Short photoperiod inhibition of growth in body mass and reproduction in ACI, BUF, and PVG inbred rats

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Abstract

Laboratory rats have been generally considered non-photoresponsive, but strains of laboratory rats have been found to be variable for this trait. Young males of both the Fischer (F344) and Brown Norway strains (BN) suppress reproductive development, food intake and body mass in short winter photoperiod (short days (SD); 8 h light:16 h darkness), and food restriction interacts with SD to enhance the effect of SD alone. Conversely, young male Harlan Sprague Dawley outbred rats, along with other outbred laboratory rats tested, have little or no response to SD except when unmasked by food restriction or other treatments, and have generally been considered nonphotoperiodic. In order to assess how widespread this trait might be among rat strains, and to test for uncoupling of reproductive and nonreproductive responses, we tested 3 additional inbred strains, including ACI, PVG and BUF rats, for photoresponsiveness and for unmasking of photoperiodic responses by food restriction. Young males of all three inbred strains exhibited photoresponsiveness in testis mass (5–20% lower in SD), seminal vesicle mass (20–50% lower in SD), and body mass (5–10% lower in SD). Food restriction also suppressed reproduction, but there was little or no interaction with the effects of photoperiod. The results are consistent with the hypothesis that laboratory rats are genetically variable for photoperiodism, and that photoresponsiveness may be widespread among inbred rat strains, as all five inbred strains tested have shown photoperiodic responses. The results are particularly important because standard research protocols may unknowingly manipulate this pathway in rats, causing unsuspected variability among or within studies.

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Introduction

Variation in reproductive status and body mass in response to short photoperiods has been observed in laboratory rats (Leadem 1988, Heideman & Sylvester 1997). Recent studies have shown that the Fischer 344 (F344) and Brown Norway (BN) inbred rat strains exhibit robust obligate photoresponsiveness, repressing reproduction, food intake, and somatic growth in the absence of light (Leadem 1988) or short photoperiods (Heideman & Sylvester 1997, Heideman *et al.* 1998, 2000, 2001, Lorincz *et al.* 2001, Shoemaker & Heideman 2002). In contrast, other strains of laboratory rats have not been considered functionally photoresponsive because unmanipulated rats of these strains show little or no marked differences in body mass, gonad size, or food intake in response to short photoperiod (Nelson *et al.* 1994). However, photoresponsiveness in rats does not fall neatly into two phenotypes; for example, in some of the rat strains considered nonphotoperiodic, including the Wistar and Sprague Dawley outbred strains, photoperiodic responses can be unmasked by treatments such as administration of androgen (Sorrentino *et al.* 1971,

Wallen & Turek 1981, Wallen *et al.* 1987). In addition, strains of rats in which photoperiodic responses occur without manipulation differed from nonphotoperiodic rats (Sizonenko *et al.* 1985, Rivest *et al.* 1986, Wallen *et al.* 1987) in having longer critical photoperiods, with effects detectable even by relatively long photoperiods (as long as 13 h light:11 h darkness, L13:D11) (Heideman *et al.* 2000, Lorincz *et al.* 2001). This variation among rat strains raises the question of whether photoresponsiveness might be widespread among strains of rats.

The presence of this variation is likely to be important. Short photoperiod can induce large shifts in endocrine and metabolic states, which might affect most organ systems in a photoresponsive rat. Thus, inadvertent manipulation of photoperiod above or below a critical value (Heideman *et al.* 2000), or pharmacological stimulation of this pathway, may have unintended effects on food intake, body mass, reproductive status, and any organ system affected by these changes. Even brief exposure to light, or the photoperiod history of the dam can affect these traits in some photoperiod sensitive rodents (reviewed by Goldman 2001). Finally, genetic variation in photoresponsiveness

in rats has been useful in studying neuroendocrine physiological variation (e.g. Heideman *et al.* 2001).

In this study, we tested young males of three inbred strains of rats, ACI, BUF, and PVG. The strains were chosen because they are distributed within the largest 'super-group' of related inbred rat strains (Canzian 1997). This supergroup also includes the F344 strain and many other inbred rat strains commonly used in research, but is distantly related to the BN strain (Canzian 1997). The objectives of the study were to test whether photoperiodic responses might be widespread in inbred strains of rats and to assess the magnitude of any photoperiodic responses found.

Materials and Methods

General

Breeder rats of the strains ACI/SegHsd, PVG/01aHsd, and BUF/SimRijHsd were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN, USA). Male rats of each strain were gestated and raised until weaning (21 ± 1 day) in a long photoperiod (16L:8D, lights on at 0500 h; LD). At weaning, experimental males were separated from their dam and housed individually in polypropylene cages ($36 \times 24 \times 19$ cm) with stainless steel wire tops and wood shavings for bedding. Food (Rat-Mouse-Hamster 3000; Southern States Cooperative, Williamsburg, VA, USA) was available *ad libitum* (ad lib) or at 70% of the *ad libitum* available level in the food restricted (FR) groups; tap water was available *ad libitum*. Rats were housed in fan-ventilated, photoperiod-controlled chambers ($86 \times 58 \times 49$ cm) in LD or short photoperiod (8L:16D, lights on at 0900 h; SD). Lighting in both photoperiod chambers was provided by two 20-watt fluorescent bulbs, reduced by a neutral gray covering to an illuminance of 100–250 lux 5 cm above the floor of each cage. Relative humidity was between 30–70%, and the temperature was maintained at $23 \pm 2^\circ\text{C}$. All experiments were conducted in accordance with the NIH Guide for Care and Use of Laboratory Animals.

Experiment 1. Photoperiod and food restriction effects on ACI rats

Male ACI rats were tested for reproductive photoresponsiveness using a 2×2 design with photoperiod and food restriction as factors. At weaning, weight-matched groups were assigned to one of 4 treatment groups: LD ad lib, SD ad lib, LD food restricted (LD FR), and SD food restricted (SD FR) ($n = 11$ – 12 /group). Males from individual litters were divided among treatment groups. The food restricted groups received 70% of the food consumed on the previous day by the ad lib group in the same photoperiod. Body mass was measured weekly.

After 4 weeks of photoperiod and food restriction treatment, the rats were euthanized with carbon dioxide gas,

body mass was measured, both testes were excised, and wet weight was recorded. In addition, both seminal vesicles were immediately excised, drained of fluid contents, and weighed.

Experiment 2. Photoperiod and food restriction effects on BUF rats

This experiment tested male BUF rats for reproductive photoresponsiveness. At weaning, weight-matched groups were assigned to one of 4 treatment groups: LD ad lib ($n = 10$), SD ad lib ($n = 8$), LD FR ($n = 7$), and SD FR ($n = 5$). As above, males from individual litters were divided among treatment groups. Treatments and data collection were conducted as in Experiment 1.

Experiment 3. Photoperiod and food restriction effects on PVG rats

This experiment tested male PVG rats for reproductive photoresponsiveness using the same methods and treatments, with weight-matched groups ($n = 14$ – 16 /treatment group), as in Experiment 1.

Statistical analysis

Body mass, testis mass, and seminal vesicle mass were analyzed by ANOVA using photoperiod (LD and SD) and food (ad lib and FR) as factors. In all statistical tests, significance levels at P values < 0.05 were considered significant. Analyses were performed using Statview + Graphics (v.1.04 A; Abacus Concepts, Berkeley, CA, USA) on a Macintosh (Apple Computer, Cupertino, CA, USA) computer. All means are presented with their standard errors.

Results

Experiment 1

For ACI rats (Fig. 1), there was a significant effect of photoperiod ($F = 8.09$, $P = 0.007$) and food restriction ($F = 5.20$, $P = 0.028$) on testis mass, but no interaction ($F = 0.70$, $P = 0.41$). Likewise, there was a significant effect of photoperiod ($F = 20.96$, $P < 0.001$) and food restriction ($F = 19.60$, $P < 0.001$) on seminal vesicle mass but no significant interaction of the two ($F = 0.15$, $P = 0.70$). Finally, there was a significant effect of photoperiod ($F = 10.91$, $P = 0.002$) and food restriction ($F = 93.10$, $P < 0.0001$) on increase in body mass but no significant interaction of the two ($F = 0.35$, $P = 0.55$).

Experiment 2

For BUF rats (Fig. 2), there was a significant effect of photoperiod ($F = 19.15$, $P = 0.002$), food restriction ($F = 32.11$, $P < 0.001$), and an interaction of photoperiod and food restriction ($F = 5.37$, $P = 0.029$) on testis mass. Also, there was a significant effect of photoperiod

Figure 1 Mean ($\pm$ S.E.) of paired testis mass (a), paired, emptied, seminal vesicle mass (b), and change in body mass (c) for male ACI rats under long day (LD) or short day (SD) photoperiods, and fed an ad lib or food restricted (FR) diet for four weeks, beginning at three weeks of age. See Materials and Methods and Results for sample sizes and statistical tests.

Figure 2 Mean ($\pm$ S.E.) of paired testis mass (a), paired, emptied, seminal vesicle mass (b), and change in body mass (c) for male BUF rats under long day (LD) or short day (SD) photoperiods, and fed an ad lib or food restricted (FR) diet for four weeks, beginning at three weeks of age. See Materials and Methods and Results for sample sizes and statistical tests.

($F = 20.96$, $P = 0.002$) and food restriction ($F = 19.60$, $P < 0.001$) on seminal vesicle mass, but there was no interaction of photoperiod and food restriction ($F = 0.15$, $P = 0.71$). Finally, there was a significant effect of photoperiod ($F = 5.17$, $P = 0.03$) and food restriction ($F = 74.6$, $P < 0.0001$) on increase in body mass but no significant interaction of the two ($F = 0.74$, $P = 0.40$).

Experiment 3

For PVG rats (Fig. 3), there was a significant effect of photoperiod ($F = 8.90$, $P = 0.004$) and food restriction ($F = 35.20$, $P < 0.001$) on testis mass, but no interaction of photoperiod and food restriction ($F = 2.45$, $P = 0.12$). Likewise, there was a significant effect of photoperiod ($F = 38.86$, $P < 0.002$) and food restriction ($F = 151.01$, $P < 0.001$) on seminal vesicle mass, but no interaction ($F = 0.08$, $P = 0.78$). Finally, there was a significant effect of photoperiod ($F = 19.08$, $P < 0.0001$) and food restriction ($F = 440.76$, $P < 0.0001$) on increase in body mass but no significant interaction of the two ($F = 0.17$, $P = 0.67$).

Discussion

The results show that in all three strains, ACI, BUF, and PVG, young males are photoresponsive. Each inbred strain showed significantly lower reproductive organ masses (Figs 1a,b, 2a,b, 3a,b) and slower increase in body mass (Figs 1c, 2c, 3c) in the short photoperiod. There were also effects of food restriction on reproduction, and, with one exception, no interaction of food restriction with photoperiod. The single exception was an interaction between photoperiod and food restriction on testis mass in BUF rats. Overall, this suggests that food restriction may unmask further reproductive responses to SD in young male BUF rats, but not in the other strains. This lack of evidence for an interaction between food and photoperiod is unlike the situation in young male BN rats (Lorincz *et al.* 2001) and F344 rats (Heideman *et al.* 1998).

Previous work on young male F344 and BN rats indicated that reproductive and body mass responses to SD were maximal, when represented as percentage difference between SD and LD treatments, between 4 weeks and 8 weeks of photoperiod treatment (Heideman & Sylvester 1997, Lorincz *et al.* 2001). By 16 weeks of treatment, reproductive responses to SD were no longer detectable, but body mass differences persisted for 10 to 16 weeks (Heideman *et al.* 1998, Lorincz *et al.* 2001). Adult male F344 rats had relatively smaller, though still significant, reductions in reproductive organ mass and body mass in SD, suggesting that adult males have weaker responses than young males (Shoemaker & Heideman 2002), and studies on other rodent species also suggest that adults can have weaker photoperiod responses than younger animals (Johnston & Zucker 1979, Rivest *et al.* 1986, Donham *et al.* 1989, Stanfield & Horton 1996). If ACI, BUF,

Figure 3 Mean ($\pm$ S.E.) of paired testis mass (a), paired, emptied, seminal vesicle mass (b), and change in body mass (c) for male PVG rats under long day (LD) or short day (SD) photoperiods, and fed an ad lib or food restricted (FR) diet for four weeks, beginning at three weeks of age. See Materials and Methods and Results for sample sizes and statistical tests.

and PVG rats are similar to these other strains in age-related changes and the time course of responses to photoperiod, then we would predict either very slight and possibly insignificant photoperiodic responses in unmanipulated adult rats of these three strains.

The three strains varied in levels of photoresponsiveness. When considered as percentage difference in mass

between LD and SD, the three strains are ordered in magnitude of response $ACI > BUF > PVG$ for reproductive measures, and $ACI > BUF = PVG$ for growth in body mass. All three strains have lower magnitude responses in both measures than F344 rats (Heideman & Sylvester 1997, Heideman *et al.* 1998) or BN rats (Lorincz *et al.* 2001). In addition, BN rats differ in photoperiodic responses from F344 rats (Lorincz *et al.* 2001). Considered together, these data suggest that photoperiod affects reproduction differently in these strains even under the same environmental conditions. This also implies that these strains differ genetically in photoperiodic responses, and that the strains have physiological differences in the pathway through which photoperiod and melatonin secretion regulate responses to photoperiod (Ebling & Cronin 2000, Heideman *et al.* 2000, Goldman 2001, Prendergast *et al.* 2001).

Canzian (1997) carried out a phylogenetic analysis of 63 inbred rat strains. While the rat strains tested in that analysis were sometimes developed by crosses of other strains, and thus Canzian's tree is not a true phylogeny, it does provide a method to estimate genetic differences among strains. Strains F344, ACI, PVG, and BUF rats are distributed across a major supergroup of rat strains that included approximately half of the strains tested by Canzian (1997). The presence of photoresponsiveness in all of these strains suggests that photoresponsiveness may be a general trait of this group. Some of the three strains tested in this study had relatively weak responses to SD, and none underwent a response in reproduction or body mass as great as F344 rats. If these three strains are representative, then most strains within this supergroup may have relatively weak responses to SD. The BN strain has responses to SD that are in the same order as those of F344 rats. BN rats are genetically an outlier distantly related to all other inbred rat strains and are an independent domestication of rats from the other strains tested (Festing & Bender 1984, Canzian 1997). No strains have been tested for photoresponsiveness in two other supergroups of rats identified by Canzian (1997), each of which includes important research strains, including WKY strains in one, and LEW strains in the other.

The results of this study lead us to hypothesize that many, and perhaps most inbred strains of rats may be photoperiodic, even without manipulation, in both reproductive characteristics and body mass. Previous studies on F344 rats suggest that photoperiodic responses of young rats may persist into adulthood (Shoemaker & Heideman 2002). The results of this study also suggest that the magnitude of response and the specific photoresponsive traits are likely to differ among strains. The fact that some non-inbred strains of rats that are not normally photoresponsive can be induced to show reproductive inhibition under SD photoperiods by treatments such as food restriction (Sorrentino *et al.* 1971, Blask *et al.* 1980), neonatal androgen treatment (Reiter *et al.* 1968), adult androgen treatment (Wallen & Turek 1981, Wallen *et al.* 1987), or olfactory bulbectomy (Reiter *et al.* 1969, Reiter *et al.* 1971, Nelson & Zucker 1981) (but see

Peiper *et al.* 1990), further adds to the complexity of photoperiodic responses among laboratory rats. Variable photoperiodic responses may be useful in the study of neuroendocrine variation in environmental control of reproduction and body mass (Bittner & Friedman 2000, Heideman *et al.* 2001). The results are particularly important because standard research protocols may unknowingly manipulate this pathway and cause unsuspected effects within or among studies on rats that are sensitive to photoperiod.

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