Effects of age and photoperiod on reproduction and the spleen in the marsh rice rat (*Oryzomys palustris*)

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Edmonds, Kent E., and Milton H. Stetson. Effects of age and photoperiod on reproduction and the spleen in the marsh rice rat (*Oryzomys palustris*). *Am J Physiol Regulatory Integrative Comp Physiol* 280: R1249–R1255, 2001.—To examine the interactions between age and photoperiod on reproduction and spleen weights, we exposed adult male and female rice rats of various ages to photoperiods of 16:8-h light-dark photoperiods (16L:8D) or 12L:12D. After 10 wk, animals were killed and the following data were recorded: weights of testes, seminal vesicles, uterus, ovaries, body, and spleen and, in addition, vaginal patency. Young adult males displayed a greater degree of testicular and seminal vesicle regression in short photoperiods than did older males; the testes of most older males did not regress in response to short photoperiods. Spleen weight was unresponsive to short photoperiods in all males, but was affected by age. Females, however, exhibited reproductive organ regression and decreased vaginal patency in response to short photoperiods at all ages examined. Body weights were affected by photoperiod in young females, and, as in males, photoperiod had no effect on spleen weights. These data suggest that the reproductive response to photoperiod in adult male rice rats declines with age, whereas in adult females it does not.

Photoperiodism; seasonality; sex differences; reproductive regression; immune function

Photoperiod (day length) is a potent environmental stimulus that affects reproduction in a number of rodent species (5). The effects of photoperiod in adults are manifested by maintenance of reproductive function on long photoperiods and regression of reproductive structures on short photoperiods (20). The pineal gland and its hormone melatonin have been implicated in the photoperiodic regulation of reproduction in all mammals studied to date (35).

Although not an environmental factor, age also has profound influences on reproductive status. In many mammals, reproductive function declines with advancing age. Decreases in sexual behavior and reproductive performance, endocrine characteristics, and reproductive tract characteristics have all been noted in aged mice compared with younger individuals (6). On the other hand, no serious decrements in reproductive function are found in aging male white-footed mice or Syrian hamsters housed on long photoperiods (34, 38).

On short photoperiods, older male hamsters and voles display reduced testicular regression compared with younger males (1, 11, 13, 33). These decreased reproductive responses to short photoperiod may be a consequence of altered circadian rhythmicity. Older animals have been shown to exhibit age-related changes in various circadian parameters such as period (significantly shorter in older animals), entrainment, and rhythm splitting (24) that may affect an animal's ability to measure photoperiodic time. On the other hand, additional recent data in Syrian hamsters suggest that the free-running circadian period in both males and females does not shorten with age (9, 12).

Decreased reproductive responsiveness to the effects of short photoperiods on gonadal regression may be due to changes in melatonin production. In hamsters, age-related changes in pineal melatonin content have been reported (22, 23, 30). In Syrian hamsters, for example, melatonin rhythms are severely dampened in 18-mo-old males and females compared with 2-mo-old animals, whereas in old gerbils (19 mo of age) there is no nocturnal rise in melatonin (32). In voles, on the other hand, no detectable differences in pineal melatonin content in older vs. younger males are observed (11). The attenuated melatonin rhythm in older hamsters and gerbils, although correlating, does not necessarily indicate a role in age-induced alterations in reproductive function. It is possible that there is a change in responsiveness to melatonin in older animals. Data from Donham et al. (11) in older vs. younger meadow voles and from Blank et al. (2) in photoperiodically responsive and nonresponsive phenotypes of deer mice of the same age in which there is no difference in pineal melatonin content suggest that differences in responsiveness to melatonin may explain differences in reproductive photoresponsiveness.

The genus (*Oryzomys*) extends its range from South America into the temperate zone (40). Field studies reveal that the marsh rice rat (*Oryzomys palustris*) exhibits an annual reproductive cycle under natural conditions (15, 25, 41). Photoperiod is an important factor regulating reproductive function in this species; long photoperiods stimulate reproductive development in juveniles and maintain gonadal function in adults.
whereas short photoperiods inhibit reproductive development and induce gonadal regression (16–18). In addition, the pineal gland and its hormone melatonin (which is regulated by photoperiod) play a significant role in reproductive function (14, 17–19).

The objective of the experiments reported here was to assess the effects of age and photoperiod on reproductive status of male and female rice rats. Specifically, we addressed whether age 1) causes any decrements in reproductive size in adult male and female rice rats housed on a long photoperiod and 2) affects the extent of reproductive organ regression in adult male and female rice rats housed on a short photoperiod. In addition, we assessed the effects of photoperiod on spleen weight to determine if a structural component of the immune system in this species is affected by age and/or photoperiod.

MATERIALS AND METHODS

The rice rats used in this study were obtained from our laboratory colony at the University of Delaware, Newark, DE. Food (Prolab Animal Diet #3000, Agway, Syracuse, NY) and tap water were provided ad libitum. Room temperature was maintained at 21 ± 2°C. Rice rats were individually housed in plastic cages (27 × 20 × 15 cm). Pine shavings were used as bedding. Males (5 mo of age) and females (3–4 mo of age) used in this study were virgins, whereas all other age groups used were retired breeders who, during their lifetimes, had been subjected to at least one photoperiodic change during breeding periods. For the studies reported here, animals were housed on 16:8-h light-dark cycles (16L:8D; lights off at 2000) for ≥3 mo before the start of the study and were, therefore, reproductively mature as assessed by palpation of the testes and the presence of vaginal patency. Because some older animals had been exposed to short photoperiods before this experiment, we were careful not to begin this study until any animals housed on short photoperiods (12L:12D) for up to 11 wk had been exposed to long photoperiods (16L:8D) for at least 11 wk before transfer to short photoperiods (12L:12D). In Syrian hamsters, exposure to long photoperiods for ~11 wk terminates the refractoriness that develops after exposure to short photoperiods (36). Because there are no data regarding the photoperiodic requirements for terminating refractoriness in rice rats, we do not know whether 11 wk was sufficient to restore photoperiodic sensitivity in these animals. However, photorefractoriness is not believed to have influenced the experimental outcome. All studies were conducted with the approval of the Institutional Animal Care and Use Committee of the University of Delaware and according to the National Research Council’s recommendations in the Guide for the Care and Use of Laboratory Animals.

Experiment 1: males. To examine whether aging causes decrements in reproductive function on long photoperiods and/or affects the extent of reproductive regression on short photoperiods, adult male rice rats 5, 15, 18, and 24 mo of age at the start of the experiment (7.5, 17.5, 20.5, and 26.5 mo of age at death) were maintained on 16L:8D or transferred to 12L:12D for 10 wk. Animals were killed by decapitation, and weights of testes, seminal vesicles, and spleen were recorded at necropsy. Tissue was debrided and weighed to the nearest 0.1 mg. Seminal vesicles were not stripped of seminal fluid before weighing, therefore seminal vesicle weights reflect both tissue and fluid weight (hereafter referred to only as seminal vesicle weight). We were careful not to lose seminal fluid during the dissections. Final body weights were not recorded in males at death, because males were killed at 2400 to collect pineal glands as part of a separate study and we did not want to potentially affect melatonin levels by measuring body weights. However, initial body weights were recorded and used to divide males into age-matched and weight-matched treatment groups.

Experiment 2: females. To examine the effects of aging and photoperiod on reproduction and to determine if female rice rats exhibit reproductive responses similar to males, adult females 3–4, 11–12, and 18–22 mo of age at the start of the experiment (5.5–6.5, 13.5–14.5, and 20.5–24.5 mo of age at death) were maintained on 16L:8D or transferred to 12L:12D for 10 wk. Animals were killed by decapitation, and weights of uterus, ovaries, spleen, and body were recorded at necropsy. Vaginal patency was also assessed. Tissue was debrided and weighed to the nearest 0.1 mg. Body weights and vaginal patency were assessed at weeks 0, 4, 6, 8, and 10; however, the data for body weight are reported as the change in body weight over the 10 wk of the study and the data for vaginal patency are only reported for weeks 0 and 10 of the study.

Statistical analyses. Data on organ weights collected at necropsy were analyzed by two-way ANOVA for the effects of age, photoperiod, and their interactions. Body weight data collected during the 10 wk of the study were analyzed by two-way ANOVA, and data on the percentage of animals exhibiting vaginal patency were analyzed by χ² with the Number Cruncher Statistical Software Package (21). Because the data were not always normally distributed in each group, they were log-transformed before statistical analyses to make the sample variances homogenous. Where significant differences were found, a Newman-Keuls post hoc test was performed. All data were considered significant at P < 0.05.

RESULTS

Males. Photoperiod and age interacted to affect testicular weights (Fig. 1, top, photoperiod, F = 28.72, P < 0.0001; age, F = 3.57, P = 0.02; interaction, F = 2.80, P = 0.048). All males maintained on 16L:8D, regardless of age, had large testes. In males housed on 12L:12D, the extent of testicular regression was greatest in young (5 mo of age) animals. However, testicular weights were significantly decreased in males (18 mo of age) housed on 12L:12D compared with males maintained on 16L:8D. In males (15 and 24 mo of age) housed on 12L:12D, testicular weights were not significantly decreased compared with males maintained on 16L:8D. It appears that a few older males do retain some photoperiodic sensitivity as gonadal regression occurred to varying degrees.

Seminal vesicle weights were also affected by photoperiod and age (Fig. 1, middle, photoperiod, F = 115.75, P < 0.0001; age, F = 6.04, P = 0.001). An interaction between photoperiod and age just missed statistical significance (F = 2.66, P = 0.057). In all age groups, seminal vesicle weights on 16L:8D were significantly greater than seminal vesicle weights on 12L:12D. However, as was the case with testicular weights, the extent of seminal vesicle regression was greatest in young males. For example, seminal vesicle weights in 5-mo-old males housed on 12L:12D were about one-sixth the size of males housed on 16L:8D, whereas the
differences in seminal vesicle weights in older males housed on 12L:12D were about one-half to one-fourth the size of males housed on 16L:8D. Regardless of age, accessory reproductive organs such as the seminal vesicles appeared to be more affected by photoperiod than were the testes, although age did influence the extent of reproductive organ regression.

Spleen weights (Fig. 1, bottom) were affected by age only \((F = 5.51, P = 0.002)\); photoperiod and the interaction of age and photoperiod were not statistically significant \((P > 0.05)\). In general, older males had larger spleens than younger males. Because we did not record body weights in males in this experiment, we do not know whether photoperiod may have influenced the weight of the spleen relative to body weight. The tremendous variability in spleen weights in the 24-mo-old age groups resulted from two animals (1 in each photoperiod) that had unusually large spleen weights for this species that did not appear to be grossly abnormal in any way.

**Females.** Uterine weights (Fig. 2, top) were affected by photoperiod \((F = 232.25, P < 0.0001)\), age \((F = 34.27, P < 0.0001)\), and an interaction between photoperiod and age \((F = 3.68, P = 0.03)\). Regardless of age, uterine weights were significantly decreased at the end of 10 wk of exposure to 12L:12D compared with animals maintained on 16L:8D.

Ovarian weights (Fig. 2, middle) were affected by photoperiod \((F = 153.61, P < 0.0001)\) and age \((F = 14, P < 0.0001)\), but not by an interaction between photoperiod and age \((F = 0.50, P > 0.05)\). As with uterine weights and regardless of age, ovarian weights were significantly decreased at the end of 10 wk of exposure to 12L:12D compared with animals maintained on 16L:8D.

Spleen weights (Fig. 2, bottom) were affected only by age \((F = 24.01, P < 0.0001)\); photoperiod and the
interaction of age and photoperiod were not statistically significant ($P > 0.05$). As in males, spleens tended to be larger in older animals than in younger animals.

Expression of uterine, ovarian, and spleen weights on a relative (mg/g body wt) basis, rather than on an absolute basis, produced similar conclusions as those derived from absolute organ weights. Therefore, data on relative organ weights are not reported.

The percentage of animals exhibiting vaginal patency was significantly affected by photoperiod (Table 1; $x^2 = 468.95, P < 0.0001$). All females housed on long photoperiods, regardless of age, maintained patent vaginas, whereas almost all females housed on short photoperiods had nonpatent vaginas by the termination of the study. Although we report only the results at weeks 0 and 10, all females who had nonpatent vaginas attained nonpatency by week 6 of the study. Three females in the oldest age group (18–22 mo) failed to exhibit decreased vaginal patency, possibly reflecting a decreased responsiveness to the effects of photoperiod.

Body weights (Fig. 3) were significantly affected by treatment ($F = 27.12, P < 0.0001$), age ($F = 36.01, P < 0.0001$), and an interaction of treatment and age ($F = 13.13, P < 0.0001$). Although middle age (11–12 mo) and older (18–22 mo) females did not significantly gain weight during the 10 wk of the study, young females gained weight throughout the study, with females housed on 16L:8D gaining an average of 8.15 g and females housed on 12L:12D gaining an average of 4.37 g. Body weights at all time points after week 0 were significantly different between younger animals housed on long and short photoperiods.

DISCUSSION

Reproductive responsiveness to photoperiod has been shown to decline with age in several species of rodents housed on short photoperiods, such as the Siberian (Djungarian) hamster, Syrian hamster, prairie vole, and meadow vole (1, 11, 13, 26, 33). Aged rice rats are also reproductively less responsive to short photoperiods, and this decreased responsiveness occurs in males, but not females. The majority of older male rice rats (>15 mo of age at the start of the study) failed to undergo gonadal regression on short photoperiods to the same extent as young (5 mo of age) male rice rats. Similar data have been obtained in adult Siberian hamsters, which fail to undergo gonadal regression after ~1 year of age (1). However, some older male rice rats still retained some degree of photosensitivity as assessed by significantly reduced average testicular weights on short photoperiods.

Seminal vesicle weights were responsive to the effects of short photoperiods at all ages, but they also appeared to be less sensitive in older males. For example, younger males housed on short photoperiods had seminal vesicles that were one-fifth to one-sixth the size of seminal vesicles from males housed on long photoperiods, whereas in older males housed on short photoperiods seminal vesicles were one-half to one-fourth the size of seminal vesicles from males housed on long photoperiods. The decrease in the ratio of seminal vesicle weights between males housed on long and short photoperiods suggests a reduced sensitivity to the effects of short photoperiods. Because the seminal vesicles are androgen dependent, testosterone levels were presumably reduced in short photoperiod-housed males at all ages even though the paired testis weights of most older rice rats were not significantly affected by short photoperiods. This suggests an effect of photoperiod primarily on pituitary luteinizing hormone release.

Age significantly affected spleen weights in both male and female rice rats (older animals had larger spleens). However, spleen weights were unaffected by photoperiod in both sexes. This contrasts with data from Syrian hamsters in which short photoperiod-housed animals had increased spleen weights relative to long photoperiod-housed animals (4, 39). In Syrian

**Table 1. Initial (week 0) and final (week 10) percentage of adult female rice rats exhibiting vaginal patency at various ages and housed on 16L:8D or 12L:12D in experiment 2**

<table>
<thead>
<tr>
<th>Age/Photoperiod</th>
<th>0</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>3–4 mo/16L:8D</td>
<td>100(11)</td>
<td>100(11)</td>
</tr>
<tr>
<td>11–12 mo/16L:8D</td>
<td>100(15)</td>
<td>100(15)</td>
</tr>
<tr>
<td>18–22 mo/16L:8D</td>
<td>100(11)</td>
<td>100(10)</td>
</tr>
<tr>
<td>3–4 mo/12L:12D</td>
<td>100(11)</td>
<td>0(11)*</td>
</tr>
<tr>
<td>11–12 mo/12L:12D</td>
<td>100(16)</td>
<td>0(16)*</td>
</tr>
<tr>
<td>18–22 mo/12L:12D</td>
<td>100(11)</td>
<td>27.3(11)*</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate sample size. One female in the 18–22 mo, 16:8-h light-dark photoperiod (16L:8D) group died just before the termination of the study and, therefore, its data are not included at the week 10 time point. *Significantly different from age-matched, 16L:8D control group.
hamsters, these differences in spleen weights were attributed, in part, to increased numbers of spleen lymphocytes and macrophages (4). Champney (8), on the other hand, found little to no difference in spleen weights in hamsters subjected to short photoperiods and various treatments consisting of melatonin and/or propranolol administration, some treatments of which were designed to mimic long photoperiods. In addition, Nelson and Blom (28) showed that spleen weights were unaffected by melatonin or by the presence or absence of estradiol in ovariectomized (ovx) deer mice. However, ovary-intact deer mice have significantly smaller spleens than ovx deer mice. Spleen weights in intact male rice rats are not significantly different from those of castrated males housed on both short and long photoperiods (K. E. Edmonds and M. H. Stetson, unpublished data), suggesting the lack of an effect of gonadal steroids on spleen weight in rice rats.

Although we found no differences in the effects of photoperiod on spleen weight in this study, effects on other immune indexes have been observed. For example, Blom et al. (3) found photoperiodic effects on white blood cell and lymphocyte counts in deer mice. In addition, spleens of rats, mice, guinea pigs, and birds have been shown to possess melatonin-binding sites (7). This suggests that photoperiod and possibly melatonin are important factors affecting immune parameters in rodents and birds. In rice rats, evening injections of melatonin inhibit growth of the spleen in unilaterally castrated males (K. E. Edmonds and L. Riggs, unpublished data). It remains possible that other immune parameters may be affected by photoperiod and/or melatonin in rice rats, but additional studies are required to address this hypothesis.

The attainment of old age is not uncommon in Oryzomys. We have allowed numerous males to reach advanced age in our animal colony. Frequently, males between 1 and 2 yr of age have successfully sired young (K. E. Edmonds and M. H. Stetson, unpublished data). Others have also reported Oryzomys reaching advanced age in both the field and the laboratory. For example, Negus et al. (25) recaptured an adult male in the field 20 mo after its first capture. C. P. Bloch (personal communication) reported that three rice rats trapped in Virginia were recaptured in the field 13, 14, and 18 mo, respectively, after their initial capture. In addition, Worth (42) reported that males of a different species (Oryzomys laticeps), which were set aside to determine longevity in captivity, died at an average age of 2 yr and 11 mo; the oldest one dying at 3 yr and 9 mo of age. The longevity of Oryzomys both in the laboratory and in the field suggests the usefulness of this animal model to study the effects of aging on reproduction (and circadian rhythms) in photoperiodic rodents.

Females, on the other hand, were reproductively responsive to short photoperiods at all ages examined. Collectively, the uteri, ovaries, and vaginas were all inhibited by exposure to short photoperiods. Spleen weights, as in males, were unaffected by photoperiod. Only age affected spleen weights in females (older females tended to have larger spleens). Body weight increased throughout the 10-wk study in the young (3–4 mo), but not the middle age or older females (11–12 and 18–22 mo, respectively). By the end of the study, young females (5.5–6.5 mo of age at that time) still had not attained the same final body weights as middle age and older females (data not reported). Three females in the 18–22 mo of age group failed to exhibit decreased vaginal patency on 12L:12D (these females also had the three largest uterine weights in the group.) It is unknown if this lack of responsiveness was due to an age-induced lack of responsiveness or a reduced responsiveness to short photoperiods in general. Had these females been examined at any age, they may have been unresponsive to short photoperiods. Variability in the gonadal response to short photoperiods has been demonstrated in individuals of several other rodent species (2, 27, 29) and may have a circadian basis (29).

From a life history perspective, the failure of older male animals to undergo gonadal regression to the same extent as younger males has been proposed to be adaptive (11). Older and, therefore, reproductively mature males may be able to continue their reproductive efforts in the winter in an attempt to produce additional offspring before they, possibly, succumb to harsher winter conditions. Although the offspring might also perish during the winter, the probability exists that a litter can be successfully reared if conditions for survival, such as adequate food or warmer temperatures, are favorable. This hypothesis, however, may be untenable in rice rats due to the observation that females are responsive to short photoperiods at all ages, but older males would be unable to breed with them during the short days of winter when the females undergo reproductive organ regression. At more northerly latitudes, such as in Delaware, female rice rats are reproductively quiescent (decreased uterine weights and decreased vaginal patency) during the winter (15). Similarly, adult male rice rats have regressed testes during the winter (15). However, it is unknown if there were any older adult males in that study, because males were classified into age groups on the basis of body weight only. Older male rice rats may be more likely to breed at southern latitudes, where a small percentage of female rice rats remain reproductively mature throughout the winter (41).

An effect on the ability of the pineal gland to synthesize melatonin has been hypothesized to account for the age-related differences in photosensitivity. In Syrian hamsters, Djungarian hamsters, gerbils, and rats, old age attenuates nighttime levels of melatonin (22, 31, 32) and decreases the activities of enzymes important in the melatonin biosynthetic pathway such as pineal N-acetyltransferase and hydroxyindole-O-methyl transferase (10, 37). On the other hand, Donham et al. (11) found no differences in the pineal melatonin rhythm in younger compared with older meadow voles. However, they only compared voles that had been housed on a short photoperiod (10L:14D) from 20 to 80 days of age vs. voles housed on 10L:14D
from 80 to 140 days of age. Unfortunately, we were unable to measure melatonin rhythms throughout the night in the rice rats in this study due to relatively small sample sizes (n = 5–11 males/group and 10–16 females/group).

In summary, both age and photoperiod affected reproductive status in male and female rice rats. Specifically, the reproductive response of male and female rice rats housed on a long photoperiod was unaffected by age, whereas the response (reproductive organ regression) to a short photoperiod in male rice rats declined with age. In female rice rats, on the other hand, the reproductive response to a short photoperiod did not decline with age. Older females retained responsiveness to a short photoperiod and underwent reproductive organ regression. In addition, photoperiod had no effect on spleen weights in either sex, whereas age did affect spleen weights.

**Perspectives**

Aging is associated with a host of physiological changes. Presumably, some of these changes are designed to enhance the fitness of an individual under natural conditions. The failure of older males to respond to the short daylengths of autumn and winter with gonadal regression is only advantageous if it, ultimately, results in the successful birth and survival of offspring. Because the average life span of the rice rat under natural conditions is probably <1 year, the evolutionary advantage of a sexually dimorphic response to short photoperiods in aged male and female rice rats remains unknown. However, it is conceivable that if older males remain reproductively active, then they may have the advantage (over individuals that undergo a cycle of gonadal regression and recrudescence) of being the first to breed in the spring when the females become reproductively active. Whatever the significance may be, the rice rat appears to be an interesting animal model to address the mechanisms involved in age-related photoperiodic responses. Whether the effects of age are dependent on altered circadian rhythmicity and, subsequently, pineal gland function is an area for additional research.

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