Butyric acid suppresses palatable food consumption in Siberian hamsters (*Phodopus sungorus*) housed in short, but not long, photoperiods

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**Abstract:** Short days induce winter-like adaptations in small mammals such as Siberian hamsters (*Phodopus sungorus* (Pallas, 1773)). Specifically, hamsters adjust food consumption, metabolic processes, and immune function to optimize energetic needs and promote winter survival. One potentially inexpensive behavioral adaptation to increase survival is avoidance of infection. We tested the hypothesis that photoperiod affects avoidance of potentially infected food. In experiment 1, hamsters were weaned into either short or long days with ad libitum food. Three weeks later, hamsters were presented with either skim milk treated with butyric acid (2%), a bacterial product that serves as a potent cue of spoilage, or unadulterated skim milk; consumption was measured. After milk presentation, blood samples were obtained to assess cortisol. In experiment 2, skim-milk consumption was again assessed after 3 weeks in either short or long days. In experiment 3, we tested the hypothesis that food avoidance was due to photoperiod-induced differential neophobia. Short-day hamsters increased milk consumption, which was blocked by butyric acid. Short days increased cortisol concentrations; neither food restriction nor butyric acid affected cortisol concentrations. Photoperiod did not alter neophobic responses. These experiments suggest that short-day hamsters avoid food treated with butyric acid possibly as an adaptive trait to avoid costly winter infections.

**Résumé :** Les jours courts induisent des adaptations de type hivernal chez les petits mammifères, tels que les hamsters nains de Dzoungarie (*Phodopus sungorus* (Pallas, 1773)). En particulier, les hamsters ajustent leur consommation de nourriture, leurs processus métaboliques et leur fonction immunitaire de manière à optimiser leurs besoins énergétiques et de favoriser leur survie durant l’hiver. Une adaptation comportementale potentielle à faible coût pour augmenter la survie est l’évitement des infections. Nous vérifions l’hypothèse selon laquelle la photopériode affecte l’évitement de nourriture potentiellement contaminée. Dans l’expérience 1, nous avons soumis des hamsters dans des conditions de jours courts ou longs et les avons nourris à volonté. Trois semaines plus tard, nous avons fourni aux hamsters ou bien du lait écrémé traité à l’acide butyrique (2%), un produit bactérien qui est un puissant indicateur d’avarie, ou alors du lait écrémé sans additif et nous avons mesuré leur consommation. Après la présentation du lait, des prélèvements sanguins ont permis de doser le cortisol. Dans l’expérience 2, nous avons de nouveau évalué la consommation de lait écrémé après 3 semaines en conditions de jours courts ou longs. Dans l’expérience 3, nous avons testé l’hypothèse selon laquelle l’évitement de la nourriture est dû à une néophobie différentielle sous l’action de la photopériode. En conditions de jours courts, les hamsters augmentent leur consommation de lait, qui est cependant enrayée par l’acide butyrique. Les jours courts font augmenter les concentrations de cortisol, mais ni les restrictions alimentaires, ni l’acide butyrique n’affectent les concentrations de cortisol. La photopériode ne modifie pas les réactions de néophobie. Ces expériences indiquent que les hamsters en conditions de jours courts évitent la nourriture traitée à l’acide butyrique, possiblement comme un trait adaptatif pour éviter les infections coûteuses en hiver.

**Introduction**

Small mammals that live in temperate or boreal habitats, including Siberian hamsters (*Phodopus sungorus* (Pallas, 1773)), have evolved strategies to cope with temporal variations in food availability. Siberian hamsters use short day lengths (i.e., photoperiod) as a relatively noise-free cue to cease breeding, which prevents energetically expensive reproductive functions (spermatogenesis, pregnancy, and lactation) from coinciding with low food availability during...
autumn and winter. Such adaptations may promote survival during harsh winters and thus allow hamsters to reproduce during the following spring when environmental conditions are more conducive to offspring survival. Specifically, short day lengths decrease food intake (Wade and Bartness 1984), body mass (Steinlechner et al. 1983), and plasma glucose concentrations (Bartness et al. 1995) in some species of rodents.

Many seasonally breeding mammals enhance some aspects of immune function in response to short day lengths (Nelson et al. 2002). This phenomenon may be an adaptation to compensate for an increased stressor load during the winter or it may be a consequence of reproductive costs during the summer, both of which may dampen immune function (Nelson 2004; Martin et al. 2008a). In addition to immune function, hamsters may also alter other physiological or behavioral processes to promote survival when at increased risk for infection. Other behaviors such as increased predator vigilance, communal huddling, or social avoidance of sick animals might also be evoked by the short days of winter and involve photoperiodic adjustments in sensory or perceptual systems (Martin et al. 2008b). For example, short-day hamsters decreased ingestion of dietary iron, a nutrient vital to bacterial replication, and short-day animals either differentially detected or avoided iron compared with long-day animals (Bilbo et al. 2002). Given the high costs of infection during the winter (when small animals are exposed to an energetic bottleneck of high thermogenic costs and low resource availability), individuals should enhance the ability to detect potentially infectious individuals and food.

The presence of butyric acid in food is consistent with a high bacterial load. Butyric acid (BA) produces a powerful odor that has been described as sharp, rancid, putrid, and sour, and elicits avoidance behavior in rodents (Fendt 1977). Typical species include Clostridium butyricum, Clostridium perfringens, and Clostridium pasteurianum. Less common but lethal species include Clostridium tetani, which causes the neurological disease tetanus, and Clostridium botulinum, which produces the nerve toxin botulin that can lead to respiratory and musculoskeletal paralysis (Martinko and Madigan 2005). Bacteria presumably evolved to produce noxious toxins to prevent consumption of shared food resources with animals. Bacteria may produce malodorous toxins in particular as an adaptation to warn animals of the dangers of consuming an infected substance (Janzen 1977). Thus, individual mammals likely evolved to be wary of food emitting such odors to reduce or eliminate the likelihood of infection (Janzen 1977).

Two distinct possibilities may exist for seasonal changes in infection avoidance in hamsters: in short day lengths, hamsters may (1) enhance the ability to detect and avoid infected food or (2) ignore BA during reduced food availability to consume the calories (a strategy that animals in summer, with presumably more abundant food, would avoid). The former would suggest short-day hamsters have a heightened sensitivity to food quality and thus elevate behavioral defense against infection. The latter would suggest that short-day hamsters blunt sensitivity to spoiled food cues, a riskier but plausible strategy. It is also possible for ad libitum food conditions in the laboratory to obscure photoperiod-related differences in spoiled food avoidance. Therefore, we also added food restriction as an experimental condition to rule out this possibility.

Materials and methods

Experiment 1

Forty-seven male Siberian hamsters from our breeding colony were used in this study (for experimental timeline see Fig. 1). Hamsters were weaned at approximately 21 days of age and transferred to either short (8 h light : 16 h dark; n = 24) or long (16 h light : 8 h dark; n = 23) days with lights off at 1500 Eastern Standard Time (EST). All procedures were approved by The Ohio State University Institutional Laboratory Animal Care and Use Committee and conducted in accordance with National Institutes of Health and United States Department of Agriculture guidelines for animal care and use. Hamsters were housed individually in polypropylene cages (28 cm × 17 cm × 12 cm) with ad libitum access to food (Harlan Teklad 8640, Indianapolis, Indiana, USA) and filtered tap water unless otherwise stated. Hamsters were randomly assigned to food-restricted (FR) or ad libitum (long day (LD), FR: n = 11; short day (SD), FR: n = 12; LD, ad libitum: n = 12; SD, ad libitum: n = 12) groups. During the first 2 weeks, all hamsters had ad libitum access to food, and food consumption was measured and calculated to determine daily food consumption in grams. After 2 weeks in photoperiod, estimated testis volume was obtained (see below for detailed methods); hamsters in the food-restricted groups received 70% of their daily food consumption for 1 week. After 1 week of food restriction, hamsters from each group were randomly assigned to receive a bottle with either skim milk or skim milk with 2% BA (Sigma-Aldrich, St. Louis, Missouri, USA) for 45 min. This time period was used to obtain blood to assess cortisol concentrations in response to BA. Owing to the pungent odor of BA, a low concentration was used. In an earlier study, 4% BA was used for mice and appeared to be unpalatable (Harrison et al. 1991). To reduce this problem, 2% BA was used, based on a pilot study that indicated hamsters consumed this concentration (E.M. Johnson, L.B. Martin, and R.J. Nelson, unpublished data).

Prior to BA presentation (at 1500), hamsters were transferred individually to a fume hood in a separate room and allowed to acclimate for 15 min. After 15 min, food and water were removed from the cage and hamsters were given either skim milk or skim milk with 2% BA. Bottles were weighed before and after placement into cages to obtain milk consumption in grams. After 45 min of milk presentation, body mass was assessed and hamsters were exsanguinated through a retro-orbital sinus bleed.

Estimated testis volume

Hamsters were lightly anesthetized with isoflurane vapor and a 1 inch × 1 inch (1 inch = 25.4 mm) patch of fur was shaved from the abdomen. The right testis was moved to the scrotum with forceps and external testis length and width

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Fig. 1. Timeline for experiment 1.

were measured with analogue calipers (±0.1 mm). The product of testis width squared times testis length provided a measure of estimated testis volume (ETV), which is highly correlated ($r > 0.95$) with testis mass (Gorman and Zucker 1995).

**Cortisol radioimmunoassay**

Blood samples were kept on ice until centrifugation for 30 min at 6000 $r/min$ (3.3g). The plasma was the drawn off and stored in polypropylene microcentrifuge tubes at −80 °C. Cortisol is the primary circulating glucocorticoid in this species (Reburn and Wynne-Edwards 1999) and plasma cortisol concentrations were determined in a single assay using a Diagnostic Systems Laboratories $^{125}$I double antibody kit (Los Angeles, California, USA). This kit has been validated previously in this species (Reburn and Wynne-Edwards 1999; Jasnow et al. 2000).

**Experiment 2**

Because we found a main effect of short day lengths on skim-milk consumption, we conducted a second experiment to determine if short days altered milk consumption independently of BA analysis. We again randomly assigned hamsters to either long or short days upon weaning (21 days; short day: $n = 6$; long day: $n = 6$). Hamsters had ad libitum access to food and water and were allowed 3 weeks to acclimate to their respective photoperiods and then presented with skim milk in the same manner as in experiment 1.

**Experiment 3**

It was possible that long-day hamsters consumed less milk because they displayed more neophobia than short-day hamsters. To determine whether photoperiod affected neophobia in Siberian hamsters, hamsters were again randomly assigned to either long or short days upon weaning (21 days; short day: $n = 6$; long day: $n = 6$). Hamsters had ad libitum access to food and water and were allowed 3 weeks to acclimate to their respective photoperiods and then presented with a novel object (a small Habitrail tube) for 5 min to determine neophobic responses (Martin et al. 2007). At 6 weeks of age (3 weeks in photoperiod), hamsters were moved to the testing room and allowed to acclimate for 15 min. The tube was placed in each cage for 5 min. Behavior was recorded on video and later scored by an observer who was uninformed about treatment conditions for (1) latency to make contact with the tube, (2) time spent touching the tube, and (3) the number of times hamsters touched the tube. The Habitrail tube was cleaned with 70% ethanol between tests.

**Statistical analyses**

Food and estimated testis volume were corrected for body mass in both experiments 1 and 2. Milk consumption was analyzed as absolute consumption in grams and as grams consumption relative to grams body mass (g/g). Milk consumption and cortisol concentrations from hamsters in experiment 1 were analyzed with photoperiod, food restriction, and BA as the independent factors using $2 \times 2 \times 2$ ANOVAs. Pairwise comparisons were performed using Fisher’s protected least significant difference (PLSD). Body mass from experiment 1 was analyzed using a $2 \times 2$ ANOVA with photoperiod and food consumption as independent measures. Food consumption data (both absolute and corrected for body mass) were analyzed with Student’s $t$ tests with photoperiod as the independent factor. For experiment 2, body mass and milk consumption data were analyzed with Student’s $t$ tests, using photoperiod as the independent factor. For experiment 3, data were again analyzed with Student’s $t$ tests with photoperiod as the independent factor. Tests were conducted using StatView version 5.0.1 software (SAS Institute Inc., Cary, North Carolina, USA). All mean differences were considered statistically significant if $p \leq 0.05$.

**Results**

**Photoperiod and food restriction effects on morphology**

Short days significantly reduced body mass ($F_{[1,43]} = 18.66, p < 0.0001$), but this effect was driven by hamsters in the food-restricted group, as post hoc tests revealed that in the ad libitum food group, there was no significant effect of day length on body mass ($t_{[22]} = 2.019, p = 0.056$). Food restriction also significantly reduced body mass ($F_{[1,43]} = 9.493, p < 0.005$), but this effect was limited to short-day hamsters, as food restriction in long-day hamsters did not significantly alter body mass ($t_{[22]} = 1.438, p > 0.1$). There was a main effect of photoperiod on estimated testis volume ($F_{[1,44]} = 45.931, p < 0.0001$; Fig. 2); short day lengths significantly reduced testis volume, but there was no effect of food restriction on estimated testis volume ($p > 0.05$). Prior to food restriction, there was no effect of day length on absolute food consumption ($t_{[45]} = 1.394, p > 0.05$) or food consumption relative to body mass ($t_{[45]} = 0.707, p > 0.05$). In experiment 2, short days significantly reduced body mass ($t_{[10]} = 2.994, p < 0.05$).
Milk consumption

In experiment 1, there was a significant interaction between photoperiod and BA–milk on relative ($F_{[1,39]} = 4.808$, $p < 0.05$; Fig. 3) and absolute ($F_{[1,39]} = 4.04$, $p = 0.05$) milk consumption. Fisher’s PLSD revealed that short days increased relative and absolute milk consumption in food-restricted hamsters ($p < 0.05$ in both cases), but not when BA was present ($p > 0.05$ in both cases). Among hamsters that had ad libitum access to food, short days did not significantly alter relative or absolute milk consumption ($p > 0.05$ in both cases). In short-day hamsters, BA presentation significantly reduced relative milk consumption, regardless of food availability ($p < 0.05$ in both food availability groups) and absolute milk consumption, regardless of food availability ($p < 0.05$ in both food availability groups). In experiment 2, short days significantly increased relative ($t_{[10]} = 2.277$, $p < 0.05$; Fig. 4), but not absolute milk consumption ($t_{[10]} = 2.076$, $p > 0.05$).

Cortisol concentrations

In experiment 1, there was a main effect of photoperiod on cortisol concentrations ($F_{[1,38]} = 17.66$, $p < 0.0005$; Fig. 5); short day lengths significantly elevated cortisol concentrations, regardless of food availability ($p < 0.05$ in both food availability groups) and absolute milk consumption, regardless of food availability ($p < 0.05$ in both food availability groups). In experiment 2, short days significantly increased relative ($t_{[10]} = 2.277$, $p < 0.05$; Fig. 4), but not absolute milk consumption ($t_{[10]} = 2.076$, $p > 0.05$).

Neophobia

In experiment 3, photoperiod did not alter the latency to touch ($t_{[10]} = 0.165$), the number of touches ($t_{[10]} = 1.382$), or the duration spent touching ($t_{[10]} = 0.495$) the novel object ($p > 0.05$ in all cases; Figs. 6A–6C).

Discussion

Winter is a particularly challenging time for seasonally breeding rodents, as they are subjected to low temperatures and reduced food availability. One important adaptation that may confer winter survival is the enhancement of some aspects of immune function (Nelson et al. 2002). This immune enhancement may be an adaptation to invest in immune function that, under natural conditions, may be impaired by challenging winter-related stressors (Nelson 2004). Conversely, reproductive regression in response to decreasing day lengths may free up energy that can be redirected to-
ward the immune system. However, it would also be adaptive and least costly to avoid infection altogether. This study supports a role of food avoidance in defending against pathogen acquisition. A similar phenomenon occurs with avoidance of parasitized individuals. Rodents can recognize whether a conspecific is infected through chemosensory signals such as major histocompatibility complex and major urinary proteins. Subsequently, rodents avoid mating with parasitized individuals, and thus avoid a potential infection and may also confer genetic resistance to infection to offspring by mating with a healthy conspecific (Kavaliers et al. 2005; Zala et al. 2008). Cessation of food consumption when in the presence of an aversive odor may likewise reduce the risk of contracting a pathogen.

Hamsters housed in winter-like conditions reduced palatable food consumption when exposed to BA, whereas hamsters housed in long days exhibited no such change in food intake. This avoidance may indicate that during winter Siberian hamsters engage behavioral defenses to preempt potential infections. Contrary to findings in experiment 1, short-day hamsters in experiment 2 (that had ad libitum access to food) increased relative milk consumption (Fig. 4) and reduced body mass compared with long-day hamsters. The short-day-induced increase in milk consumption may be a consequence of adjusting the raw measurement by body mass. Absolute milk consumption in experiment 2 did not significantly differ between short-day and long-day hamsters (although there was a trend in that short-day hamsters had a higher mean milk consumption; \( p = 0.065 \)). When considered together, data from experiments 1 and 2 suggest that short photoperiod may elicit a subtle increase of palatable food consumption at 3 weeks of exposure, but food restriction elicits a significantly greater increase. Data from experiment 3 indicate that day length does not alter neophobic responses, which suggests that although long-day hamsters consumed less milk, they were not likely exhibiting a neophobic response (Figs. 6A–6C).

The presence of BA elicited no difference in milk consumption among long-day hamsters under both ad libitum and food-restricted conditions (Fig. 3). This may indicate that long-day hamsters sampled both BA and non-BA milk, and after that did not consume more. If this is the case, then it suggests that long-day hamsters are less motivated to consume unspoiled food than short-day hamsters. This may seem counterintuitive given hamsters reduce body mass and food consumption in short days (Steinlechner et al. 1983; Wade and Bartness 1984; Bartness et al. 1995). However, short days (especially when coupled with food restriction) may increase the motivation to consume unspoiled food when it is available. Short-day hamsters have higher thermo-
regulatory demands compared with long-day hamsters (Steinlechner et al. 1983), so increasing palatable food consumption when it is available would increase the probability of survival in a food-scarce environment. It is also important to note that although hamsters typically reduce body mass in response to short days, it is not a consequence of reduced food consumption. Rather, lowering body mass reduces energy demands and absolute food consumption (Steinlechner et al. 1983). In experiment 1, short days increased absolute, as well as relative, milk consumption which indicates that short-day increases in relative milk consumption are not elevated simply because of a reduction in body mass. Short day lengths significantly elevated cortisol concentrations, but neither food restriction nor BA altered cortisol concentration which suggests that avoidance of the BA milk is not likely mediated by glucocorticoids (Fig. 5).

Whether short-day hamsters avoid BA because it is aversive or they have evolved defenses to avoid the sharp-smelling odor (as many rodent species have with predator odor) remains to be tested. This study is the first step in determining whether Siberian hamsters avoid food-related stimuli that may lead to infection. Although Siberian hamsters may avoid spoiled food merely because it smells bad, aversive odorants may yet elicit an innate, adaptive response (avoidance). BA may be a “semiochemical” — a molecule that elicits an innate behavioral response by activating the vomeronasal organ (as opposed to only the main olfactory system). It is possible that neurons in the vomeronasal organ (as opposed to only the main olfactory system) detect BA and subsequently activate limbic areas to initiate avoidance of sour-smelling food. Future studies should also investigate the brain mechanisms responsible for such avoidance.

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References


