

**Rapid #: -3320346**

**Ariel**  
**IP: 130.58.88.95**

---

**CALL #:** [http://0-www3.interscience.wiley.com.luna.wellesley.edu/cgi- ...](http://0-www3.interscience.wiley.com.luna.wellesley.edu/cgi-...)  
**LOCATION:** **WEL :: Main Library :: inet**  
TYPE: Article CC:CCL  
JOURNAL TITLE: Journal of pineal research  
USER JOURNAL TITLE: Journal of pineal research  
WEL CATALOG TITLE: Journal of pineal research  
ARTICLE TITLE: NELSON,R "REPRODUCTIVE AND NONREPRODUCTIVE RESPONSIVENESS TO PHOTOPERIOD IN LABORATORY RATS  
ARTICLE AUTHOR:  
VOLUME: 17  
ISSUE: 3  
MONTH:  
YEAR: 1994  
PAGES: 123-131  
ISSN: 0742-3098  
OCLC #:  
CROSS REFERENCE ID: 64623634  
VERIFIED:

**BORROWER:** **PSC :: Main Library**  
**PATRON:** **Sara M. Hiebert Burch (SC)**  
PATRON ID: .p1013452  
PATRON ADDRESS: \*  
PATRON PHONE: \*  
PATRON FAX: \*  
PATRON E-MAIL:  
PATRON DEPT: \*  
PATRON STATUS: \*  
PATRON NOTES: \*



This material may be protected by copyright law (Title 17 U.S. Code)  
System Date/Time: 4/8/2010 7:40:26 AM MST

---

# Reproductive and nonreproductive responsiveness to photoperiod in laboratory rats

Nelson RJ, Moffatt CA, Goldman BD. Reproductive and nonreproductive responsiveness to photoperiod in laboratory rats. J. Pineal. Res. 1994;17:123-131

**Abstract:** Laboratory rats (*Rattus norvegicus*) have been traditionally considered nonphotoperiodic because reproductive function is unaffected by day length. However, at least three experimental manipulations of rats—perinatal androgen injection, peripubertal androgen implants, and peripubertal olfactory bulbectomy—have been reported to unmask reproductive responsiveness to photoperiod. The physiological means by which early testosterone treatment or olfactory bulbectomy affect the expression of photoperiodism were hypothesized to operate through similar underlying mechanism(s) that involved gonadotropin and prolactin blood levels. Short day lengths reduce blood levels of gonadotropins in so-called photoperiodic rodent species. Reduced prolactin levels result in virtually all reproductively photoperiodic species housed in short day lengths. In Experiment 1, male weanling rats either were olfactory-bulbectomized or received a sham-procedure and housed for 10 weeks in long (LD 16:8) or short (LD 8:16) days. Short-day rats reduced body mass, testicular sperm counts, and the size of their reproductive systems; olfactory bulbectomy amplified this inhibitory effect for some parameters including testicular and epididymal sperm counts. However, neither short days nor olfactory bulbectomy affected blood titers of follicle stimulating hormone (FSH) or prolactin. Pelage density was also unaffected by photoperiod, but rats retained their juvenile fur color; i.e., short-day rats remained white, but long-day rats became yellowish. In Experiment 2, male rats were injected with testosterone at 3 days of age, then housed in long or short days until 10 weeks of age. Day length alone did not affect any experimental parameter measured in Experiment 2 except fur color; again, short-day rats retained their juvenile fur color. Perinatal testosterone treatment combined with short day lengths suppressed reproductive organ size and function. Blood plasma concentrations of FSH, but not prolactin were reduced at 6 and 10 weeks of age by early testosterone injection. In Experiment 3, male offspring of rats born in our lab were weaned at 21 days of age and implanted with a Silastic capsule that was either empty or filled with testosterone. Animals were housed for 10 weeks in long or short days. Photoperiod did not affect reproductive organ size or function in this experiment; however, pelage color was again affected by day length. There were no significant interactions between photoperiod and hormonal treatment. Rats bearing testosterone capsules displayed reduced reproductive organ size and function. Blood levels of FSH were reduced at 6, but not 10, weeks of age in these animals. Prolactin concentrations were unaffected by testosterone treatment. Taken together, these results suggest that laboratory rats retain some vestiges of photoperiodic responsiveness, indicating that the physiological capability to measure day length is extant, but that the effects of photoperiodic regulation on reproduction are minor and probably of limited functional significance in the laboratory setting.

**Randy J. Nelson,<sup>1</sup>  
Christopher A. Moffatt,<sup>1,2</sup> and  
Bruce D. Goldman<sup>3</sup>**

<sup>1</sup>Department of Psychology, Behavioral Neuroendocrinology Group, The Johns Hopkins University, Baltimore, MD 21218 U.S.A.; and

<sup>3</sup>Department of Physiology and Neurobiology, University of Connecticut, Storrs, CT 06268 U.S.A.

**Key words:** testes function—day length—reproduction—prolactin—follicle stimulating hormone—seasonal cycles—pelage—adipose—body mass—sperm

Address reprint requests to Randy J. Nelson, Department of Psychology, Johns Hopkins University, Baltimore, MD 21218 USA.

<sup>2</sup>Current address: Neuroscience and Behavior Program, University of Massachusetts, Amherst, MA 01003.

Received April 6, 1994; accepted June 22, 1994.

## Introduction

Laboratory rats (*Rattus norvegicus*) are relatively unresponsive to photoperiod. Although female rats of some strains display reproductive responsiveness to day length [Leadem, 1988], male rats have been reported to maintain large testes, mate, and sire offspring despite chronic maintenance in short photoperiods or continuous darkness [Kinson and Robinson, 1970; Reiter et al., 1969; 1971; Turek et al., 1976]. In contrast, individuals of many nontropical rodent species display profound reproductive regression in response to short day exposure [Goldman and Nelson, 1993]; blood levels of gonadotropins, prolactin, and reproductive steroid hormones decline, reproductive organ masses decrease, and mating as well as spermatogenesis stop [Goldman and Elliott, 1988; Yellon and Goldman, 1987]. Three experimental manipulations performed on male laboratory rats have been reported to exhibit interactive relations to photoperiod in their effects on the reproductive system, viz., long-term peripubertal testosterone implants, perinatal testosterone injections, and peripubertal olfactory bulbectomy.

Male rats bearing subcutaneous testosterone implants displayed some testicular regression when housed in short days, but not in long photoperiods [Wallen and Turek, 1981]. Similarly, neonatal injections of testosterone slowed gonadal growth in short-day reared male rats as compared to their long-day counterparts [Vanecek and Illnerova, 1982]. Removal of the olfactory bulbs also unmasked reproductive responsiveness to blinding and short photoperiods [Reiter et al., 1969; Nelson and Zucker, 1981]. Olfactory-bulbectomized rats discriminated long from short-day lengths via an endogenous circadian mechanism; among bulbectomized rats, as among individuals of photoperiodic rodent species, the phase of light rather than its duration determined the response of the reproductive system [Nelson et al., 1982]. Sensory functions of the olfactory bulbs are apparently not involved in the mediation of photoperiodic responsiveness [Nelson et al., 1985]. Prior removal of the pineal gland blocked reproductive responsiveness to short days in all three paradigms [Nelson et al., 1985].

The physiological means by which early testosterone treatment or olfactory bulbectomy affect the expression of photoperiodism are unknown. We hypothesized that the three experimental manipulations unmasking reproductive responsiveness to photoperiod in rats operated through a similar underlying mechanism(s). Gonadotropin levels are not usually affected by day length among laboratory rats [Wallen and Turek, 1981; Ronnekleiv and

McCann, 1975a,b]. Because reductions in blood concentrations of prolactin and gonadotropin levels are observed among species that typically regress their reproductive systems in response to short days, we presumed that mechanisms that regulated prolactin and gonadotropin secretion would be involved as possible common physiological pathways inducing rat reproductive photoperiodism.

Reduced prolactin levels are observed in virtually all reproductively photoperiodic species when they are maintained in short day lengths [Goldman and Nelson, 1993]. If short days suppressed plasma prolactin levels in rats, then any manipulation that reduced gonadotropin levels would result in an endocrine profile resembling that of a so-called photoperiodic animal chronically maintained in short days; i.e., reduced blood levels of both prolactin and gonadotropins [Goldman and Nelson, 1993]. It was hypothesized at the onset of the study, therefore, that olfactory bulbectomy and early testosterone treatment in previous experiments caused either temporary or chronic reductions in blood levels of follicle stimulating hormone (FSH). We believed that when these experimental manipulations were paired with short-day exposure, plasma levels of prolactin would also be reduced and the reproductive system would fail to develop as quickly as those without both hormonal deficits. This proposition was tested in the present study.

## Materials and methods

### Experiment 1

**Housing conditions.** Eighty 22-day-old male Sprague-Dawley rats (Charles River) were shipped to our laboratory and housed 5–6 per cage (45 × 24 × 20 cm) in LD 16:8 (16 hr light/day; lights on 0600 h Eastern Standard Time [EST]) photoperiods at  $21 \pm 2^\circ\text{C}$  and a relative humidity of  $50 \pm 10\%$  for 2 days. Food (Agway Prolab 1000; Syracuse, NY) and tap water were provided ad libitum. At 24 days of age, rats were subjected either to olfactory bulbectomy or a sham-procedure. After surgery, animals were housed individually until the end of the study. The procedures used in Experiment 1 were based on the methods described in Nelson and Zucker (1981).

**Surgical procedures.** At 24 days of age, male rats were anesthetized with an intraperitoneal (i.p.) injection of a mixture of ketamine (50 mg/kg), rompun (5 mg/kg), and acepromazine maleate (5 mg/kg) and randomly assigned to one of two surgical manipulations: olfactory bulbectomy or a sham operation. After surgery, animals either re-

mained in long days or were transferred to a room programmed to be illuminated for 8 hr/day (LD 8:16; lights on 1000 h EST) and maintained at  $21 \pm 2^\circ\text{C}$  and  $50 \pm 10\%$  relative humidity.

Olfactory bulbectomies were performed by drilling a 2.5 mm hole medially just posterior to the nasal-frontal suture. After the bulbs were visualized, they were removed by aspiration. The resulting hole was packed with Gelfoam (Upjohn, Kalamazoo, MI) and the skin was sutured. The sham-operated rats received a 2.5 mm hole drilled medially and posterior to the nasal-frontal suture. The bulbs were visualized, hemostasis was achieved by direct pressure, and the scalp incision was sutured. Animals recovered from surgery in their new home cages warmed to  $35^\circ\text{C}$  by thermostatically controlled heating pads. This resulted in 20 animals per surgical condition in each photoperiodic treatment condition.

Six weeks post-surgery, all animals were anesthetized by methoxyflurane vapors (Metofane; Pitman-Moore, Ft. Washington, NJ) and a blood sample was obtained from the retro-orbital sinus 1 min or less after introduction into the anesthesia jar [Riley, 1960]. The blood samples were centrifuged (3,500 rpm) for 1 hr at  $4^\circ\text{C}$ , the plasma separated, and stored frozen ( $-80^\circ\text{C}$ ) until radioimmunoassays were performed.

*Autopsy procedures.* Ten weeks after the surgery, all animals were again anesthetized by methoxyflurane vapors and another blood sample was obtained from the retro-orbital sinus; rats were then killed by lethal injection of sodium pentobarbital. The animals were weighed. Paired testes, epididymides, seminal vesicles, as well as intrascapular brown fat and gonadal fat pads were dissected at autopsy and weighed.

Paired testes (capsule removed) and epididymides were minced with dissecting scissors, separately transferred to an Eberbach blender, and homogenized for 60 and 90 sec, respectively. The number of sperm-shaped cells resistant to homogenization was determined in duplicate for each homogenate in a hemocytometer under phase-contrast microscopy. The average value was used to compute the final number of sperm per paired organ.

Pelage quality and color was also assessed. Fur depth and length of underhair and guard hair were measured with calipers to the nearest 0.1 mm. Pelage density was obtained by shaving and weighing ( $\pm 0.01$  mg) a  $1\text{ cm}^2$  patch of fur obtained from the posterior dorsal surface of rats fitted with a template. Two colleagues, uninformed to the treatment conditions of the rats, rated the fur color of each animal on a 10-point scale; 1 was considered

white (Munsell color chip = 5Y 9/1) and 10 was considered yellow (Munsell color chip = 2.5Y 8/6). The average color rating for each rat was computed.

*Hormone assays.* Plasma FSH and prolactin were measured using RIA kits obtained from the National Institute of Arthritis, Metabolic and Digestive Diseases. For the FSH assay, anti-rat FSH-S-7 was the primary antibody and rat FSH-RP-1 was the standard. For the prolactin assay, the primary antibody was anti-rat prolactin-S-8 and the standard was rat prolactin-RP-1. The amounts of plasma used in these assays were 100  $\mu\text{l}$  for FSH and 30  $\mu\text{l}$  for prolactin. A single determination was performed for each sample, and all determinations for a given hormone were made in a single assay.

*Statistical analyses.* Comparisons among groups for each experimental parameter were analyzed with an overall analysis of variance (SAS General Lineal Model for unbalanced ANOVA, Version 5, 1985). Individual pair-wise comparisons were analyzed with independent two-tailed t-tests. The treatment effects were considered statistically significant if  $P < 0.05$ . All analyses involved planned comparisons; consequently, no post-hoc correction factors were deemed necessary (Keppel, 1982).

## Experiment 2

*Housing conditions.* Adult male and female Sprague-Dawley rats (Charles River) were shipped to our laboratory. Mating pairs were established and housed in LD 16:8 (16 hr light/day; lights on 0600 hr EST) photoperiods at  $21 \pm 2^\circ\text{C}$  and a relative humidity of  $50 \pm 10\%$ . Food (Agway Prolab 1000; Syracuse, NY) and tap water were provided ad libitum. Animals were housed in polycarbonate cages ( $45 \times 24 \times 20$  cm). Two weeks after insemination (assessed by visualized sperm plug), pairs were maintained either in LD 16:8 or LD 8:16 (lights on 1000 EST) photoperiods. Male offspring were assigned to one of the two treatment conditions described below. The procedures for Experiment 2 were based on the methods described by Vanecek and Illnerova (Vanecek and Illnerova, 1982).

*Testosterone treatment.* Male rats were injected with testosterone (1 mg) (Sigma, St. Louis, MO) suspended in sesame seed oil (0.1 cc) or the oil vehicle alone at 3 days of age. Males were weaned at 21 days of age, individually housed, and remained thereafter in the photoperiodic conditions in which they were reared until 10 weeks of age. This resulted in the following experimental groups: LD

TABLE 1. Body and reproductive organ masses, and sperm counts at 10 weeks of age for olfactory-bulbectomized and sham-bulbectomized male rats (Experiment 1)

Group	Body mass (g)	Paired testes mass (mg)	Paired epididymal mass (mg)	Seminal vesicle mass (mg)	Testicular sperm count ( $\times 10^6$ )	Epididymal sperm count ( $\times 10^6$ )
16:8 Sham	507.6 $\pm$ 10.7	3580 $\pm$ 150	1150 $\pm$ 50	510 $\pm$ 30	391 $\pm$ 29	377 $\pm$ 31
8:16 Sham	415.7 $\pm$ 14.7	3120 $\pm$ 70	1030 $\pm$ 20	460 $\pm$ 20	332 $\pm$ 16	416 $\pm$ 31
16:8 Obx	422.9 $\pm$ 11.0	3190 $\pm$ 50	1020 $\pm$ 20	440 $\pm$ 20	435 $\pm$ 47	447 $\pm$ 24
8:16 Obx	356.3 $\pm$ 11.9	2920 $\pm$ 80	930 $\pm$ 40	400 $\pm$ 20	316 $\pm$ 18	347 $\pm$ 21

16:8; testosterone-injected:  $n = 20$ ; LD 16:8; oil-injected:  $n = 20$ ; LD 8:16; testosterone-injected:  $n = 20$ ; LD 8:16; oil-injected:  $n = 20$ .

Blood samples were drawn at six and ten weeks of age. Other autopsy procedures, hormone assays, and statistical analyses were conducted as in Experiment 1 except that animals were killed at 10, rather than 13, weeks of age. This change was made to facilitate comparisons to previous studies in which rats were killed at 75 days of age [Vanecek and Illnerova, 1982].

#### Experiment 3

**Housing conditions.** Adult male and female Sprague-Dawley rats (Charles River) were shipped to our laboratory. Mating pairs were established and housed in LD 16:8 (lights on 0600 hr EST) photoperiods at  $21 \pm 2^\circ\text{C}$  and a relative humidity of  $50 \pm 10\%$ . Food (Agway Prolab 1000; Syracuse, NY) and tap water were provided ad libitum. Animals were housed in polypropylene cages ( $45 \times 24 \times 20$  cm). Male offspring were weaned at 21 days of age and assigned to one of the two treatment conditions described below and housed either in LD 16:8 or LD 8:16 photoperiods. The procedures used for Experiment 3 were based on Wallen and Turek [1981].

**Testosterone treatment.** At weaning, males were weighed, anesthetized with methoxyflurane vapors, and implanted subcutaneously with Silastic capsules that were either empty or filled with testosterone (Sigma: St. Louis, MO) (10 mm of testosterone was packed into 15 mm capsules; OD = 0.318 cm; ID = 0.157 cm). This resulted in four experimental groups: LD 16:8; testosterone-implanted:  $n = 20$ ; LD 16:8; empty implant:  $n = 20$ ; LD 8:16; testosterone-implanted:  $n = 20$ ; LD 8:16; empty implant:  $n = 20$ .

Blood samples were drawn at six and ten weeks after implant. Animals were killed after the second blood sample was obtained. Other autopsy procedures, hormone assays, and statistical analyses were conducted as in Experiment 1.

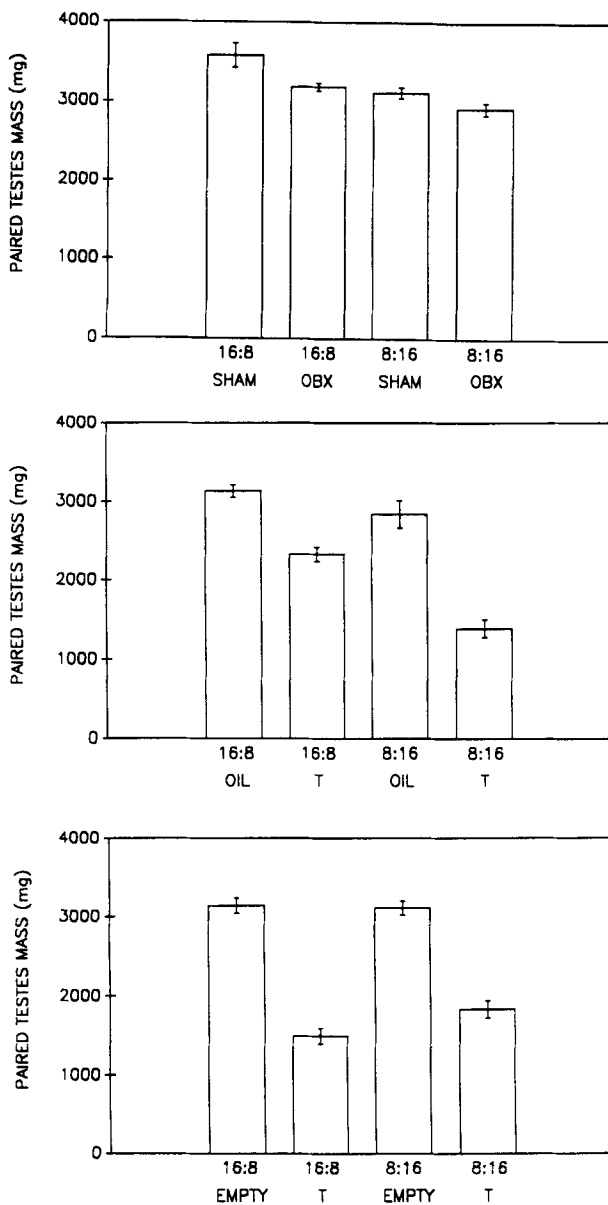
The three experiments were conducted sequentially. There were no failures associated with the lighting schedules; however, for two days during week 5 of Experiment 3 the thermostat failed and animals in both lighting conditions were exposed to ambient temperatures between  $27$  and  $30^\circ\text{C}$ . Because no photoperiodic effects were obtained, Experiment 3 was repeated with new animals using the same experimental protocol and sample sizes.

## Results

### Experiment 1

In contrast to previous studies, short photoperiods inhibited reproductive function in the Sprague-Dawley strain of laboratory rats. Short-day exposure led to reduced paired absolute and relative testes and epididymal masses, as well as absolute seminal vesicle mass ( $P < 0.05$  in each case; Table 1 and Fig. 1). Although epididymal sperm counts were unaffected by photoperiod ( $P > 0.05$ ), testicular sperm numbers were reduced among short-day rats ( $P < 0.05$ ; Table 1). Gonadal fat pad and intrascapular brown adipose tissue weighed less in short than in long-day rats ( $P < 0.05$  in each instance). Day length did not affect blood concentrations of FSH or prolactin either at 6 or 10 weeks of age ( $P > 0.05$  in every comparison; Table 2). Day length also did not affect pelage development ( $P > 0.05$  in each case), but did affect fur color. The fur of short-day rats was rated to be whiter than long-day animals ( $P < 0.05$ ; Fig. 2). Consistent with previous reports, short days reduced body mass growth as compared to long-day animals ( $P < 0.05$ ; Table 1).

Olfactory bulbectomy seemed to amplify the photoperiodic effects for some, but not all, experimental parameters. For example, short-day bulbectomized rats weighed less than long-day bulbectomized rats ( $P < 0.05$ ; Table 1). Similarly, testicular and epididymal sperm numbers were reduced among short-day, as compared to long-day, bulbectomized rats ( $P < 0.05$  in both cases; Table 1). However, olfactory bulbectomy did not affect some other experimental parameters. For instance, when



**Fig. 1. Upper panel:** Mean ( $\pm$  standard error of the mean [S.E.M.]) paired testes mass (mg) of adult rats that were housed in long (LD 16:8) or short (LD 8:16) days and received olfactory bulbectomies (OBX) or sham-operations (SHAM). **Middle panel:** Mean ( $\pm$  S.E.M.) paired testes mass (mg) of adult males housed in long or short days and injected either with testosterone dissolved in sesame seed oil (T) or the oil alone (OIL) when 3 days of age. **Bottom panel:** Mean ( $\pm$  S.E.M.) paired testes mass (mg) of adult males housed in long or short days and implanted at 21 days of age either with an empty Silastic capsule (EMPTY) or one filled with testosterone (T).

corrected for body mass, seminal vesicle mass did not differ among the groups. Likewise, olfactory bulbectomy did not affect pelage development.

#### Experiment 2

Day length alone did not affect any experimental parameter measured in Experiment 2 except testic-

ular mass and fur color (Figs. 1 and 2; other data submitted for review, but not shown). There were no significant differences in testicular mass between long and short-day animals, but short-day rats treated on day 3 with testosterone displayed greater reproductive inhibition than long-day rats treated with early testosterone ( $P < 0.01$ ; Fig. 1). Fur color was judged to be whiter in short- than in long-day rats ( $P < 0.05$ ; Fig. 2).

Early testosterone treatment induced significant suppression of reproductive organ size and function. For example, absolute and relative paired testes and epididymides mass, as well as seminal vesicle mass, were less in short- than in long-day rats ( $P < 0.05$  in each case; Table 3). Both testicular and epididymal sperm counts, were lower in testosterone-injected rats at 3 days of age than in oil-injected rats ( $P < 0.05$  in both cases; Table 3). Blood plasma concentrations of FSH, but not prolactin were reduced at 6 and 10 weeks of age by early testosterone treatment ( $P < 0.05$  for FSH at both ages;  $P > 0.05$  for prolactin at both ages; data submitted for review, but not shown). Body mass and absolute gonadal fat pad and intrascapular brown adipose tissue masses were unaffected by early testosterone treatment ( $P > 0.05$ ; Table 3); however, gonadal fat pad and intrascapular brown fat pad masses were reduced in short day rats subjected to early testosterone treatment ( $P < 0.05$  in both cases; data submitted but not shown).

#### Experiment 3

Photoperiod did not affect reproductive organ size or function among rats in either iteration of this experiment (data submitted, but not shown). However, short-day exposure suppressed both absolute and relative intrascapular brown fat masses ( $P < 0.05$ ). Pelage color was also affected by day length. Again, fur color was judged to be whiter in short- than in long-day rats ( $P < 0.05$ ; Fig. 2).

Implants of Silastic capsules containing testosterone affected reproductive organ size and function. Both absolute and relative paired testes and epididymides masses were reduced in animals bearing testosterone implants as compared to rats bearing empty capsules ( $P < 0.05$  in both cases; Table 4). Relative, but not absolute, seminal vesicle mass was reduced by testosterone treatment. Both testicular and epididymal sperm numbers were reduced among rats bearing testosterone capsules ( $P < 0.05$  in both cases; Table 4). Blood plasma levels of FSH at 6 weeks of age were reduced among animals bearing testosterone implants ( $P < 0.05$ ), but other hormone levels were unaffected by content of the capsule ( $P > 0.05$ ; Table 5). Body mass, but nei-

TABLE 2. Plasma FSH and PRL levels at 6 and 10 weeks of age for olfactory bulbectomized and sham-bulbectomized male rats (Experiment 1)

Group	FSH 6 weeks (ng/ml)	FSH 10 weeks (ng/ml)	PRL 6 weeks (ng/ml)	PRL 10 weeks (ng/ml)
16:8 Sham	9.17 ± .82	7.69 ± .65	12.61 ± 2.02	18.40 ± 1.56
8:16 Sham	8.35 ± .51	7.68 ± .51	11.53 ± 1.48	15.51 ± 1.14
16:8 Obx	6.42 ± .72	6.24 ± .36	14.67 ± 2.71	15.41 ± 2.45
8:16 Obx	8.23 ± .61	6.63 ± .33	11.46 ± 1.58	13.49 ± 1.50

ther gonadal fat pad nor intrascapular brown adipose tissue depot masses, were affected by testosterone treatment (data not shown). Both underhair length and fur density were increased by testosterone exposure ( $P < 0.05$  in both cases).

## DISCUSSION

Reproductive function was reduced slightly by short-day exposure in Experiment 1. Olfactory bulbectomy in short-day animals appeared to slow maturation rate relative to short-day sham-bulbectomized animals or long-day animals. Similarly, perinatal testosterone exposure delayed testicular development, but had minimal effects on other reproductive organ sizes. In contrast, short day animals in Experiment 3 did not delay maturation of any reproductive function. However, other nonreproductive functions were suppressed in short- but not in long-day animals. Taken together, these results suggest that laboratory rats retain some vestiges of photoperiodic responsiveness, but that the effects of photoperiodic regulation on reproductive activities are minor and of questionable functional significance in the laboratory setting. In all studies, short-day animals maintained the soft, white juvenile coat, whereas long-day rats developed the yellowish, coarse adult coat by the same age. Thus, all animals seemed to process photoperiodic information, but differed in the extent to which reproductive function was coupled to photoperiod. Earlier work suggested that the transition from the juvenile to the adult pelage might be triggered by an increase in prolactin secretion at puberty [Rennels and Callahan, 1959; Duncan and Goldman, 1984]. However, differences in plasma concentrations of prolactin corresponding to the differences in pelage quality were not detected in the present study.

The primary goal of this study was to discern the common physiological mechanisms underlying photoperiodic responsiveness among laboratory rats. This goal was not achieved, in part, because rats failed to respond to short days after experimen-

tal manipulations in Experiment 3. In any case, photoperiodic effects on prolactin were not observed among short-day rats. Furthermore, experimental manipulations that were selected to reduce gonadotropin levels did not affect gonadotropin levels differentially in the two photoperiods. This failure to replicate previous reports completely [e.g., Wallen and Turek, 1981; Vanecek and Illnerova, 1982], as well as to replicate the photoperiodic effects observed in Experiment 1, requires explanation. The most obvious difference between Experiment 1 and Experiments 2 and 3 was that animals in Experiment 1 were reared at the supplier and shipped to our facility when 120–160 g in weight. Animals in Experiments 2 and 3 were reared from conception in our laboratory. How this difference may have accounted for the observed changes in photoperiodic responsiveness is not clear. Hormonal levels among the three experiments did not differ significantly.

Previous studies indicated that early testosterone manipulations paired with short-day exposure impaired reproductive function [Wallen and Turek, 1981; Vanecek and Illnerova, 1982; Wallen et al., 1987]. We did not replicate some of these findings, although some nonreproductive functions were affected by photoperiod after perinatal or prepubertal testosterone manipulation. The main difference between the published accounts of earlier studies and the present study was the strain of rats used. Sprague-Dawley rats provided by Charles River were used in all of the present experiments. Although this strain and supplier were used in previous studies of the interaction between olfactory bulbectomy and rat photoperiodism [Reiter et al., 1969; Nelson and Zucker, 1981] ARS Sprague-Dawley rats from Gibco [Wallen and Turek, 1981], and Wistar-Konarovice rats [Vanecek and Illnerova, 1982] were used in previous studies of early testosterone treatment and rat photoperiodism. Strain differences in photoperiodic responsiveness of reproductive function have been reported previously for golden hamsters (*Mesocricetus auratus*) [Eskes and Zucker, 1978; Ogilvie et al., 1992], as well as laboratory rats (Leadem, 1988).

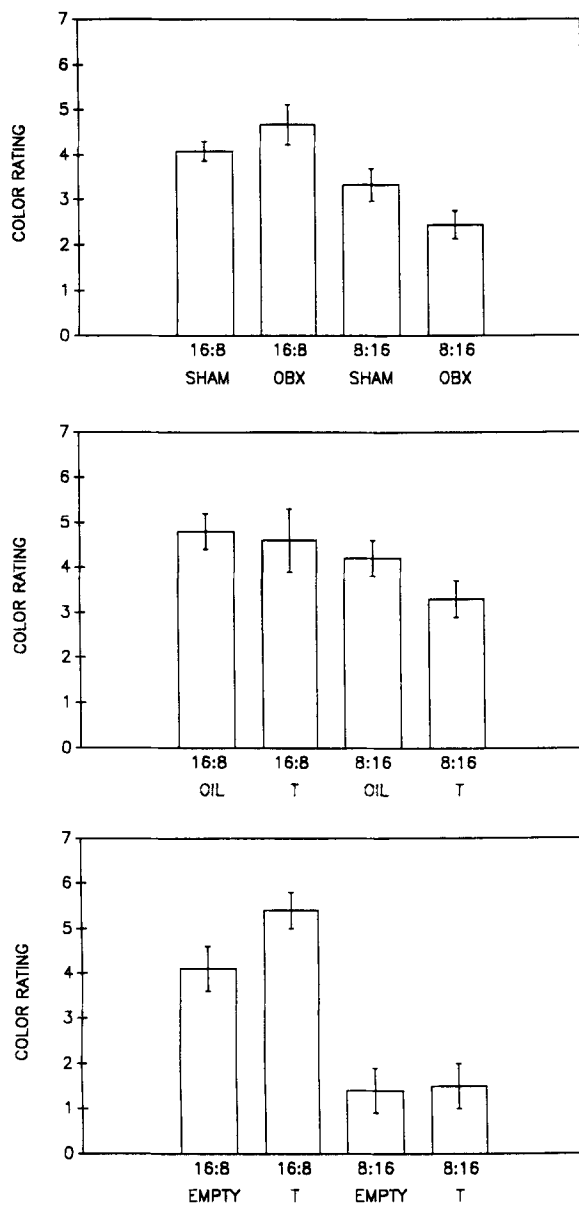


Fig. 2. **Upper panel:** Mean ( $\pm$  S.E.M.) color rating on a 10 point scale (only 1–7 shown) where 1 was considered white (Munsell color chip = 5Y 9/1) and 10 was considered yellow (Munsell color chip = 2.5Y 8/6) for the fur of adult male rats that were housed in long (LD 16:8) or short (LD 8:16) days and received olfactory bulbectomies (OBX) or sham-operations (SHAM). **Middle panel:** Mean ( $\pm$  S.E.M.) color rating of the fur of adult males housed in long or short days and injected either with testosterone dissolved in sesame seed oil (T) or the oil alone (OIL) when 3 days of age. **Bottom panel:** Mean ( $\pm$  S.E.M.) color rating of the fur of adult males housed in long or short days and implanted at 21 days of age either with an empty Silastic capsule (EMPTY) or one filled with testosterone (T).

These strain differences may affect one or more components of reproductive responsiveness to day length among rats, which typically requires experimental manipulation to be revealed. But even within these special experimental circumstances,

reproductive responsiveness to photoperiod is usually observed only among young rats; i.e., reproductive maturation is slowed by short days. This is similar to some other so-called "photoperiodic" species that display reproductive responsiveness only during a specific phase of development (e.g., cotton rats [*Sigmodon hispidus*] [Johnston and Zucker, 1979]). Also, the critical photoperiod necessary to suppress reproductive function in short days (i.e., the minimum daily number of hours/day that maintains reproductive function) is between 8 and 9 hr among rats [Wallen et al., 1987]. The critical photoperiod among so-called photoperiodic species usually ranges between 11 and 14 hr [Goldman and Elliott, 1988]. However, an individual may possess a range of critical photoperiods for different seasonal adaptations (e.g., reproductive function and coat color [Duncan et al., 1985]).

The most consistent photoperiodic effect observed in our three experiments involved coat color. Rat pups develop a soft, white coat several days after birth that is replaced after puberty by a coarse yellowish coat. Rats housed in short days maintained their white, juvenile coats as compared to long-day animals which developed the adult-type yellowish pelage. Thus, short days appear to slow maturation of the pelage. It is possible that laboratory husbandry practices have selected against reproductive responsiveness to day length. Nonbreeding individuals were likely culled from laboratory colonies and a trait for reproductive unresponsiveness to photoperiod could be fixed in a population quickly. However, pelage development and color would not be subject to these same artificial selection pressures and could remain responsive to day length. Thus, the physiological mechanisms associated with photoperiodism remain extant in this species. However, the reproductive system seems to be uncoupled from photoperiodic regulation except during very specific conditions. This finding could make laboratory rats a useful model in studies of reproductive coupling to photoperiodic time measurement systems.

The usefulness of labeling species, or even individuals, as photoperiodic or nonphotoperiodic may be limited. The ability to measure day length in order to discern time of year may be as fundamental as maintaining a circadian clock, and tightly conserved over many taxa. Individuals appear to differ in which physiological, morphological, and behavioral processes are coupled to photoperiodic regulation. Although reproductive function appears to be uncoupled from photoperiodic regulation in rats, mice, and humans, the extent to which various nonreproductive traits are influenced by day length remains largely unspecified.

TABLE 3. Body and reproductive organ masses and sperm counts at 10 weeks of age for male rats injected with testosterone or oil at 3 days of age (Experiment 2)

Group	Body mass (g)	Paired testes mass (mg)	Paired epididymal mass (mg)	Seminal vesicle mass (mg)	Testicular sperm count ( $\times 10^6$ )	Epididymal sperm count ( $\times 10^6$ )
16:8 Test	450.3 $\pm$ 9.7	3130 $\pm$ 80	1100 $\pm$ 30	480 $\pm$ 30	379 $\pm$ 25	436 $\pm$ 32
8:16 Test	417.7 $\pm$ 13.6	2840 $\pm$ 170	990 $\pm$ 40	570 $\pm$ 50	300 $\pm$ 34	366 $\pm$ 48
16:8 Oil	437.0 $\pm$ 6.3	2330 $\pm$ 90	920 $\pm$ 40	360 $\pm$ 20	256 $\pm$ 16	284 $\pm$ 25
8:16 Oil	426.3 $\pm$ 16.7	1390 $\pm$ 110	840 $\pm$ 70	350 $\pm$ 40	271 $\pm$ 19	264 $\pm$ 43

TABLE 4. Body and reproductive organ masses and sperm counts at 10 weeks of age for male rats implanted with Silastic capsules of testosterone or empty capsules when 21 days of age (Experiment 3)

Group	Body mass (g)	Paired testes mass (mg)	Paired epididymal mass (mg)	Seminal vesicle mass (mg)	Testicular sperm count ( $\times 10^6$ )	Epididymal sperm count ( $\times 10^6$ )
16:8 Test	354.0 $\pm$ 8.0	1490 $\pm$ 100	620 $\pm$ 20	510 $\pm$ 40	107 $\pm$ 15	111 $\pm$ 32
8:16 Test	352.3 $\pm$ 12.7	1830 $\pm$ 110	690 $\pm$ 30	520 $\pm$ 30	117 $\pm$ 14	88 $\pm$ 17
16:8 Empty	442.5 $\pm$ 14.2	3140 $\pm$ 100	1130 $\pm$ 40	490 $\pm$ 50	421 $\pm$ 23	432 $\pm$ 25
8:16 Empty	417.5 $\pm$ 15.0	3110 $\pm$ 90	1070 $\pm$ 40	540 $\pm$ 30	358 $\pm$ 13	397 $\pm$ 31

TABLE 5. Plasma FSH and PRL levels at 6 and 10 weeks of age for males implanted with testosterone-filled or empty capsules when 21 days of age (Experiment 3)

Group	FSH 6 weeks (ng/ml)	FSH 10 weeks (ng/ml)	PRL 6 weeks (ng/ml)	PRL 10 weeks (ng/ml)
16:8 Empty	4.59 $\pm$ .81	4.68 $\pm$ .62	15.56 $\pm$ 2.33	15.84 $\pm$ 2.36
8:16 Empty	4.66 $\pm$ .31	4.16 $\pm$ .48	16.11 $\pm$ 1.52	17.00 $\pm$ 2.34
16:8 Test	1.75 $\pm$ .00	6.18 $\pm$ 1.69	8.84 $\pm$ 2.14	17.78 $\pm$ 1.98
8:16 Test	1.75 $\pm$ .00	6.35 $\pm$ .78	14.68 $\pm$ 2.01	19.62 $\pm$ .82

### Acknowledgments

We thank Jill Rhyne-Grey, Talia Adams, Michael Chider, Mariko Kita, Glynis Dales, and Joe Shiber for expert technical assistance. This research was supported in part by grant HD 22201 awarded by the National Institute of Child Health, NIH.

### Literature Cited

- DUNCAN M.J., B.D. GOLDMAN (1984) Hormonal regulation of the annual pelage color cycle in the Djungarian hamster, *Phodopus sungorus*. II. Role of prolactin. *J. Exp. Zool.* 230:97-103.
- DUNCAN M.J., B.D. GOLDMAN, M.N. DiPINTO, M.H. STETSON (1985) Testicular function and pelage color have different critical daylengths in the Djungarian hamster, *Phodopus sungorus*. *Endocrinology* 116:424-430.
- ESKES G.A., I. ZUCKER (1978) Photoperiodic control of hamster testis: Dependence on circadian rhythms. *Proc. Natl. Acad. Sci. USA* 75:1034-1037.
- GOLDMAN B.D., J.A. ELLIOTT (1988) Photoperiodism and seasonality in hamsters: Role of the pineal gland. In: *Processing of Environmental Information in Vertebrates*, M.H. Stetson ed. Springer-Verlag: New York, pp. 203-218.
- GOLDMAN B.D., R.J. NELSON (1993) Melatonin and seasonality in mammals. In: *Melatonin: Biosynthesis, Physiological Effects and Clinical Applications*, H.S. Yu, and R.J. Reiter, eds. CRC Press: Boca Raton, pp. 225-252.
- JOHNSTON, P.G., I. ZUCKER (1979) Photoperiodic influences on gonadal development and maintenance in the cotton rat, *Sigmodon hispidus*. *Biol. Reprod.* 21:1-8.
- KEPPEL, G. (1982) *Design & Analysis*. Englewood Cliffs, NJ: Prentice Hall.
- KINSON G.A., S. ROBINSON (1970) Gonadal function of immature rats subjected to light restriction, melatonin administration and removal of the pineal gland. *J. Endocrinol.* 47:391-392.
- LEADEM, C.A. (1988) Photoperiodic sensitivity of prepubertal female Fisher 344 rats. *J. Pineal Res.* 5:63-70.
- NELSON, R.J., I. ZUCKER (1981) Photoperiodic control of reproduction in olfactory-bulbectomized rats. *Neuroendocrinology* 32:266-271.
- NELSON, R.J., M.J. BAMAT, I. ZUCKER (1982) Photoperiodic regulation of testis function in rats: Mediation by a circadian mechanism. *Biol. Reprod.* 26:329-335.
- NELSON, R.J., A.S. FLEMING, C.J. WYSOCKI, T.W. SHINDER, I. ZUCKER (1985) Chemosensory and neural influences on photoperiodic responsiveness of laboratory rats. *Neuroendocrinology* 40:285-290.
- OGILVIE K.M., R.S. DONHAM, M.H. STETSON (1992) Daily rhythms of follicle-stimulating hormone in adult anestrus and prepubertal female Turkish hamsters (*Mesocricetus brandti*). *Biol. Reprod.* 46:279-283.
- REITER R.J., D.C. KLEIN, R.J. DONOFRIO (1969) Prelimin-

- ary observations on the reproductive effects of the pineal gland in blinded, anosmic male rats. *J. Reprod. Fertil.* 19:563-565.
- REITER R.J., S. SORRENTINO JR, C.L. RALPH, H.J. LYNCH, D. MULL, E. JARROW (1971) Some endocrine effects of blinding and anosmia in adult male rats with observations on pineal melatonin. *Endocrinology* 88:895-900.
- Rennels E.G., W.P. Callahan (1959) The hormonal basis for pubertal maturation of hair in the albino rat. *Anat. Rec.* 135:21-26.
- RILEY, V. (1960) Adaptation of orbital bleeding technic to rapid serial blood studies. *Proc. Soc. Exper. Biol. Med.* 104:751-754.
- RONNEKLEIV, O.K., S.M. MCCANN (1975a) Effects of pinealectomy, anosmia and blinding on serum and pituitary prolactin in intact and castrated male rats. *Neuroendocrinology* 17:340-353.
- RONNEKLEIV, O.K., S.M. MCCANN (1975b) Effects of pinealectomy, anosmia and blinding alone or in combination on gonadotropin secretion and pituitary and target gland weight in intact and castrated male rats. *Neuroendocrinology* 19:97-114.
- TUREK, F.W., C. DESJARDINS, M. MENAKER (1976) Differential effects of melatonin on the testes of photoperiodic and nonphotoperiodic rodents. *Biol. Reprod.* 15:94-97.
- VANECEK J., H. ILLNEROVA (1982) Effect of photoperiod on the growth of reproductive organs and on pineal N-acetyltransferase rhythm in male rats treated neonatally with testosterone propionate. *Biol. Reprod.* 27:517-522.
- WALLEN, E.P., M.A. DEROSCH, A. THERBERT, S. LOSEE-OLSON, F.W. TUREK (1987) Photoperiodic response in the male laboratory rat. *Biol. Reprod.* 37:22-27.
- WALLEN, E.P., F.W. TUREK (1981) Photoperiodicity in the male albino laboratory rat. *Nature* 289:402-404.
- YELLON S.M., B.D. GOLDMAN (1987) Influence of short days on diurnal patterns of serum gonadotrophins and prolactin concentrations in the male Djungarian hamster, *Phodopus sungorus*. *J. Reprod. Fertil.* 80:167-174.