Genetic variation in photoperiodism among naturally photoperiodic rat strains

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Lorincz, Anakka M., M. Benjamin Shoemaker, and Paul D. Heideman. Genetic variation in photoperiodism among naturally photoperiodic rat strains. Am J Physiol Regulatory Integrative Comp Physiol 281: R1817–R1824, 2001.—Rattus norvegicus has been considered nonphotoperiodic, but Fischer 344 (F344) rats are inhibited in growth and reproductive development by short photoperiod (SD). We tested photoresponsiveness of the genetically divergent Brown Norway (BN) strain of rats. Peripubertal males were tested in long photoperiod or SD, with or without 30% food reduction. Young males were photoresponsive, with reductions in testis size, body mass, and food intake in SD and with enhanced responses to SD when food restricted. Photoperiods ≥11 h of light inhibited reproductive maturation and somatic growth, whereas photoperiods of 12 h or more produced little or no response. F344/BN hybrids differ from both parent strains in the timing, amplitude, and critical photoperiod of photoperiodic responses, indicating genetic differences in photoperiodism between these strains. This is consistent with the hypothesis that ancestors of laboratory rats were genetically variable for photoperiodism and that different combinations of alleles for photoperiodism have been fixed in different strains of rats.

reproduction; seasonal breeding; critical day length; photoperiod

SEASONALLY BREEDING RODENTS in the temperate zones use environmental cues such as photoperiod to time sexual maturity and reproductive attempts (2, 4). This increases the probability that offspring will be born in conditions that are optimal for survival. However, occasional reports of wild rodents that do not suppress reproduction in winter have been followed by studies that have demonstrated genetic variation in responses to photoperiod both among and within populations of photoperiodic rodents (6, 11, 18, 20, 27, 29, 33). It has been hypothesized that genetic variation in photoperiodism may be typical of temperate zone populations of rodents (13).

Variation in photoresponsiveness has also been observed in laboratory rats. Recent studies have shown that the Fischer 344 (F344) inbred rat strain exhibits robust obligate photoresponsiveness, repressing reproduction, somatic growth, and food intake in short photoperiods (SD) (9, 10, 14, 15, 19). In contrast, other strains of laboratory rats have not been considered functionally photoresponsive because unmanipulated rats of these strains do not show marked differences in body mass, gonad size, or food intake (22) compared with traditional photoresponsive rodents such as Phodopus sungorus, Mesocricetus auratus, or Peromyscus leucopus (3). However, even in rat strains considered nonphotoperiodic, such as the Wistar and Sprague-Dawley outbred strains, procedures such as neonatal androgen treatment and food reduction have been shown to unmask facultative photoresponsiveness (26, 30, 31). Those strains of rats that can be induced to become photoperiodic also differ from F344 rats in having a much shorter critical photoperiod, the photoperiod below which reproduction is inhibited (24, 25, 30). The presence of this variation among rat strains allows comparisons of rat strains to identify the neuroendocrine and genetic basis for photoperiodism and also raises the question of how widespread photoresponsiveness might be among strains of rats.

Two hypotheses have been proposed to account for the existence of photoperiodic and nonphotoperiodic laboratory strains of rats. First, it has been hypothesized that wild Rattus norvegicus, ancestral to laboratory rats, was not photoperiodic but retained in vestigial form some of the elements of the complex neuroendocrine pathway for photoperiodism (21–23, 31). Under this hypothesis, functional photoresponsiveness in particular rat strains has arisen due to a mutation(s) that restored partial function to the pathway. Such mutations should be rare, and, therefore, we would predict that photoresponsiveness in rats should be based on changes at a single locus or a small set of loci. If so, photoresponsive strains should have a common genetic basis for their photoresponsiveness; in other words, the allele(s) that causes photoresponsiveness should be identical in each photoresponsive strain of rats. In addition, such rare mutant alleles should have occurred in only a single ancestor or a few of the ancestors of laboratory rats. Therefore, one would predict also that photoresponsiveness should be present in very few strains, perhaps only a single strain or a single clade of related inbred strains derived from the same founder population. The second hypothesis is

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that the ancestors of laboratory rats were photoperiodic, but photoresponsiveness has been lost in some strains of laboratory rats due to artificial selection or genetic drift during domestication (21). This hypothesis implies that the ancestral founders of different strains of laboratory rats were variable at multiple loci for photoresponsiveness, as appears to be typical of temperate-zone rodent species (4, 13). According to the latter hypothesis, the process of domestication and inbreeding might have fixed different combinations of alleles for photoresponsiveness in different strains of rats (9). If this hypothesis is correct, then one would predict that photoresponsiveness might be common among distantly related strains of rats and that photoresponsive strains would differ in alleles that affect parameters such as critical photoperiod, specific nonreproductive and reproductive responses to photoperiod, and the amplitude and duration of photoperiodic responses. Thus this hypothesis predicts complex genetic variation in photoresponsiveness among strains.

The goals of this study were to test these hypotheses by examining photoresponsiveness in an inbred rat strain that is genetically distant from most other strains, identifying the critical photoperiod of this strain, and performing a test for genetic variation in photoresponsiveness among strains. We chose the Brown Norway (BN) inbred strain of rats because phylogenetic studies comparing genetic and biochemical markers of laboratory rats have consistently characterized BN rats as the most genetically different from most other strains (1, 5, 7). In addition, a pilot experiment on F344/BN rat female hybrids suggested that the strains differ in genes for photoresponsiveness and that the BN strain might be either nonphotoperiodic or only very weakly photoperiodic.

MATERIALS AND METHODS

General. Breeder rats of the strain BN/RijHSD and F344/NHsd were obtained from Harlan Sprague Dawley (Indianapolis, IN). All rats were held individually in polyethylene cages (36 × 24 × 19 cm) with stainless steel wire tops and pine shavings for bedding. Food (LM-485 Mouse/Rat Diet 7012; Harlan Teklad, Madison, WI) and tap water were provided ad libitum. Breeder rats were kept on a 16:8-h light-dark [long photoperiod (LD)] photoperiod with lights on at 0500. SD treatment was an 8:16-h light-dark photoperiod with lights on at 0900. Light intensity was 80–250 lx.

Experiment 1: Test for obligate and facultative photoperiodic responsiveness. This experiment tested the somatic growth and reproductive maturation of male BN rats in response to LD or SD photoperiods. In addition, this experiment tested whether BN rats have the capacity for stronger photoperiodic response when faced with food restriction (FR). Male BN rats were gestated, born, and raised in LD until weaning at 21 days of age, at which time they were caged individually and assigned to weight-matched treatment groups. Rats were transferred from LD into one of five photoperiod treatments: 10:14-h light-dark photoperiod (L10; n = 6); 11:13-h light-dark photoperiod (L11; n = 5); 12:12-h light-dark photoperiod (L12; n = 6); 13:11-h light-dark photoperiod (L13; n = 6); or 14:10-h light-dark photoperiod (L14; n = 5). Rats were held in fan-ventilated photoperiod chambers with light provided by two standard 20-W fluorescent light bulbs (F20T12 Cool White; General Electric) dimmed with neutral density filters to 100–300 lx as measured 5 cm above the floor. Photoperiod treatments were terminated after exactly 5 wk because in experiment 1, this treatment duration produced the maximal effects of SD on testicular growth in terms of percent difference from LD. At the end of treatment, rats were euthanized by carbon dioxide gas, body mass was recorded, testes were excised and weighed, and paired seminal vesicles, including their fluid contents, were excised and weighed.

Experiment 2: Critical photoperiod of BN rats. After finding significant effects of SD on BN rats, we conducted a second experiment to test whether the critical photoperiods for reproductive development and for somatic growth of BN rats would result in functional photoperiodism for a rat in natural conditions in the wild. Male BN rats were gestated, born, and raised in LD until weaning at 21 days of age, at which time they were caged individually and assigned to weight-matched treatment groups. Rats were transferred from LD into one of five photoperiod treatments: 10:14-h light-dark photoperiod (L10; n = 6); 11:13-h light-dark photoperiod (L11; n = 5); 12:12-h light-dark photoperiod (L12; n = 6); 13:11-h light-dark photoperiod (L13; n = 6); or 14:10-h light-dark photoperiod (L14; n = 5). Rats were held in fan-ventilated photoperiod chambers with light provided by two standard 20-W fluorescent light bulbs (F20T12 Cool White; General Electric) dimmed with neutral density filters to 100–300 lx as measured 5 cm above the floor. Photoperiod treatments were terminated after exactly 5 wk because in experiment 1, this treatment duration produced the maximal effects of SD on testicular growth in terms of percent difference from LD. At the end of treatment, rats were euthanized with carbon dioxide gas, body mass was recorded, both testes were excised and weighed, and paired seminal vesicles, including their fluid contents, were excised and weighed.

Experiment 3: Photoperiodic response of male F344/BN hybrids. This experiment tested the photoperiodic response of F1 male F344/BN hybrids. F344/BN hybrid males produced in our breeding colony by mating male F344 rats with female BN rats were gestated and raised in LD. At the age of 21 days, rats were weaned, caged individually, weight matched, and placed into either LD (n = 16) or SD (n = 15). Testis width and length were measured for each rat at exact 2-wk intervals by one person (M. B. Shoemaker) blind with respect to treatment. Testis volume was estimated using the formula for the volume of a prolate spheroid (width² × length × 0.523). Food intake and body mass of all rats were also measured at precise 2-wk intervals. At week 12, data were obtained on only eight rats in each group.

Experiment 4: Photoperiodic response of female F344/BN hybrids. This experiment tested the photoperiodic response of F1 female F344/BN hybrids. F344/BN hybrid
females were taken from the same litters as in experiment 3. At the age of 21 days, rats were weaned, caged individually, weight matched, and placed into either LD or SD (n = 15 for each). Beginning at 21 days of age, rats were checked daily for vaginal opening by swabbing the vaginal area with distilled water followed by visual inspection. Food intake was measured at precise 2-wk intervals through 8 wk of treatment. Body mass measurements were recorded at weeks 4, 6, and 8, with one additional measurement at week 12 in an extension of the experiment to test the persistence of body mass differences.

Statistical analysis. In this study, negative results were potentially as valuable as positive results for experiments 1, 3, and 4. Therefore, in these three experiments, sample sizes were relatively large to provide high statistical power to detect subtle effects and to provide high confidence in any negative results. Power analyses were used to estimate sample sizes necessary to provide statistical power (1 - β) of 0.8 to detect 10% differences between group means. In other words, if there were true differences between group means of at least 10%, tests using these sample sizes would be expected to result in P < 0.05 in 80% of such tests. Statistical tests were carried out using Statview 4.5 (Abacus Concepts, Berkeley, CA) with significance set at P < 0.05. Attained levels of significance are presented to provide maximal information on probabilities.

For experiment 1, data on reproduction and body mass from each time point were analyzed using two-factor ANOVA. Data on food intake for each time point were analyzed using t-tests. For experiment 2, all data were analyzed using one-factor ANOVA, followed by paired comparisons using the Bonferroni-Dunn method. For these paired comparisons with the Bonferroni-Dunn method, the experiment-wise significance level (the probability that any of the multiple pairwise comparisons is falsely significant) was set at P < 0.05, equivalent in this particular experiment to a significance level of P < 0.005 for each individual paired comparison. Although this method is conservative in the probability of type I error for the entire set of comparisons, it has a fairly high probability of type II error. In other words, it is likely that some pairs of groups that would be considered significantly different if compared by t-test were not identified as significantly different using this method. This conservative approach was chosen because we felt it was particularly important for our hypotheses to avoid a potential risk of incorrectly concluding that critical photoperiods are different for the measures of reproductive development or somatic growth. For experiments 3 and 4, data on testis volume, body mass, and food intake were analyzed at each time point with unpaired t-tests. Data on vaginal opening were analyzed using an unpaired t-test.

RESULTS

Experiment 1: Photoperiodic response of BN rats. SD caused reproductive inhibition of BN rats from 2.5 wk of treatment until the end of this experiment. Male BN rats in SD had significantly smaller testis volume than rats in LD after 2.5 and 5 wk of treatment (P = 61.3, \( P < 0.0001 \) and \( F = 111.9, P < 0.0001 \), respectively; Fig. 1A). At 10 wk of treatment, rats in SD had significantly smaller paired testis mass than rats in LD (\( F = 38.1, P < 0.0001 \); Fig. 1B) and significantly smaller paired seminal vesicle mass than rats in LD (\( F = 45.8, P < 0.0001 \); Fig. 1C). BN rats in SD also had lower body mass than rats in LD (\( F = 12.8, P < 0.001 \); Fig. 2A) and lower food intake than rats in LD (\( F = 21.79, P < 0.005 \); Fig. 2B).

FR caused reproductive inhibition of BN rats from 2.5 wk of treatment until the end of this experiment. Food-restricted male BN rats had significantly smaller testis volume than ad libitum-fed rats after 2.5 and 5 wk of treatment (\( F = 50.3, P < 0.0001 \) and \( F = 142.8, P < 0.0001 \), respectively; Fig. 1A). At 10 wk of treatment, FR rats had significantly smaller paired testis mass (\( F = 45.8, P < 0.0001 \); Fig. 1B) and paired seminal vesicle mass than ad libitum-fed rats (\( F = 11.3, P < 0.01 \); Fig. 1C). FR BN rats had lower body mass than ad libitum-fed rats (\( F = 144.0, P < 0.0001 \); Fig. 2A).

During the course of this experiment, there were significant interactions between photoperiod and FR treatments for reproductive measures but not for body mass. After 2.5 wk of treatment, however, there was no
interaction between photoperiod and food treatment on testis volume ($F = 0.19$, $P = 0.50$). By 5 wk of treatment, there was a statistical trend ($F = 3.28$, $P = 0.079$) for an interaction between FR and SD to suppress testis growth below the additive effects of SD and FR (Fig. 1A). After 10 wk of treatment, the interaction between FR and SD to further enhance the effects of SD on testis mass was statistically significant ($F = 4.48$, $P = 0.05$; Fig. 1B). This interaction was not significant for seminal vesicle mass ($F = 2.56$, $P = 0.11$). The combined effect of SD and FR on body mass was purely additive, with no significant interaction ($F = 0.01$, $P > 0.90$; Fig. 2A).

**Experiment 2: Critical photoperiod.** BN rats treated with different photoperiods for 5 wk differed significantly in testis mass ($F = 9.86$, $P < 0.0001$; Fig. 3A), seminal vesicle mass ($F = 9.11$, $P < 0.002$; Fig. 3B), and body mass ($F = 4.18$, $P = 0.01$; Fig. 3C). After 5 wk of treatment, BN rats in L11 were reproductively inhibited and showed significantly smaller paired testis mass compared with rats in all longer photoperiod treatments ($P < 0.005$ for all; Fig. 3A) and significantly smaller paired seminal vesicle mass compared with rats in L13 and L14 ($P < 0.05$; Fig. 3B). At the final week of treatment, BN rats in L11 weighed significantly less than rats in L14 ($P < 0.005$; Fig. 3C).

**Experiment 3: Photoperiodic response of male F344/BN hybrids.** SD caused reproductive inhibition of F344/BN F$_1$ rats from 2 wk of treatment until the end of this experiment (Fig. 4A). Male F344/BN F$_1$ rats in SD had significantly smaller testis volume after 2, 4, 6, 8, and 10 wk of treatment ($P < 0.005$ for all), but testis volume in the two groups did not differ after 12 wk of treatment ($P = 0.53$). Male F344/BN F$_1$ rats in SD and LD were similar in body mass after 2 wk of treatment ($P = 0.11$), but rats in SD had lower body mass than rats in LD after 4, 6, 8, 10, and 12 wk of treatment ($P < 0.01$ for all; Fig. 4B). Male F344/BN F$_1$ rats in SD and LD had similar food intake during the first 2 wk of treatment ($P = 0.81$), but rats in SD had lower food intake than rats in LD at 4, 6, 8, and 10 wk of treatment ($P < 0.05$ for all; Fig. 4B). During the last 2 wk of treatment, the two groups did not differ in food intake ($P = 0.48$).

**Experiment 4: Photoperiodic response of female BN/ F344 hybrids.** Female F344/BN F$_1$ rats in LD and SD underwent vaginal opening at the same age (34.07 and 34.13 days, respectively; $t = 0.07$, $P = 0.93$; Fig. 5A). However, SD inhibited somatic growth of female hybrids (Fig. 5B). There was no difference in body mass after 4 wk of treatment ($t = 1.49$, $P = 0.15$), but body
mass was significantly lower in SD after 6, 8, and 12 wk of treatment (t = 3.08, P < 0.005; t = 3.54, P < 0.005; and t = 4.81, P < 0.0001, respectively) (Fig. 5B). SD had not affected food intake significantly after 2 and 4 wk of treatment (t = 0.26, P = 0.82 and t = 1.12, P = 0.27, respectively), but food intake was significantly lower in SD at 6 and 8 wk of treatment (t = 2.16, P < 0.05 and t = 2.26, P < 0.05, respectively) (Fig. 5C).

**DISCUSSION**

Our results show that BN rats are photoresponsive. Young males repressed reproductive development, somatic growth, and food intake in response to SD alone. The amount of suppression of reproduction and somatic growth in BN rats by SD was similar to that seen in F344 rats. BN rats in SD for 5 wk had testes that weighed 40% less than those of LD controls (Fig. 3A), within the range of the 30–60% decrease reported for F344 rats in several experiments (9, 14, 15). In F344 rats, a slower rate of testicular growth in SD corresponded to slowed, but not halted, spermatogenesis (15). In BN rats, seminal vesicle mass was much lower in SD than in LD after 10 wk of treatment (Fig. 1C). This suggests that androgen levels were still low and fertility would be impaired in SD-treated BN rats, even though testis size was no longer depressed (Fig. 1B). BN rats in SD were 20% lower in body mass, and decreased food intake by 15%; responses very similar to those of F344 rats (9, 14, 15). The duration of photoperiodic responses of young male BN and F344 rats to SD was very similar (Figs. 1 and 2) (14, 15).

As in F344 rats (14), the inhibition of reproduction by SD was not due simply to smaller body size. The much smaller body size of FR BN rats in LD did not reduce testis mass or seminal vesicle mass proportionately. After 2.5 and 5 wk of treatment, food-restricted BN rats in LD weighed much less than ad libitum-fed rats in SD (Fig. 2A), but they had testes similar in size (Fig. 1A and B).

Unlike F344 rats, BN rats apparently possess only a moderate capacity for enhanced facultative photoperiod response due to mild FR. Although a statistical trend showing an interaction between FR and SD was present after 5 wk of treatment (P = 0.079; Fig. 1), statistically significant interactions between FR and SD to further repress reproduction were not evident until 10 wk of treatment, and even then the affect was

**Fig. 4.** Means ± SE of estimated testis volume (A), body mass (B), and daily food intake (C) for male Fischer 344 (F344)/BN hybrid rats held in LD or SD for 12 wk from weaning. *Significant differences (P < 0.05) for particular weeks.

**Fig. 5.** Means ± SE for age at vaginal opening (A), body mass (B), and daily food intake (C) for female F344/BN hybrid rats held in LD or SD from weaning. *Significant differences (P < 0.05) for particular weeks.
slight. In F344 rats, in contrast, the combination of SD and FR at 70% of ad libitum intake has a rapid and strong effect, further enhancing reproductive inhibition starting at 1 wk of treatment and continuing through 13 wk of treatment (14).

Here we use the term “critical photoperiod” for photoperiods below which inhibitory effects occur, but it is important to state two caveats. First, the critical photoperiod concept implies a narrow photoperiod range above which photoperiod treatment is not inhibitory, and below which there is maximal inhibition, a situation which may not hold for BN rats, especially in the effects of SD on somatic growth (Fig. 3C). Second, the concept of a single “critical photoperiod” is only meaningful in the context of some specific treatment or class of treatments, because in photoresponsive rodents, inhibitory responses to particular photoperiods can vary with light intensity, the direction of photoperiod change (16), and gradual or abrupt changes in photoperiod (8, 9, 12).

In BN rats, inhibitory photoperiods for reproductive effects were ~11 or fewer hours of light. Rats in L11 for 5 wk had significantly smaller testis mass and seminal vesicle mass than those of any longer photoperiod treatment. The L11 and L10 groups had reproductive responses similar to that of BN rats in SD in experiment 1. This indicates that, under these conditions, the critical photoperiod for reproduction is less than 12 h of light. In contrast, the critical photoperiod for reproductive effects in young male F344 rats in similar conditions is 13.5 h of light (9), and the critical photoperiod of adult male Harlan Sprague-Dawley rats with photoresponsiveness induced by androgen treatment is 9 h of light (30). Our data indicate that the critical photoperiod for somatic growth in BN rats is between 11 and 14 h of light (Fig. 3C), but the data do not allow us to resolve the critical photoperiod more precisely. In fact, the data suggest the possibility that shorter photoperiods may have progressively stronger effects over that range of photoperiods. If so, then for somatic growth in BN rats, there may not be a critical photoperiod above which there are no effects, but below which there are complete effects.

The BN inbred strain of rats is the second reported to have robust responses to SD alone and to have critical day lengths that would make the trait functional in nature. In published estimates of genetic relatedness among inbred rat strains, F344 rats have been positioned genetically within a large group of fairly closely related strains. In contrast, BN rats have been found to be the most genetically divergent from other strains, consistent with the derivation of the BN strain from an independent domestication of rats (1, 5, 7). The finding of photoresponsiveness in unmanipulated individuals of these two genetically distant rat strains might be due to independent mutations uncovering a vestigial photoperiod pathway in these two strains. We feel that it is more likely that the founders of these and other laboratory rat strains were variable for photoresponsiveness and that some strains inherited a functional photoperiodic pathway from these founders. However, the data available thus far are not sufficient to firmly reject either hypothesis. Even so, our results suggest that a functional photoperiod pathway may be present in other inbred strains of rats as well. Thus photoperiod should be given more attention in studies that measure reproductive function or feeding and body mass in rats. For example, in recent publications on the BN rat strain to test testicular regression during aging (17, 28), photoperiod was not specified.

Our findings that F344 and BN rats respond differentially to FR in SD, that their critical photoperiods are slightly different, and that the offspring of crosses between F344 and BN rats differ in photoresponsiveness from parent strains indicate that the two parent strains differ in the alleles and/or loci of genes that affect photoresponsiveness. In experiment 3, testis growth of the first generation male BN/F344 hybrids was less inhibited by SD (15% smaller testes in SD than LD) than either parent strain (30–60% smaller testes in SD than LD). However, inhibition of body mass and food intake were similar to both parent strains. Female hybrids were inhibited in food intake and body mass, but they did not delay vaginal opening in SD. In contrast, peripubertal F344 females showed a 1- to 2-wk delay in vaginal opening when blinded (19).

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we began this study with the hope of identifying a nonphotoperiodic inbred strain that we could use as a comparison with photoperiodic F344 rats in studies on variability in the photoperiod pathway. Most studies on photoperiodic responses in rats have used outbred strains, especially Wistar or Sprague-Dawley, two strains that have very weak responses to photoperiod and in which photoperiodism is usually considered a vestigial trait (22, 31). This genetic variation for the presence or absence of photoperiodic responses, as well as variation in critical day length and other parameters of photoperiodism, indicates that the photoperiod pathway in rats should be a useful model for the study of within-species variation in the brain.

Perspectives

One major hypothesis in evolutionary physiology is that complex physiological systems have been optimized by the process of evolution by natural selection. The competing hypothesis is that in physiological systems controlled by complex interactions among multiple genes, considerable genetic variation will persist because selection on many of the alleles affecting a complex trait is too weak to eliminate all alleles contributing to nonoptimal phenotypes (see papers collected in Ref. 32). The mammalian genome projects are currently initiating major studies on individual variation at the level of gene sequences in a “bottom-up” approach (gene to phenotype). However, complexity theory suggests that, particularly for phenotypes with complex genetic bases for variation, bottom-up approaches are likely to fail to find many important sources of variability because individual genes have undetectably small effects on the phenotype. Thus top-down approaches will be a necessary part of this emerging field. Inbred strains of rats and mice can be a useful tool in top-down studies of normal, nonpathological within-species variation. Because individuals within a particular inbred strain are nearly genetically identical, each inbred strain can be treated as a genetic individual, providing the power of replication in studies of variation. In the current study, we found evidence for genetic variation in the regulation of photoperiodism, consistent with the second hypothesis above. We speculate that variation may be common in complex physiological pathways in wild mammals and retained in domesticated mammals. Persistent variation in complex physiological pathways may explain why artificial selection is so effective during domestication.

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