Photoperiod and Temperature Interact to Affect the GnRH Neuronal System of Male Prairie Voles

(*Microtus ochrogaster*)

Lance J. Kriegsfeld, Nathan J. Ranalli, Marie A. Bober, and Randy J. Nelson

Behavioral Neuroendocrinology Group, Departments of Psychology, Neuroscience, and Division of Reproductive Biology, Johns Hopkins University, Baltimore, MD 21218-2686, USA

Abstract  Individuals of numerous species limit energy expenditure during winter by inhibiting reproduction and other nonessential functions. To time these adaptations appropriately with the annual cycle, animals rely on environmental cues that predict, well in advance, the onset of winter. The most commonly studied environmental factor that animals use to time reproduction is photoperiod. Rodents housed in short photoperiods in the laboratory or in naturally declining day lengths exhibit pronounced alterations in reproductive function concomitant with alterations in the hypothalamic gonadotropin-releasing hormone neuronal system. Because animals in their natural environment use factors in addition to photoperiod to time reproduction, the present study sought to determine the independent effects of photoperiod and temperature, as well as the interaction between these factors, on reproductive parameters and the GnRH neuronal system. Male prairie voles were housed in either long (LD 16:8) or short (LD 8:16) day lengths for 10 weeks. Animals in each photoperiod were further subdivided into groups housed in either mild (i.e., 20°C) or low (i.e., 8°C) temperatures. As shown with immunohistochemistry, voles that underwent gonadal regression in response to short photoperiods and long-day voles housed in low temperatures (and maintained large gonads) exhibit higher GnRH-immunoreactive (GnRH-ir) neuron numbers in the preoptic area/anterior hypothalamus (POA/AH) relative to all other groups. In addition, voles that underwent gonadal regression in response to both short days and low temperatures did not exhibit an increase in GnRH-ir neuron numbers compared to long-day, mild-temperature controls. These data suggest that photoperiod and temperature interact to influence reproductive function potentially by alterations of the GnRH neuronal system.

Key words  seasonal, reproduction, testosterone, day length, gonad, arvicoline, rodent

Winter represents a time when peak energetic demands coincide with minimal levels of energy (i.e., food) availability. To survive during the winter, animals inhabiting temperate and boreal latitudes must...

1. To whom all correspondence should be addressed: Randy J. Nelson, Department of Psychology, Behavioral Neuroendocrinology Group, Johns Hopkins University, Baltimore, MD 21218-2686, USA.
2. Present address: Lance J. Kriegsfeld, Department of Psychology, Columbia University, New York, NY 10027, USA.
partition scarce, assimilated energy into those demands directly relevant to survival (Bronson, 1989). Because small mammals have a large surface-to-volume ratio and are unable to store large amounts of fat, most assimilated calories are used for thermoregulation during winter (Bronson and Heideman, 1994). In turn, nonessential functions, such as reproduction, are inhibited (Bronson and Heideman, 1994; Nelson et al., 1990). Cessation of reproduction and the initiation of numerous other seasonal adaptions often require a significant amount of time to accomplish. Thus, animals rely on cues that predict, well in advance, the onset of winter to initiate these changes prior to the onset of harsh winter conditions.

The most reliable proximate cue that animals use to time breeding is the annual cycle of changing photoperiod. Prolonged exposure to short-day lengths in the laboratory induces gonadal regression in many species of small mammals (Bronson and Heideman, 1994; Goldman and Nelson, 1993). Gonadal regression is preceded by a decline in gonadotrophin (luteinizing hormone and follicle-stimulating hormone) secretion (Jallageas et al., 1994; Swann and Turek, 1988). Reduced secretion of the gonadotrophins suppresses gametogenesis and steroidogenesis and eventually leads to regression of the reproductive system (Bartke et al., 1980; Bronson and Heideman, 1994; Gaston and Menaker, 1967; Goldman and Nelson, 1993).

Generally, GnRH-immunoreactive (GnRH-ir) cell numbers are increased in discrete brain regions of rodents housed in short days relative to long-day conspecifics; a similar pattern is seen in wild deer mice (Peromyscus maniculatus) exposed to natural short photoperiods (Glass, 1986; Korytko et al., 1995; Ronchi et al., 1992; Shiotani et al., 1985). In addition, the number and density of GnRH-ir fibers extending into the median eminence are increased in animals housed in inhibitory photoperiods (Glass, 1986; Ronchi et al., 1992). Increased cell numbers and fiber/cellular optical density may indicate inhibited GnRH release. For example, when animals are injected (icv) with colchicine (a potent inhibitor of microtubule polymerization and a blocker of axoplasmic transport), the optical density of immunoreactivity in axons and cell bodies is increased, and more immunoreactive neurons are visualized (e.g., Nishio et al., 1994). Taken together, these data suggest that in rodents, short-day lengths may inhibit reproduction, in part, by suppressing GnRH release sufficiently to prevent the maintenance of normal gonadal function.

In natural populations of rodents, there is considerable variation in the onset and termination of breeding from year to year (reviewed in Nelson, 1987). Although photoperiod is the most reliable predictor of the time of year, given the variability of the breeding season in wild populations of rodents, it is likely not the only cue used to time reproduction (Bronson, 1989; Bronson and Heideman, 1994). Because ambient temperature changes seasonally, animals may also use temperature as a proximate factor to “fine-tune” the year-to-year timing of breeding. In accord with this supposition, low temperatures can inhibit testicular function alone (e.g., deer mice, P. maniculatus) (Dejardins and Lopez, 1983) or in combination with other inhibitory extrinsic factors, including short-day lengths (reviewed in Bronson, 1989 [deer mice, P. maniculatus]; Dejardins and Lopez, 1983 [prairie voles, Microtus ochrogaster]; Nelson et al., 1989).

Most rodent species (e.g., hamsters [P. sungorus sungorus], deer mice [P. maniculatus], meadow voles [M. pennsylvanicus], white-footed mice [P. leucopus]) examined exhibit considerable variation in reproductive responsiveness to photoperiod (Gorman and Zucker, 1997; Nelson, 1987; Prendergast and Freeman, 1999). Prairie voles (M. ochrogaster) are seasonally breeding arvicoline rodents that show phenotypic variation in reproductive responsiveness to photoperiod in the laboratory. During winter in the field, or when maintained on short-day lengths in the laboratory, most male prairie voles undergo gonadal regression (Adams et al., 1980; Charlton et al., 1975; Negus et al., 1977; Nelson, 1985; Nelson et al., 1989; Valentine and Kirkpatrick, 1970). However, in common with several other rodent species (see above), 20% to 40% of male voles do not undergo reproductive regression in response to short days (Nelson, 1987). This phenotypic polymorphism in response to photoperiod appears to be modulated by the GnRH neuronal system; male voles that inhibit reproduction in response to short photoperiods exhibit increased GnRH cell numbers in the preoptic area/anterior hypothalamus compared to long-day voles, while short-day, reproductively competent voles resemble long-day animals (Kriegsfeld and Nelson, 1999).

The general goal of this study was to investigate the mechanism(s) by which extrinsic factors interact to terminate reproduction in a seasonally breeding species. Specifically, this study sought to determine the effects of photoperiod and temperature on reproductive function in male prairie voles. In addition, this
study examined the extent to which reproductive inhibition caused by short photoperiods and/or low temperatures is modulated by the alterations in the GnRH neuronal system. Prairie voles that respond to short days or low temperatures with reproductive inhibition may exhibit increased numbers of GnRH-immunoreactive (GnRH-ir) neurons compared to long-day, reproductively competent animals and voles that do not respond to short days or low temperatures with reproductive inhibition. This pattern of GnRH-ir staining would be suggestive of a decrease in GnRH release in regressed animals. Animals exposed to both inhibitory proximate factors may show a similar pattern of staining, or these animals may exhibit a pattern indicative of a decrease in GnRH synthesis and/or release of GnRH peptide compared to long-day voles or nonresponders. This pattern would be reflected as a decrease in neuron numbers and/or decreased staining intensity of neurons. Finally, alterations of the GnRH system may not be dependent on responsiveness to inhibitory environmental factors; animals exposed to short days and/or low temperatures may exhibit alterations at the level of the GnRH system independent of reproductive status. These possibilities were examined in this study.

MATERIALS AND METHODS

Animals

Eighty-five adult male prairie voles (M. ochrogaster) (> 60 days of age) were used in this study. Animals were housed individually in polycarbonate cages (28 × 17 × 12 cm) with ad libitum access to food (Agway Prolab 2000, Syracuse, NY) and tap water for the duration of the study. All animals were born and maintained on long-day lengths (light:dark [LD] 16:8, lights on 0700 h Eastern Standard Time [EST]) until the beginning of the experimental treatment. Rooms were maintained with an ambient temperature of 21 ± 2°C and relative humidity of 50% ± 5% until the beginning of the study.

Treatment

At the onset of the experiment, animals were moved to one of four experimental conditions: (1) long (LD 16:8, lights on 0700 h EST) days and mild (20°C) temperatures (LD20; n = 7), (2) long days and low (8°C) temperatures (LD8; n = 25), (3) short (LD 8:16, lights on 0700 h) days and mild temperatures (SD20; n = 25), or (4) short days and low temperatures (SD8; n = 28) for 10 weeks. After 10 weeks of exposure to the experimental conditions, voles were injected with a lethal dose of sodium pentobarbital and perfused transcardially with 50 ml of 0.9% saline followed by 200 ml of 4% paraformaldehyde in phosphate-buffered saline (PBS, pH = 7.4). Ten weeks of experimental treatment was chosen to allow adequate time for the reproductive system to fully regress, while ensuring that the animals would not be in a state of photo-refractoriness. The brains were removed, placed into 4% paraformaldehyde for a 2-h postfixation, and subsequently cryoprotected in a 20% sucrose solution overnight. Brains were then frozen on crushed dry ice and stored at –70°C until processed.

Paired testes, paired epididymides, and seminal vesicle weights were recorded at autopsy from all animals. Paired testes weights were used to separate short-day animals into responders (R) and nonresponders (NR). Animals with testes weights > 500 mg were considered nonresponders, while voles with testes weights < 300 mg were considered responders (Nelson et al., 1989). Animals with intermediate paired testes weights were not used in the study. Based on testis mass, animals were assigned to one of the following groups: (1) long-day voles housed in mild temperatures (LD20; n = 7), (2) long-day voles housed in low temperatures (LD8, n = 7), (3) short-day voles maintained in mild temperatures that were reproductively competent (SD20NR; n = 7), (4) short-day voles in mild temperatures that regressed their reproductive systems (SD20R; n = 7), and (5) short-day voles in low temperatures that regressed their reproductive systems (SD8R; n = 7). All animals in LD20 and LD8 maintained reproductive function, while all animals in SD8 inhibited reproductive function (see below).

Immunohistochemical Procedures

Immunohistochemistry was performed in seven replications (5 brains in each “run” × 7 replications) as previously described (Kriegsfeld and Nelson, 1999). One brain from each experimental group was processed in parallel to ensure that any slight variation in each run of immunohistochemistry would affect each group similarly. All incubation times were strictly controlled across replications. Briefly, brains were sectioned in the coronal plane at 40 µm using a cryostat.
Every fourth section was collected into 0.1 M PBS (pH 7.4). Following preincubation in normal goat serum, the sections were incubated for 48 h at 4°C in rabbit antimammalian GnRH (LR-1, gift of R. Benoit, McGill University, Montreal, Canada), diluted 1:60,000 with 0.3% Triton X-100 (Sigma) in PBS. LR-1 is raised against [D-\text{Lys} 6] GnRH conjugated with glutaraldehyde to ovalbumin. The antigenic determinants are amino acids 3-4 and 7-10 (R. Benoit, personal communication, 1998). Sections were then sequentially incubated in biotinylated goat antirabbit IgG (1:250, Vector Laboratories) and avidin-biotin-HRP complex (Vector Laboratories). HRP label was demonstrated using nickel-intensified 0.04% diaminobenzidine (Polysciences, Inc.) in 0.1 M PBS as the chromogen and 0.01% hydrogen peroxide as the substrate.

Cell Counts, Size, and Optical Density

Slides were examined under bright field illumination on a Nikon Optiphot microscope by two independent observers unaware of the experimental conditions to which the animals had been exposed. The distribution of GnRH-immunoreactive cells was mapped onto a mouse brain atlas (Slotnick and Leonard, 1975). In every fourth tissue section, each neuron was assessed for cytoarchitectonic location, two-dimensional cell area, and optical density of staining. This counting procedure is in contrast to previously published reports on prairie voles in our laboratory (Kriegsfeld and Nelson, 1999), in which alternate sections were quantified. We elected to count every fourth section in this study because a post hoc power analysis of data obtained from previous studies suggested that counting every fourth section would provide adequate statistical power for the effect size seen in immunohistochemical evaluation of GnRH in voles. Cells were counted in the medial and lateral septum (MS and LS, respectively), diagonal band of Broca (DBB), preoptic area (lateral and medial; POA), and the anterior hypothalamus (AH). For purposes of analysis, some areas were combined (i.e., MS/DBB, POA/AH). Cells were counted by observers who were unaware of the experimental conditions to which the animals were exposed.

Soma size and optical density (OD) measurements were taken from the two sections with the highest cell counts for each brain region. In previous studies on the GnRH system of male voles in our laboratory, we initially measured all GnRH neurons in all sections counted. Measures across sections were highly, positively correlated. Post hoc analyses indicated that averaging the data from the two brain sections with the highest cell counts resulted in statistically equivalent results to those obtained by measuring every neuron counted. All cell size and OD analyses were performed under 400x magnification. Cells were measured by sending microscopic fields of view onto a power Macintosh 7600 computer using a Sanyo CCD video camera (Model VCC-3972) connected to a Nikon Optiphot microscope. Cell bodies were outlined, and the two-dimensional area was calculated using NIH Image 1.61. If the whole perimeter of the cell was not clearly visible, then it was not measured. This criterion excluded cells that could not be focused in one plane or cells that were not intact. Optical density measures were performed on all cells for which soma area was taken. Each pixel in the gray-scale image capture has a measurable specific intensity. Each pixel has a value ranging from 0 (white) to 256 (black). The average value for all pixels in an outlined area is taken as the mean intensity of staining for a given region of the image. Optical density measures were normalized to minimize differences between replications of immunohistochemistry. First, a background measurement was taken by placing a square outline, eight times, on nonoverlapping, unstained areas of each section. The mean of these eight measures provided the background optical density for each section. The optical density for each cell body was assessed by outlining the cell body, obtaining a density measure using NIH Image, and subtracting the background optical density from the optical density of each cell.

Median Eminence Optical Density Measurements

The optical density of GnRH staining in the median eminence was measured by two naive observers in the region of the main tract of fiber input, rather than in the region of axon terminals, because high-staining density where axons converge results in inaccurate optical density measures (Figure 1). For each brain, three successive sections (of every fourth section) comprising the median eminence were measured. Optical density measures were normalized to minimize differences between replications of immunohistochemistry. First, a background measurement was taken by placing a square outline, eight times, on nonoverlapping, unstained areas of each section. The
mean of these eight measures provided the background optical density for each section. This procedure was repeated on the same section for eight nonoverlapping, stained regions of the section. The mean for these eight measures served as the mean density measure for a particular section. All mean background optical density measures were subtracted from the mean value for each section. For statistical purposes, one measure was calculated for each animal by taking the mean for all three sections measured.

Statistics

Cell body size and optical density measures were averaged separately for each brain region, so that a single value could be analyzed for each animal for each brain region evaluated. Data for body weight, reproductive organs, cell counts, cell size, and cellular optical density were analyzed using a series of one-way ANOVAs. All pairwise comparisons were conducted using the Tukey-HSD test and planned comparisons where appropriate. Comparisons were considered statistically significant when \( p \leq 0.05 \).

RESULTS

Reproductive Measures

When animals were held in long day lengths, all voles maintained reproductive function in both mild and low temperatures. As expected, voles housed in short day lengths and mild temperatures exhibited a phenotypic polymorphism in reproductive responsiveness. When voles were maintained in a combination of short days and low temperatures, virtually all of the animals were classified as responders and inhibited reproductive function (Figure 2).

Seven voles were randomly selected from each condition using a table of random digits, and only the data from the brains/reproductive organs of these animals were analyzed. Although 7 animals were initially assigned to the LD20 condition, and all other groups were created by random assignment of 7 animals, it is unlikely that including more animals in the LD20 group and randomly selecting a subset from this group would have resulted in different results for the LD20 voles. Testis size and cell counts of animals in the LD20 condition exhibited variability between animals similar to \( (p > 0.05) \) the variability between animals in all other conditions. In addition, the mean GnRH-ir
cell counts obtained in LD20 animals in the present study are similar to those obtained in previous studies of male prairie voles in our laboratory (e.g., Kriegsfeld and Nelson, 1999).

Reproductive organ and body weights are presented in Figure 3. Paired testes, epididymides, and seminal vesicles weighed significantly less in SD20R and SD8R voles compared to all other conditions ($p < 0.05$ in each case; Figure 3). LD8 and LD20 voles had significantly larger paired testes weights compared to all other groups ($p < 0.05$ in each case), while LD20 animals had seminal vesicle weights greater than all other groups ($p < 0.05$). LD8 voles had significantly larger epididymides compared to all other groups ($p < 0.05$ in each case). Body weight was significantly lower in both SD20R and SD8R voles compared to all other groups ($p < 0.05$ in each case).

**GnRH Neuronal Assessment**

Representative staining of GnRH-immunoreactive (GnRH-ir) neurons is pictured in Figure 4. GnRH-ir neurons were counted from the medial septum to the anterior hypothalamus. Cell counts for the lateral septum were not analyzed because few cells were seen in this region. LD8 voles exhibited increased GnRH-ir cell numbers in the MS/DBB relative to both LD20 and SD20NR animals ($p < 0.05$ in each case; Figure 5, top panel). No other group comparisons were significant in this region. In the POA/AH, LD8 voles and SD20R...
voles exhibited increased numbers of GnRH-ir neurons relative to all other groups ($p < 0.05$ in each case; Figure 5, bottom panel). There were no other differences among groups in this region.

The optical density of GnRH-ir fibers extending into the median eminence was unaffected by photoperiod, temperature, or reproductive condition (Table 1). Two-dimensional cell area and the relative optical density of GnRH-ir cells for each experimental group, in each brain area quantified, are presented in Figures 6 and 7. In the MS/DBB, cell area was increased in SD20NR voles compared to LD20 and SD8R animals ($p < 0.05$ in each case). In the POA, cell area was greater in SD20NR voles compared to LD20, SD20R, and SD8R animals ($p < 0.05$ in each case). Photoperiod, temperature, and reproductive condition did not affect the relative optical density of GnRH-ir neurons in any brain areas examined ($p > 0.05$).

**DISCUSSION**

The results from this study suggest that temperature and photoperiod interact to affect reproductive function and the GnRH neuronal system. Low temperatures alone do not inhibit reproductive function when male prairie voles are housed in long day lengths. However, when voles are housed in short day lengths and low temperatures, virtually all animals exhibit regressed reproductive systems. As reported in previ-
ous studies (reviewed in Negus et al., 1977; Kriegsfeld and Nelson, 1999), voles maintained in short days and mild temperatures exhibit a range of reproductive responsiveness.

Recent studies indicate that photoperiodic history may affect reproductive responsiveness to short day lengths (Gorman and Zucker, 1997). Exposure to very long day lengths (LD 18:6) for 10 weeks prior to transfer to short day lengths (i.e., LD 10:14) increases the proportion of reproductively nonresponsive Siberian hamsters (*P. sungorus sungorus*) relative to hamsters exposed to a 14:10 LD cycle prior to exposure to short days (Gorman and Zucker, 1997). It has been suggested that this dependency on photoperiodic history

<table>
<thead>
<tr>
<th>Group</th>
<th>Fiber Density</th>
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<tbody>
<tr>
<td>LD20</td>
<td>28.27 (± 2.68)</td>
</tr>
<tr>
<td>LD8</td>
<td>27.10 (± 4.37)</td>
</tr>
<tr>
<td>SD20NR</td>
<td>27.20 (± 2.20)</td>
</tr>
<tr>
<td>SD20R</td>
<td>29.53 (± 2.96)</td>
</tr>
<tr>
<td>SD8R</td>
<td>29.61 (± 5.98)</td>
</tr>
</tbody>
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Figure 1. Relative optical density (± SEM) (higher values represent more intense staining) of GnRH-ir neurons in the brains of voles housed for 10 weeks in long day lengths (LD 16:8) in either mild (20°C) or low (8°C) temperatures or voles maintained on short days (LD 8:16) in mild or low temperatures. Voles were separated into either responders (R) or nonresponders (NR) based on paired testes weight.

Figure 2. Two-dimensional cell area (± SEM) of GnRH-ir neurons in the brains of voles housed for 10 weeks in long day lengths (LD 16:8) in either mild (20°C) or low (8°C) temperatures or voles maintained on short days (LD 8:16) in mild or low temperatures. Voles were separated into either responders (R) or nonresponders (NR) based on paired testes weight. * Significantly larger than both LD20 and SD8R animals (p < 0.05).

Figure 3. Relative optical density (± SEM) (higher values represent more intense staining) of fiber staining in the main fiber tract to the median eminence in the brains of voles housed for 10 weeks in long day lengths (LD 16:8) in either mild (20°C) or low (8°C) temperatures or voles maintained on short days (LD 8:16) in mild or low temperatures. Voles were separated into either responders (R) or nonresponders (NR) based on paired testes weight.

Figure 4. Mean cell area (µm²) of GnRH-ir neurons in the brains of voles housed for 10 weeks in long day lengths (LD 16:8) in either mild (20°C) or low (8°C) temperatures or voles maintained on short days (LD 8:16) in mild or low temperatures. Voles were separated into either responders (R) or nonresponders (NR) based on paired testes weight. * Significantly larger than both LD20 and SD8R animals (p < 0.05).

Figure 5. Mean cell area (µm²) of GnRH-ir neurons in the brains of voles housed for 10 weeks in long day lengths (LD 16:8) in either mild (20°C) or low (8°C) temperatures or voles maintained on short days (LD 8:16) in mild or low temperatures. Voles were separated into either responders (R) or nonresponders (NR) based on paired testes weight. * Significantly larger than both LD20 and SD8R animals (p < 0.05).
may represent a means by which animals in nature evaluate the potential reproductive benefits of developing the reproductive system versus delaying reproduction until the following season (Gorman and Zucker, 1997). However, the results of this study suggest that in their natural environment, voles with a propensity to maintain reproductive function during short-day exposure may nonetheless inhibit reproductive function when ambient temperature is low. If voles born late in the breeding season maintain reproduction, but ambient temperature is too low for pup survival, voles may compromise their own survival with little chance of producing a viable litter. Thus, rather than evaluating only the costs of maintaining reproduction based on photoperiodic information, prairie voles (M. ochrogaster) may use several proximate factors to weigh the costs and benefits of maintaining or inhibiting reproduction during winter (Bronson and Heideman, 1994; Nelson et al., 1989).

These data confirm and extend previous findings on the interaction between photoperiod and temperature on reproductive function. For example, in male prairie voles (M. ochrogaster), a combination of low temperatures (i.e., 5°C) and short photoperiods decreases testicular mass to a greater degree than either factor alone (Nelson et al., 1989). Likewise, low ambient temperatures facilitate gonadal involution resulting from exposure to short days in deer mice (P. maniculatus) (Dejardins and Lopez, 1983). Low temperatures may also act to alter the “critical” photoperiod necessary to support reproduction in Djungarian hamsters (Phodopus sungorus campbelli). Juvenile Djungarian hamsters (P. sungorus campbelli) maintained in day lengths greater than 13 h fully develop their reproductive apparatus by 75 days of age. However, a decrease in temperatures from 22°C to 5°C requires longer day lengths for the reproductive system to develop (Steinlechner et al., 1991).

In addition to alterations in reproductive physiology, exposure to short day lengths led to alterations in the GnRH neuronal system. In this study, voles that inhibited reproduction in short day lengths and mild temperatures increased GnRH-ir neurons in the POA/AH compared to all other groups (excluding LD8 animals) (Figure 5, bottom panel). These data extend previous findings demonstrating that short-day, responsive voles have increased numbers of GnRH-ir neurons in the POA/AH compared to long-day animals (Kriegsfeld and Nelson, 1999).

The data from this study suggest that exposure to a single inhibitory proximate factor may result in the inhibition of GnRH release (i.e., more GnRH-ir neurons visualized with immunocytochemistry). However, inhibition of reproduction due to a combination of proximate factors results in GnRH-ir cell numbers similar to those seen in long-day, reproductively competent voles. In a previous study using an identical experimental design, in situ hybridization for mRNA for proGnRH suggested that GnRH synthesis is unaffected by exposure to a single inhibitory proximate factor, while GnRH synthesis is decreased in animals exposed to both short days and low temperatures (Kriegsfeld et al., 2000). Thus, although the GnRH-ir profile of LD20 and LD8 voles appears similar in the present study, the underlying alterations accounting for these profiles likely differ between these groups. Whereas the in situ hybridization data suggest that GnRH synthesis is inhibited in LD8 but not LD20 voles, the immunocytochemistry data obtained in this study provide further information suggesting that GnRH secretion is also likely inhibited in SD8 voles. If GnRH secretion for LD8 animals was equivalent to that of LD20 voles, whereas GnRH synthesis is inhibited in LD8 voles, there would likely be a decrease in GnRH-ir cells in LD8 voles compared to LD20 animals. Thus, together with the data on GnRH synthesis (Kriegsfeld et al., 2000), the observation that LD8 voles have similar numbers of GnRH-ir cells to LD20 animal suggests that exposure to short days and low temperatures leads to a reduction in GnRH synthesis concomitant with a decrease in GnRH release. Further studies using radioimmunooassay for GnRH in hypotalamic homogenates will help to clarify the means by which photoperiod and temperature interact to alter GnRH synthesis and peptide content.

In this study, LD8 and SD20R animals exhibited more GnRH-ir cell numbers in the POA/AH compared to all groups and did not differ from each other, although LD8 voles had testes weights indicative of reproductive competence while SD20R animals did not (Figure 5, bottom panel). Low temperatures are capable of reducing neuronal secretion rates in vitro (Majundar et al., 1995). Likewise, there is some evidence that short photoperiods decrease pituitary sensitivity to GnRH compared to long-day controls (Fowler et al., 1992; Horan et al., 1993; Martin et al., 1977; Xu et al., 1992). Thus, LD8 voles that have a GnRH-ir neuronal profile similar to SD20R animals may maintain reproductive function because pituitary responsiveness to smaller amounts of GnRH is sufficient to support reproduction.
Cell size was minimally affected by photoperiod, temperature, or reproductive condition in this study (Figure 6). There is some evidence that hamsters (M. auratus; P. sungorus sungorus) and reproductively responsive deer mice (P. maniculatus) increase GnRH-ir cell size after exposure to inhibitory photoperiods (Jallageas et al., 1994; Steinlechner et al., 1991), whereas other studies fail to report cell size (Bittman et al., 1991; Glass, 1986; Ronchi et al., 1992; Yellon 1994). Likewise, mean cellular optical density was unaffected by photoperiod, temperature, or reproductive condition (Figure 7). In similar studies, mean optical density is increased in the POA/AH of white-footed mice (P. leucopus) after prolonged short-day exposure (Glass, 1986), whereas deer mice (P. maniculatus) housed in short photoperiods actually exhibit decreased staining compared to long-day animals (Korytko et al., 1995). These conflicting data, combined with the failure to show an effect of photoperiod, temperature, or reproductive condition on mean optical density, indicate the difficulty in evaluating subtle changes in peptide content by immunocytochemical means. Future studies using a more sensitive assay of GnRH production are necessary to specify the mechanisms regulating differential reproductive responsiveness to photoperiod.

Taken together, the data from this study suggest that day length and temperature interact to affect reproductive function and the GnRH neuronal system of male prairie voles. Future studies are necessary to determine whether photoperiodic history affects responsiveness to short days in prairie voles as in Siberian hamsters (P. sungorus sungorus). Likewise, additional studies are necessary to determine if reproductive nonresponsiveness to short days caused by prior exposure to very long day lengths is abolished by concomitant exposure to low temperatures. These data would help to determine if previous photoperiodic information in nature is ignored during particularly harsh winters.

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