Testicular Apoptosis Is Down-Regulated during Spontaneous Recrudescence in White-Footed Mice (Peromyscus leucopus)

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Abstract Among individuals of many nontropical species, seasonal breeding is timed by tracking changes in the daily photoperiod. Transfer of rodents to short (< 12 h of light/day) day lengths for 6 to 14 weeks can induce regression of the testes by apoptosis. After 16 to 20 weeks of short day exposure, reproductive function is “spontaneously” initiated, and testicular recrudescence is observed. The gonadal mechanisms that underlie testicular recrudescence are not fully understood. If the onset of testicular regrowth that occurs during spontaneous recrudescence reflects a down-regulation of apoptotic signals, then a decline in apoptosis should be noted concurrent with increased testis mass. This experiment sought to assess the role of apoptosis in the restoration of reproductive capacity to photoperiod-inhibited white-footed mice. Males were assigned to long (16:8 LD) or short (8:16 LD) photoperiods for 0, 14, 18, 22, 26, or 30 weeks. At each of these time points, testis mass and testosterone concentrations were assessed. In addition, apoptotic activity was measured using both in situ terminal deoxynucleotidyl transferase dNTP end labeling (TUNEL) and DNA laddering. Short photoperiod exposure induced maximal decreases in testicular parameters after 14 weeks (p < 0.05). After 26 weeks of short days, testis mass was no longer different between males housed in long days and those housed in short days. In contrast, the high incidence of apoptotic TUNEL labeling and DNA laddering observed at 14 weeks was reduced to long day values after 22 weeks of short day exposure. Together, our results establish that a decrease in testicular apoptosis coincides with testicular recrudescence in white-footed mice. The current study demonstrates a decline in the incidence of testicular cell death concomitant with changes in testis mass or length, elucidating a timeline of changes at the cellular level related to the onset of recrudescence.

Key words recrudescence, apoptosis, photoperiod, TUNEL, seasonal reproduction, testis, Peromyscus

Seasonal breeding serves to synchronize energetically expensive reproductive activities to the most favorable annual environmental conditions (Zucker, 2001). For nontropical rodents, breeding is optimized...
during spring and summer seasons when environmental conditions are most conducive to reproductive success. In many species, reproductive timing is achieved by measuring and tracking changes in the daily photoperiod (Bronson and Heideman, 1994). Annual changes in day length are transduced into physiological signals through the nocturnal secretion of the pineal indolamine, melatonin (Goldman and Nelson, 1993; Arendt, 1995; Blank and Freeman, 1991). In many nontropical rodents, short photoperiods or long durations of nightly melatonin secretion decrease reproductive function. Reproductive quiescence results from a reduction in gonadotropin releasing hormone (GnRH) synthesis and secretion, followed by a decline in follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), and gonadal steroid concentrations (Bronson, 1999). In males, these endocrine events result in seasonal atrophy of the testes and subsequent decline in spermatogenic and steroidogenic function (Goldman and Elliott, 1988). Transfer of rodents such as white-footed mice (Peromyscus leucopus) to short (< 12 h of light/day) day lengths can induce regression of testicular tissue within 8 to 14 weeks. The physiological and behavioral quiescence of the nonbreeding season is eventually followed by gonadal recrudescence. After 15 to 20 weeks of short day exposure, the hypothalamic-pituitary-gonadal axis of seasonally breeding rodents is no longer responsive to the inhibitory short photoperiod signal, and reproductive function is reinstated (Schlatt et al., 1995; Matt and Stetson, 1979; Berkowitz and Heindel, 1984).

The neuroendocrine correlates mediating spontaneous gonadal regrowth or recrudescence have been well described for many rodent species (Bartke et al., 1980; Berkowitz and Heindel, 1984; Gram et al., 1982). Recrudescence is preceded by increased pituitary release of FSH and LH and a concomitant rise in serum PRL and testosterone (Bartke et al., 1980; Berkowitz and Heindel, 1984). Such photoperiod-independent endocrine changes induce active cellular regeneration within the testis, including spermatogonial proliferation and angiogenesis (Berkowitz and Heindel, 1984; Mayerhofer et al., 1989).

The cellular mechanisms in testes that underlie the physiological changes that eventually restore reproductive capacity are not fully understood (Schlatt et al., 1995). In the testes of hamsters and white-footed mice, regression induced by short day lengths is a result of apoptotic processes (Furuta et al., 1994; Young et al., 1999; Young et al., 2000). If the testicular regrowth that occurs during spontaneous recrudescence reflects a down-regulation of apoptotic signals, then a decline in both TUNEL-labeled germ cells and testicular DNA fragmentation should be noted prior to obvious changes in testis mass. It is also possible that changes in apoptotic activity could be confined only to the period of testicular regression, or atrophy. In this scenario, apoptosis would peak during regression and subside completely. No decreases in apoptotic cell death would be noted concomitant with the onset of testicular recrudescence; instead, spontaneous regrowth of the testis might be the sole result of increased expression of growth factors. This pattern would mimic pubertal development, where testicular growth is not associated with a decrease in apoptotic activity. Alternatively, the onset of testicular recrudescence could be independent of ongoing apoptotic cell death. In this situation, significant changes in testicular mass would precede clear decreases in testicular apoptosis. Assessing apoptotic activity during spontaneous recrudescence should illuminate the time course of testicular responses during this period of regrowth and correlate changes at the testicular level with seasonal fluctuations in gonadal steroid hormones. The current experiment sought to assess the role of apoptosis in the restoration of reproductive capacity to photoperiod-inhibited white-footed mice.

**MATERIALS AND METHODS**

**Animals**

Eighty adult (> 60 days of age) male white-footed mice (Peromyscus leucopus) were obtained from the Peromyscus Stock Breeding Center (University of South Carolina, Columbia, SC, USA). Animals were housed individually in polypropylene cages (28 × 7.5 × 13 cm) at 21 ± 2 °C and 50% ± 5% relative humidity. All experiments were conducted at Johns Hopkins University in Association for Assessment and Accreditation of Laboratory Animal Care–approved facilities, in accordance with National Research Council guidelines for use of laboratory animals. Tap water and food (Agway Prolab 2000, Syracuse, NY, USA) were available ad libitum for the duration of the experiment. Mice were assigned to long (16:8 LD) or short (8:16 LD) photoperiods for 0, 14, 18, 22, 26, or 30 weeks. From Week 12 of experimental photoperiod treatment, and every subsequent 7 days, males were removed from their cages and weighed. After Week 12 of experimen-
tal photoperiod treatment and every 14 days thereafter, animals were anesthetized with methoxyflurane vapors (Metofane; Medical Developments, Springvale, Melbourne, Australia), weighed, and testes manually expressed into the scrotum for measurement. To determine photoperiodic responsiveness, testis length was measured with calipers to the nearest mm. Animals that failed to respond to inhibitory photoperiod with testicular regression were discarded from the study to leave a final sample size of 5 males per group.

**Testosterone RIA**

After Week 14 of experimental photoperiod exposure and every subsequent 4 weeks thereafter, a blood sample was collected from all animals. Males were lightly anesthetized using methoxyflurane anesthesia (Metofane). Blood was obtained by retro-orbital puncture collected into heparinized tubes and centrifuged for 30 min at 2500 rpm at 4 °C. Plasma was separated and stored at –70 °C until plasma hormone values were determined in duplicate in two 125I double antibody radioimmunoassays (RIAs) (ICN Biomedicals, Costa Mesa, CA, USA). The ICN testosterone assay is specific; cross-reactions with other steroids are < 0.1% to 7.8%. A single pooled sample was run as a standard throughout both RIA runs. Interassay variability was 2.58%; sensitivity is to 0.025 ng/ml. The 125I kit has been validated for use in small rodents in our laboratory (e.g., Kriegsfeld and Nelson, 1996).

**Tissue Processing**

Each month following collection of the blood sample, a group of males (final *n* = 5 from each photoperiod) were further anesthetized with Metofane and the left testis isolated, removed, weighed, and snap-frozen in a bed of finely crushed dry ice. Testes were stored at –70 °C until Week 30, allowing all tissue to be processed for DNA isolation simultaneously. Hemicastrated males were then perfused through the left ventricle with 0.9% heparinized saline followed by 150 to 200 ml of 10% neutral buffered formalin (Electron Microscopy Sciences, Fort Washington, PA, USA) as a fixative. After fixation, the right testis and epididymis were removed, weighed, and post-fixed in 10% formalin for 1 week. Fixed tissue was then washed in PBS and dehydrated in 70% ethanol prior to paraffin embedding.

The TUNEL assay was used to stain for apoptotic activity on 6 µm sections collected every 50 µm of tissue and mounted onto glass slides. After all tissue was cut and mounted, sections were deparaffinized and endogenous peroxidases were quenched with incubation in 3% H2O2 and incubated in a 1:1500 dilution of terminal deoxynucleotidyl transferase (1 unit TdT:3.3 µl labeling buffer) (Trevigen TACS™ 2TdT, Gaithersburg, MD, USA) to label fragmented 3′ OH termini. Biotinylated nucleotides indicating DNA fragmentation were visualized using streptavidin-horseradish peroxidase, and they were colorimetrically detected with TACS Blue Label. Apoptotic activity was quantified by counting the number of cells positive for TUNEL staining within each testis cross section. For each animal, six entire testis crosssections were analyzed. Cells that incorporated the labeled biotinylated nucleotides were considered TUNEL positive (apoptotic) and were counted under bright field illumination (40 ×) on a Zeiss Axioplan 2 microscope (Thornwood, NY, USA) using Stereoinvestigator software (Microbrightfield, Colchester, VT, USA). Negative control sections were processed without TdT and did not show positive labeling. Positive control slides were also processed alongside experimental sections and labeled TUNEL positive (Trevigen, Gaithersburg, MD, USA). To control for reduction of testis size, the number of TUNEL-positive cells was expressed as number of apoptotic cells per total number of seminiferous tubules within each testis cross, as done previously (Young et al., 1999; Young et al., 2000).

**Spermatogenic Index**

To assess reproductive competence, spermatogenic activity was evaluated in five seminiferous tubules per animal (*n* = 5/group) to determine reproductive competence using the spermatogenic index developed by Grocock and Clarke (1974). This index rates the extent of spermatogenic activity in the seminiferous epithelium. Scores assigned ranged from 1 to 5. A value of 5 was given to large tubules displaying complete spermatogenesis; a score of 1 was assigned to small tubules that contained primarily Sertoli cells, spermatogonia, and few primary spermatocytes.

**DNA Isolation and Analysis**

DNA was isolated from fresh-frozen testis tissue by homogenization in a membrane lysing-buffer of 0.1 M
sodium chloride, 0.01 M EDTA, 0.3 M Tris HCl, 0.2 M sucrose. 10% SDS and potassium acetate were added, and DNA was extracted with phenol/chloroform/isoamyl alcohol separations. Total DNA was quantified spectrophotometrically based on absorbance at 260 nm. Individual aliquots of DNA (0.5 µg) were processed for 3′ end labeling with [α-32P] dideoxy-ATP (10 µCi/µl; ICN Biomedicals, Costa Mesa, CA, USA) using 5 U/µl Klenow enzyme (Trevigen, Gaithersburg, MD, USA). Labeled samples (n = 5/group) were separated on a 1.5% Trevigel, dried without heat for 2 h on a gel dryer (BioRad, Hercules, CA, USA), and exposed to Hyperfilm-MP (Amersham Life Sciences, Arlington Heights, IL, USA) for 3 h. After autoradiography, lanes were excised from the gel, and areas that corresponded to low (< 15 Kb) and high (> 15 kb) DNA fractions were counted in a β-counter to determine the extent of apoptotic activity. Counts for high and low molecular weight radioactivity were summed for total counts per minute (CPM) per lane, total CPM counts were compared for variance (ANOVA), and no significant differences were found (p > 0.05). As apoptosis creates low molecular weight DNA fractions, averages of low molecular CPM were used for comparison between groups.

Statistical Analysis

Statistical evaluation of the mean differences between experimental groups was performed by ANOVA, and, for measurements lacking equal variance, a Kruskal-Wallis ANOVA on ranks with significance level set at 0.05 using the Sigma Stat software package (Jandel Scientific, San Rafael, CA, USA) was used. To isolate significant differences between groups, the Student-Newman-Keuls method was used for the pairwise multiple comparisons for all tests except paired testis length, where unequal sample sizes required Dunn’s method for the ANOVA on ranks.

RESULTS

Body Mass

Exposure to long (16:8 LD) or short (8:16 LD) photoperiods for up to 30 weeks had no significant effect on body mass in male white-footed mice (p > 0.05 in all cases). At autopsy, body mass in males ranged from 19.45 g to 24.17 g (Table 1).

<table>
<thead>
<tr>
<th>Week</th>
<th>LD 16:8</th>
<th>LD 8:16</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>20.57 ± 1.1</td>
<td>—</td>
</tr>
<tr>
<td>14</td>
<td>20.29 ± 1.3</td>
<td>19.45 ± 0.9</td>
</tr>
<tr>
<td>18</td>
<td>23.18 ± 0.7</td>
<td>21.95 ± 0.9</td>
</tr>
<tr>
<td>22</td>
<td>24.17 ± 1.7</td>
<td>23.26 ± 0.9</td>
</tr>
<tr>
<td>26</td>
<td>22.23 ± 1.5</td>
<td>22.28 ± 1.1</td>
</tr>
<tr>
<td>30</td>
<td>23.75 ± 0.9</td>
<td>22.19 ± 1.3</td>
</tr>
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NOTE: Body mass: F = 1.66, df = 10, p = 0.123.

Testis Mass and Length

Short photoperiod exposure for 14 to 18 weeks also reduced paired testis mass as compared with males housed in long days (Fig. 1A). No differences in paired testis mass were noted among males housed in long days for up to 30 weeks (Fig. 1A; p > 0.05). With 14 weeks of short photoperiod exposure, testis mass was reduced by 62% as compared with the average mass recorded for long day males and was significantly reduced as compared with all groups (Fig. 1A; p < 0.05). After 18 weeks of short days, initial evidence of testicular recrudescence was apparent; although testis mass was reduced 36% as compared with the average long day value, this decrease was not significant. At 22 weeks of short day exposure, testis mass was significantly reduced by 46% as compared with long day males (p < 0.05). By 26 and 30 weeks, the trend toward testicular recrudescence was evident and testis mass in short day males was no longer significantly reduced as compared with males housed in long photoperiods (Fig. 1A; p > 0.05). After 18 weeks of short days, initial evidence of testicular recrudescence was apparent; although testis mass was reduced 36% as compared with the average long day value, this decrease was not significant. At 22 weeks of short day exposure, testis mass was significantly reduced by 46% as compared with long day males (p < 0.05). By 26 and 30 weeks, the trend toward testicular recrudescence was evident and testis mass in short day males was no longer significantly reduced as compared with males housed in long photoperiods (Fig. 1A; p > 0.05). Significant recrudescence as assessed by increases in testis lengths were observed in males housed for 22, 26, and 30 weeks in short days; lengths in these males were significantly greater than in regressed males housed in short days for 14 or 18 weeks (Fig. 1B; post hoc ANOVA; p < 0.01 in all means comparisons).

Reproductive Competence

The spermatogenic index, a measure of reproductive competence, was reduced after exposure to short photoperiods (Fig. 1C; p < 0.05, n = 5/group) and returned to values not significantly different from long day values after 22 weeks (p > 0.05). Recrudescence was evident in males housed in short days for 22, 26, and 30 weeks; spermatogenic activity was sig-
Significantly increased in these mice as compared with mice housed in short days for 14 or 18 weeks (Fig. 1C; post hoc ANOVA; \( p < 0.01 \) in all cases).

**Testosterone**

Testosterone concentrations did not vary among males housed in long (16:8 LD) days (Fig. 1D; \( p > 0.05 \)). In contrast, short day exposure significantly affected plasma testosterone concentrations in white-footed mice (Fig. 1D). Testosterone concentrations in males housed in short days for 14 weeks were significantly lower than concentrations measured in all long day males and in males housed in short days for 18, 26, and 30 weeks (Fig. 1D; \( p < 0.05 \)). After 18 and 22 weeks in short days, plasma testosterone concentrations had increased substantially as compared with males at 14 weeks, though values were still reduced and differed significantly from males housed for 0, 26, and 30 weeks of long days (\( p < 0.05 \)). The restoration of testosterone values continued with further short day exposure. Testosterone concentrations after 26 and 30 weeks of short days were significantly different from concentrations in short day males at 14 weeks (post hoc ANOVA; \( p < 0.05 \) for both means comparisons). Moreover, at Weeks 26 and 30, plasma testosterone concentrations returned to long day values and did not differ from long day males (Fig. 1D; \( p > 0.05 \)).
Measures of Apoptosis

DNA Fragmentation

Whole-testis apoptosis was assessed using agarose gel electrophoresis of testicular DNA from males in long (16:8 LD) and short (8:16 LD) photoperiods. The extent of DNA ladder patterns resulting from low molecular weight (< 15 kb) fragments did not differ among males housed in long photoperiods (Fig. 2A). In contrast, differences in the extent of apoptotic fragments were noted among males housed in short days (Fig. 2A).

The extent of DNA laddering (CPM of low [<15 kb] molecular weight [α-32P] dideoxy-ATP labeled testicular DNA) in response to extended exposure to short or long day photoperiods was quantified by a β-scintillation counter (Fig. 2B). Among males housed in long days, no significant differences in low molecular weight DNA were noted (Fig. 2B; p < 0.05). The amount of low molecular weight DNA differed among males chronically housed in short days; counts observed after 14 weeks of short photoperiod exposure were significantly higher than counts of recrudescing males housed in short days for 26 and 30 weeks (Fig. 2B; p < 0.05). CPM of low molecular weight DNA were also significantly higher in males housed in short days for 14 weeks as compared with males housed in long photoperiod conditions for 0, 18, 22, 26, and 30 weeks (Fig. 2B) (p < 0.05).

In Situ Labeling

The extent and localization of apoptotic cell death were assessed in cross sections of formalin-fixed testis tissue using the TdT enzyme to end-label fragmented DNA (TUNEL). Figure 4 shows typical staining in males chronically exposed to short or long days. The amount of TUNEL-positive cells per seminiferous tubule was consistent among males housed in long days (Fig. 3A). Large, active seminiferous tubules with spermatozoa and epididymides replete with spermatozoa were typical in males housed in long days (Fig. 3A). After 14 weeks of short day exposure, TUNEL-labeled cells were prominent throughout the degenerated seminiferous tubules (Fig. 3B). Tubule diameter was reduced, and many tubules lacked the full complement of germ cells (Fig. 3B). Sperm concentration also appeared reduced in the atrophied epididymides of short day males after 14 weeks. High incidence of labeling was reduced with further exposure to short day photoperiods. By week 18, the extent of TUNEL-positive cells was substantially decreased (Fig. 3C). TUNEL-labeled cells were identified predominantly as spermatocytes and spermatids due to the size and appearance of the nuclei and their position within the seminiferous epithelium. Spermatogonia also labeled, but with less frequency than other germ cells. Little apoptotic staining was noted in peritubular connective tissue, endothelial cells, Leydig cells, or Sertoli cells.

Apoptosis was quantified within each testis cross section by counting the number of TUNEL-positive
cells per seminiferous tubule (Fig. 4). Counts of TUNEL-positive cells did not differ among males housed in long days through 30 weeks (Fig. 4). Similar to the pattern observed in DNA laddering data, short day exposure for 14 weeks resulted in high levels of apoptotic cell death; males in this group had significantly higher numbers of TUNEL-positive cells as compared with every group (Fig. 4) \((p < 0.05)\). High levels of short photoperiod–induced cell death were reduced as short day exposure continued. Males at 18 weeks of short day conditions showed significantly less TUNEL labeling as compared with males at 14 weeks; however, the number of TUNEL-positive cells was still increased as compared with all long day males and males housed in short days for 22, 26, and 30 weeks. The extent of TUNEL labeling continued to decrease with short day exposure, and the number of apoptotic cells in males housed in short days for 22, 26, and 30 weeks did not differ from values quantified in long day males (Fig. 4) \((p > 0.05)\).

**DISCUSSION**

Assaying the testis for evidence of apoptotic activity during spontaneous recrudescence revealed decreases in both TUNEL-labeled germ cells and testicular DNA fragmentation that corresponded with the recovery of testis during recrudescence. Our results suggest that in *P. leucopus*, the relatively high levels of apoptotic cell death observed during testicular regression peak after 14 weeks of short (8:16 LD) photoperiod exposure, decline sharply between 14 and 18 weeks, and continue to decrease between 18 and 30 weeks of short days. This decline in apoptosis precedes and/or coincides with general measures of reproductive recrudescence. Significant increases in both testis length and spermatogenic activity occurred in SD males after 22 weeks of short days (Fig. 1). After declining with 14 weeks of short photoperiod exposure, testis mass began to increase after 18 and 22 weeks of short days and returned to values not significantly different from long day males after 26 weeks of short photoperiods (Fig. 1). A similar time course of recrudescence has been reported in Syrian hamsters (Berndtson and Desjardins, 1974). Testosterone concentrations, another indication of reproductive recrudescence, also increased significantly among short day males after 26 weeks in short photoperiod (Fig. 1).

The role of programmed cell death during testicular regression has been examined previously in
white-footed mice (Young et al., 1999). Quantification of TUNEL-labeled cells per seminiferous tubule in males housed in long days for 2 to 10 weeks was consistent with the results observed for long day males in the current study. In contrast to testicular regression during which cell death is increased above long day baseline values for approximately 10 weeks, the onset of recrudescence is characterized by a relatively abrupt (4-week) return to low levels of apoptosis. This low level of apoptosis is concomitant with the general return of reproductive competence to long day values.

In the present study, testicular apoptosis decreased prior to significant increases in serum testosterone concentrations. Apoptotic activity decreased significantly between Weeks 14 and 18 in short day males and was reduced to values typical of males housed in long days after 22 weeks. Plasma testosterone concentrations, reduced with initial short day exposure, were significantly increased to long day values after 26 weeks in short days. Both TUNEL labeling and DNA laddering were restored to long day levels 2 weeks prior to the full recovery of testosterone concentrations. In contrast to these findings, withdrawal of testosterone, a known cell-survival factor in the testis, can induce germ cell death, and its reinstatement can attenuate apoptosis in the testis (Woolveridge et al., 1999; Tapanainen et al., 1993). It is possible that testosterone concentrations in testis tissue increased prior to the increases observed in the plasma in short day males. Testosterone concentrations in the testes were not assayed in this study, and plasma T concentrations measured may not fully reflect concentrations of testosterone in the testis. Indeed, the rise in plasma T at Weeks 26 and 30 is preceded by increases in both testis length and spermatogenic index recovery, both T-dependent processes. These potential early increases in testicular testosterone concentrations may influence decreases in apoptosis that occur after 14 to 18 weeks in short days. Alternatively, serum concentrations of testosterone might reflect concentrations in the testes. In this scenario, the protective effects of testosterone may not be involved with the reduction of apoptosis during the regrowth of the testis. Initiation of high levels of apoptotic cell death in the testis during regression may be regulated in a similar manner, as plasma testosterone remains at high concentrations in males at the time of initial increases in apoptotic cell death (Young et al., 1999). Also, other variables, such as expression of 3β-hydroxysteroid dehydrogenase (3β-HSD), aromatase activity, estradiol production, and cofactor regulation, can affect testis concentrations of testosterone (Sharpe, 1990) and thus affect T-regulated processes. In bank voles (Clethrionomys glareolus), testicular immunostaining for aromatase differs seasonally (Bilinska et al., 2000), as do testicular 3β-hydroxysteroid dehydrogenase concentrations in Japanese black bears (Ursus thibetanus japonicus) (Komatsu et al., 1997). It is possible that expression of these steroidogenic enzymes and other regulatory factors changes in white-footed mice housed in long versus short days.

The extent of gonadal regression observed in the males in this study was typical of white-footed mice from similar latitudes (Gram et al., 1982). The P. leucopus used in this study were derived from mice originally wild-caught in Linville, NC (37° 04′N, 81° 52′W). Unlike strains derived from higher latitudes (>40°N) where testicular regression reduces testis mass by 60% to 80%, testis mass in our study decreased by 30% to 60% in reproductively active males (Gram et al., 1982). The timeline of regression observed in this study was also consistent with previous studies; maximal regression occurs at approximately 14 weeks in P. leucopus derived from Williamsburg, VA, USA (37° 15′N), with recrudescence evident at 20 to 25 weeks (Gram et al., 1982). This long duration of recrudescence is typical of laboratory-maintained P.
leucopus (Gram et al., 1982); reproductive function can be regained within 2 to 4 weeks in field populations (Vogt, 1981). Although not significantly different from long day values, testis mass measurements from recrudescing males did not equal long day values after 30 weeks of short days. Testis length and spermatogetic activity, both measures of reproductive competence (Gram et al., 1982; Grocock and Clarke, 1974), were, however, significantly increased in males housed for 22, 26, and 30 weeks of short days when compared with regressed males from 14 and 18 weeks of short photoperiods.

The incidence of programmed cell death in the seminiferous epithelium appears to decrease to long day values in advance of full recrudescence. High levels of apoptosis induced by short day exposure were significantly reduced after 18 to 22 weeks of short days, while testis mass was continuing to increase from 26 to 30 weeks. This suggests that to achieve reproductive competence, recrudescence onset may involve a two-step process: an initial, acute cessation of widespread apoptosis, followed by an extended period of cellular proliferation and differentiation. Levels of apoptosis were significantly reduced from Week 14 to Week 18; significant reproductive recrudescence occurred after Week 22. Mitotic figures were observed at the light level in SD mice housed for 26 to 30 weeks in the present study, although well-defined evidence of proliferation was rare and thus not quantifiable. The apparent separation of notable testis growth from widespread apoptotic activity observed in white-footed mice may serve to enhance the survivability of rapidly dividing and growing testicular cells during recrudescence. Examination of stem cell mitosis during recrudescence, possibly using BrdU labeling assays, would be important future studies to more fully examine the two-stage nature of recrudescence. These assays for cellular growth and proliferation would complement assays for cell death and further extend our knowledge of cellular events occurring during testicular regression and recrudescence.

The role that apoptosis plays during recrudescence may differ among species with different intervals of testicular regrowth, or between cycles of stimulated or spontaneous recrudescence. For example, peak testis mass is regained after 2 to 3 weeks in a stimulatory photoperiod in European starlings, a species whose reproductive response to photoperiod is distinct from that of small rodents. In this species, levels of apoptosis are consistently low throughout the period of reproductive regeneration (Young et al., 2001). It is possible that in some species apoptotic activity above baseline values is only evident during a brief period of testicular regression, and cellular growth is entirely responsible for recrudescence.

The cellular events mediating onset of recrudescence in photoperiodic rodents are relatively unexplored. We hypothesized that regrowth of the testis during recrudescence would be characterized by a decrease in apoptotic cell death. Our results demonstrate that a decrease in testicular apoptosis precedes gonadal recrudescence in white-footed mice. The current study demonstrates a decline in the incidence of testicular cell death prior to changes in testicular activity, testis length, and testosterone concentrations, elucidating a timeline of cellular changes related to onset of recrudescence.

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