open arms of CAST +T mice. D) Effect of surgery on number of rears in elevated plus maze. * p < 0.05.
Figure 9.6. Effects of photoperiod on (mean ± SEM) body mass and relative testes mass in deer mice.

Effects of 13 weeks of photoperiod treatment on A, body mass and B, relative testes mass in deer mice from Experiment 2. * p < 0.05.
CONCLUSIONS

Seasonal adaptations have evolved in animals and are hypothesized to promote survival and reproduction during yearly changes in the environment. These seasonal responses are physiological, morphological, and behavioral and are coordinated by day length (photoperiod) information in most non-tropical rodents. Intricate neuroendocrine interactions coordinate many of these changes, however, few studies have examined the effects of photoperiod on the adult brain. In light of recent evidence that the adult brain retains some degree of plasticity (Breedlove & Jordan, 2001; Kempermann, 2005; Matus, 2005), I hypothesized that photoperiod alters the adult brain resulting in seasonally-appropriate behaviors. Thus, this dissertation was designed to examine the effects of photoperiod on adult plasticity of physiological systems, brain, and behavior in male white-footed mice (*Peromyscus leucopus*) that may represent seasonal adaptations to the changing environment.

Although phenotypic plasticity is primarily studied during development and in the periphery, recent evidence suggests that significant plasticity occurs in adult systems, including the central nervous system. The first two studies examined the effects of photoperiod on one type of adult plasticity, angiogenesis, in the periphery and the brain. In Chapter 1, I predicted that short days would alter expression of angiogenesis genes in the testes throughout testicular regression and spontaneous recrudescence compared with
the static morphology of long-day testes. In agreement with my prediction, short-day mice displayed relatively high expression levels of Hif1α, Tgfβr3, and Serpine1 (anti-angiogenic or pro-apoptotic genes) during testicular regression and high expression levels of Tnf (blood vessel stabilizing gene) during testicular recrudescence. Based on evidence that similar, but more subtle, decreases in brain mass occur during the winter or in response to short photoperiods in rodents (Dark et al., 1987; Yaskin, 1984), Chapter 2 was designed to test the effects of photoperiod on cerebral blood flow and angiogenesis gene expression in the hypothalamus, hippocampus, and olfactory bulbs. Indeed, short days decreased cerebral blood flow and altered Vegf and/or Hif1α expression in the hippocampus and olfactory bulbs compared with long days. Taken together, these results suggest that photoperiod modifies the vascular network supporting the brain and testes of adults.

The next set of studies was based on evidence that short days specifically decreased hippocampal volume (or mass) and impaired hippocampal-based spatial learning and memory compared with long days (Galea et al., 1999; Perrot-Sinal et al., 1998; Yaskin, 1984). Because the hippocampus is involved in spatial learning and memory and is also one of the few brain regions associated with significant adult plasticity, I designed studies to investigate the potential effects of photoperiod on three types of hippocampal plasticity: 1) dendrite and spine complexity, 2) neurogenesis, and 3) long-term potentiation. Other studies suggest that extra-photoperiodic environmental conditions (e.g., environmental enrichment, exercise, social environment, and stressors) can influence these types of adult plasticity in the hippocampus (Fowler et al., 2002; Kempermann et al., 1997; McEwen, 1999; van Praag et al., 1999a) which, in turn, affect
spatial learning and memory performance. Chapter 3 discusses the effects of photoperiod on hippocampal dendritic and spine complexity and spatial learning and memory. Indeed, short-day impairment of water maze performance was associated with decreased hippocampal volume and altered hippocampal dendritic spine density in the CA1 and CA3 regions of the hippocampus. Photoperiodic-modification of neurogenesis within the dentate gyrus was examined in Chapter 4. In addition to replicating the impairment of spatial learning in short days, results from this study suggest that photoperiod modulates the effects of learning and memory experience on hippocampal neurogenesis. Additionally, acute long-day exposure increased gene expression of hippocampal brain-derived neurotrophic factor (Bdnf), a growth factor involved in neurogenesis, relative to short days. The third type of adult hippocampal plasticity was examined in Chapter 5: long-term potentiation (LTP). The data from this study suggest that electrophysiological potentiation within the dentate gyrus is greater in long-day mice compared with short-day mice following high-frequency stimulation of the perforant pathway. Taken together, photoperiod affects adult hippocampal spine density, neurogenesis, and LTP which may underlie the photoperiodic differences in spatial learning and memory performance.

A well-characterized photoperiodic modification in rodents is altered reproductive physiology. Photoperiod regulates breeding seasons in most non-tropical mammals (Goldman, 1999). Along with inducing changes in reproductive behavior and morphology, photoperiod affects reproductive hormone production (e.g., testosterone in males) via changes in the feedback loop of the hypothalamic-pituitary-gonadal (HPG) axis. Few studies have examined the effects of photoperiod on another hormonal axis, the hypothalamic-pituitary-adrenal (HPA) axis, which regulates production of hormones
(e.g., corticosterone) involved in stress responses and energy balance. Both testosterone and corticosterone can bind to receptors in the hippocampus and therefore alter hippocampal-based behaviors (McEwen, 1994; Xiao & Jordan, 2002). Chapters 6 and 7 test the direct effects of photoperiod-evoked alterations in testosterone and corticosterone concentrations (via surgery, pharmacological agents, or stressors) on spatial learning and memory. In Chapter 6, removal of long-day typical concentrations of testosterone did not alter spatial learning and memory performance in long-day mice, but testosterone supplementation in short-day mice significantly improved spatial learning and memory in the water maze. The photoperiodic differences in behavioral responses to testosterone were not manifested in the amount of androgen or estrogen receptor gene expression in the hippocampus. Corticosterone altered spatial learning and memory based on photoperiod and duration of exposure (acute versus chronic) in Chapter 7. Specifically, stressors, such as repeated injections, complicated the effects of glucocorticoids on water maze performance such that daily saline injections improved learning in short-day mice. In contrast, inhibition of corticosterone synthesis impaired spatial learning in mice from both photoperiods. The differential effects of stressors on water maze performance between long- and short-day mice may be explained by photoperiodic differences in HPA regulation. Short days increased HPA responsivity to various stressors (restraint, water maze, injection), as well as increased sensitivity to HPA negative feedback illustrated by increased GR and MR expression in the hippocampus, faster return to basal corticosterone concentrations following restraint, and increased sensitivity to dexamethasone suppression of corticosterone. In sum, photoperiodic changes in both the HPG and HPA
neuroendocrine axes may trigger the types of adult hippocampal plasticity described in previous chapters and underlie photoperiod-induced changes in learning and memory.

Finally, the last two chapters investigate non-hippocampal photoperiodic targets for adult brain and behavioral plasticity. Besides the hippocampus, the olfactory bulbs are considered the other brain region characterized by significant adult plasticity. Olfaction in rodents is tightly linked to reproduction and other social behaviors (Rekwot et al., 2001) known to be modified seasonally. Thus, in Chapter 8, the effects of photoperiod on olfactory bulb plasticity and olfaction were tested. Aside from increased neurogenesis in the caudal olfactory bulbs of short-day mice, photoperiod did not significantly alter olfactory bulb dendritic or spine plasticity, Bdnf expression, or olfactory behavior. In the final chapter, the potential for similarities between Seasonal Affective Disorder (SAD) in humans and photoperiodic modification of affective behaviors in rodents was tested. Evidence in non-human animals supports the hypothesis that depressive- and anxiety-like behaviors may be adaptive under certain environmental conditions (Prendergast & Nelson, 2005; Price, 1967). Similar adaptive hypotheses have been proposed for humans with symptoms of depression and anxiety (Nesse, 2000; Nesse & Williams, 1996; Wehr et al., 1988). Seasonal regulation of depression in humans with SAD, in particular, suggests that there may be benefits to seasonal modulation of affective behaviors in other animals (Eagles, 2004). In Chapter 9, I predicted that short days would increase affective-like behaviors compared with long days. I tested this hypothesis in two Peromyscus species from varying latitudes (P. leucopus and P. maniculatus) and also examined the potential interaction between testosterone and photoperiod on these behaviors. Testosterone concentrations and latitude of residence
negatively and positively, respectively, correlate with the prevalence of SAD in humans (Orengo et al., 2004; Rosenthal et al., 1984). In contrast to my predictions, photoperiod, latitude, or testosterone did not consistently alter depressive- or anxiety-like behaviors in these species. However, further investigation of photoperiodic changes in olfactory neurogenesis, olfaction, and affective behaviors may be appropriate in a different photoperiod-responsive species.

Collectively, the studies in this dissertation provide evidence for substantial photoperiodic modification of the adult brain, neuroendocrine systems, and behavior. Photoperiodic modifications of the adult brain include changes in structure, volume, neuron production, synaptic strength, and neurochemistry. Photoperiod also modulates the sensitivity and hormone production of multiple neuroendocrine systems. Finally, these changes in brain and physiology may have robust functional consequences by affecting behaviors. The photoperiod-evoked behavioral, physiological, and morphological changes described in this dissertation may confer considerable adaptive benefits during yearly changes in the environment. Further exploration of the adaptive significance of these photoperiodic modifications requires field testing and examination of fitness outcomes. The broader implications of this work are that the potential adaptive significance of these photoperiod-induced changes in adult brain, physiology, and behavior may be residually displayed in humans and underlie seasonal cognitive and affective disorders.
LIST OF REFERENCES


(Peromyscus leucopus) at different photoperiods and temperatures. *Physiological Zoology, 61*(1), 26-33.


