NOTE / NOTE

Photoperiod alters pain responsiveness via changes in pelage characteristics

Zachary M. Weil, Michelle Gatien Hotchkiss, and Randy J. Nelson

Abstract: Small mammals use day length to adjust morphology and physiology to anticipate seasonal changes in environmental conditions. The canonical photoperiod-mediated annual adaptation is seasonal breeding. However, increasing evidence suggests that day-length information can induce plasticity in the nervous system, and thus provoke behavioral plasticity that can aid in winter survival. We hypothesized that low temperatures and reduced food availability in the winter would necessitate the evolution of increased pain tolerance mediated by short day lengths. Siberian hamsters (*Phodopus sungorus* (Pallas, 1773)) housed in short days regressed their reproductive tracts and molted to winter pelage. Short-day hamsters also displayed elevated latencies of nociceptive responses in the hot-plate test, suggesting reduced pain responsivity. Prior to assessing potential neuronal or neuroendocrine mediators of altered pain responses, however, we investigated the possibility that changes in fur characteristics mediated photoperiod differences in pain responsivity. Removal of fur with a depilatory cream eliminated photoperiod differences in pain responsivity. Taken together, these data indicate that day length regulates thermal pain responses via changes in fur properties; also, changes in pelage properties have both thermoregulatory and thermal insulatory properties.

Résumé : Les petits mammifères utilisent la longueur du jour afin d'ajuster leur morphologie et leur physiologie en anticipation des changements saisonniers des conditions du milieu. L'adaptation saisonnière par excellence contrôlée par la photopériode est la reproduction annuelle. Cependant, des indications de plus en plus nombreuses laissent croire que les informations sur la longueur du jour peuvent induire une plasticité du système nerveux et provoquer ainsi une plasticité comportementale qui peut favoriser la survie à l'hiver. Nous avançons l'hypothèse selon laquelle les basses températures et la disponibilité réduite de la nourriture en hiver requièrent l'évolution d'une tolérance accrue à la douleur sous l'influence des courtes photopériodes. Des hamsters nains de Dzoungarie (*Phodopus sungorus* (Pallas, 1773)) gardés dans des conditions de jours courts subissent une régression de leurs voies reproductrices et acquièrent par mue leur pelage d'hiver. Les hamsters gardés en jours courts affichent aussi des latences élevées des réactions nociceptives dans le test de sensibilité à la chaleur, ce qui laisse croire à une réactivité réduite à la douleur. Cependant, avant d'évaluer les médiateurs neuronaux ou endocriniens potentiels de ces réactions modifiées à la douleur, nous avons étudié la possibilité que les changements dans les caractéristiques de la fourrure soient responsables des différences de réactivité à la douleur reliées à la photopériode. Le retrait de la fourrure avec une crème épilatoire élimine les différences de réactivité à la douleur reliées à la photopériode. Dans leur ensemble, ces données indiquent que la photopériode contrôle les réactions à la douleur thermique par le biais de changements dans les propriétés de la fourrure; de plus, les changements dans les caractéristiques du pelage ont des propriétés de thermorégulation et d'isolement thermique.

[Traduit par la Rédaction]

Introduction

Most animals living outside of the tropics experience significant yet predictable annual fluctuations in environmental conditions, including ambient temperature, food and water availability, and predation pressures (Bronson 1985, 1989). As such, many animals have evolved mechanisms to adjust their physiological and morphological characteristics to cope with the challenges represented by the changing seasons (Goldman and Nelson 1993). For instance, individuals of many small-mammal species restrict their breeding activities to the early spring and summer when environmental conditions are relatively mild (Bronson 1989). At other times of the year, energetic investments are shunted towards processes that aid in overwinter survival, including immunological, metabolic, and thermoregulatory adaptations (Nelson

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Received 7 July 2008. Accepted 24 July 2008. Published on the NRC Research Press Web site at cjz.nrc.ca on 3 October 2008.

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doi:10.1139/Z08-098

and Demas 1997; Prendergast et al. 2002). Photoperiod (day length) is a simple, reliable environmental cue that can provide precise calendar information (Goldman 2001). Thus, because many physiological processes require significant time to adjust to new environmental conditions, animals (and other organisms across many taxa) have evolved the ability to monitor photoperiod as an indicator of upcoming seasonal changes (Goldman and Nelson 1993). In mammals, photoperiod information is transduced from an environmental factor into a physiological signal by the duration of the nighttime secretion of pineal melatonin (Lincoln 2006).

Day-length information can also induce plasticity in the nervous system and serves to maximize survival at different times of the year (Prendergast et al. 2002). In addition to the traditional reproduction-related processes including mating behaviors and territorial defense, day length can alter the neural substrates underlying affective responses, learning, and memory, as well as sickness responses (Demas et al. 2004; Prendergast and Nelson 2005; Pyter et al. 2005; Pyter and Nelson 2006; Trainor et al. 2007). We hypothesized that short photoperiods could also alter the neural systems involved in pain perception, specifically during exposure to extreme temperatures, because many small mammals are likely to experience low temperatures during winter. Furthermore, because food is more limited during the winter than at other times of year, it might be necessary for animals to endure painful stimuli when foraging, or risk starvation.

In mammals, photoperiod information is transduced from an environmental signal into a physiological one by the nighttime secretion of pineal melatonin (Goldman and Nelson 1993). There are data to suggest that day length and melatonin can alter pain perception via central mechanisms. Melatonin, for instance, can act as an analgesic via interactions with the opioid pain modulation system (Lakin et al. 1981; Lissoni et al. 1986). Additionally, swim-stress modulation of nociceptive responses was modulated by day length in deer mice (*Peromyscus* Gloger, 1841), although no basal differences in pain responsiveness were reported (Kavaliers and Galea 1995).

We hypothesized that short day lengths would reduce the nociceptive threshold to a thermal stimuli in a standard assay of pain sensitivity in Siberian hamsters (*Phodopus sungorus* (Pallas, 1773)). Although testing of responses to low temperatures might be more ecologically valid for hamsters that live in Siberia, no nociceptive responses were observed to cold-plate tests in Siberian hamsters (Z.M. Weil and R.J. Nelson, unpublished observations). Our preliminary data with the hot-plate task indicated that short day lengths substantially increased the latency to display a pain-related behavior. However, before examining the neuronal mechanisms that could potentially underlie photoperiod differences in pain responses, we tested the possibility that the molt to winter fur insulated the hind paws from the heat stimulus. This experiment was designed with the following goals: (*i*) to test the hypothesis that short day lengths would increase pain threshold to a heat stimulus and (*ii*) to determine whether any observed photoperiod differences reflect direct or indirect effects of melatonin or other neuroendocrine systems on the pain system.

Materials and methods

Siberian hamsters used in this study were bred in our laboratory at The Ohio State University. Animals were individually housed in polypropylene cages in colony rooms with constant temperature (21 ± 4 °C) and humidity (50% ± 10%) and ad libitum access to food (Harlan Teklad #8640; Harlan, Indianapolis, Indiana) and filtered tap water. All experimental protocols were approved by the Institutional Lab Animal Care and Use Committee at The Ohio State University and were in accordance with the guidelines established by the US National Institutes of Health. All hamsters were gestated in long photoperiod (LD; 16 h light : 8 h dark) and remained there until ~60 days of age when they were randomly assigned to either remain in long days or were transferred to short-day (SD; 8 h light : 16 h dark) conditions. Hamsters remained in short days for 10 weeks prior to testing.

Hot-plate testing was conducted during the active phase (lights-off) for each photoperiod group. Twenty-four hours prior to testing, hamsters were acclimated to the hot plate (IITC, Woodland Hills, California) at room temperature inside a clear Plexiglas® cylinder (internal diameter = 8 cm; height = 12 cm). The following day, testing was conducted with the hot plate at 60 °C. Hamsters remained on the hot plate until they either displayed a nociceptive response, defined as lifting or shaking one of their hind paws (Wiesenfeld-Hallin et al. 1990; Rubinstein et al. 1996), or for a maximum of 5 min. Latency to exhibit these behaviors was recorded.

In a second experiment, separate groups of LD and SD hamsters were tested following removal of the fur on their hind paws. The hamsters were deeply anesthetized with ketamine or xylazine and then a depilatory cream (NAIR®; Church and Dwight, Princeton, New Jersey) was used to remove all hair on both hind paws. Testing began 1 week after hair removal.

Following the conclusion of all experimental procedures, hamsters were deeply anesthetized with isoflurane vapors (to effect), weighed, and decapitated. Paired testes were removed, cleaned of connective tissue, and weighed to the nearest 0.1 mg. Any SD hamsters whose paired testes masses fell within two standard deviations of the long-day mean were classified as photoperiod nonresponders (SDNR, $n = 13$) and removed from further analyses. Pelage color was scored in whole numbers from 1 to 4 (1 is dark, “summer” coat color; 4 is white, “winter” coat color) for each group, according to a scale established previously (Duncan and Goldman 1985).

Trunk blood was collected in heparinized microcentrifuge tubes and spun at 3000 r/min (800*g*) at 4 °C for 30 min. The resulting sera were removed and stored at –80 °C until further analysis. Testosterone concentrations were assessed using a Diagnostic Systems Laboratory (Webster, Texas) double-antibody radioimmunoassay kit. The trunk blood samples were assayed in duplicate according to the manufacturer’s instructions. Statistical analyses were conducted using one-way (photoperiod) or two-way (photoperiod × fur) ANOVAs. Post hoc tests were conducted using Fisher PLSD test for planned comparisons. All differences were considered statistically significant if $p < 0.05$.

Table 1. Short day lengths induced regression of the reproductive tract and molt to the winter pelage color in short-day (SD) but not short-day nonresponsive (SDNR) Siberian hamsters (*Phodopus sungorus*).

	LD	SD	SDNR
Paired testes mass (mg)	610±35	79±14*	652±35
Testosterone (ng/mL)	2.37±0.89	0.07±0.01*	4.0±1.26
Pelage score	1.0±0.00	2.14±0.09*	1.14±0.14
Body mass (g)	39.13±0.83	30.24±0.89*	41.01±1.128

Note: Data are presented as means ± SE.

*Significantly different from long-day (LD) hamsters.

Results

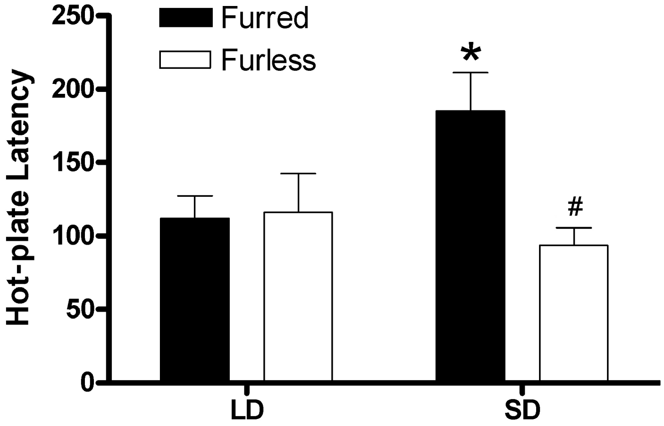
Hamsters housed in short days had significantly inhibited reproductive characteristics compared with those housed in long days, with the exception of the SDNR group which was not different from LD-housed animals on any measure (summarized in Table 1). Paired testes mass ($F_{[1,57]}$ = 194.77, $p < 0.00001$) and body mass ($F_{[1,57]}$ = 38.62, $p < 0.00001$) were significantly reduced in SD hamsters. Similarly, circulating testosterone concentrations were also reduced by chronic exposure to short day lengths ($F_{[2,58]}$ = 5.51, $p < 0.05$). Pelage color score was higher (lighter and more winter-like) in SD hamsters compared with those housed in long days ($F_{[2,30]}$ = 118.16, $p < 0.00001$).

SD hamsters that retained the fur on their paws displayed significantly longer latency to a nociceptive response when exposed to the hot-plate stimulus than those in all other groups ($F_{[3,56]}$ = 5.391, $p < 0.05$; see Fig. 1). Post hoc tests revealed that reduced responsiveness to painful stimuli in SD hamsters was mediated by altered pelage characteristics. Fur removal decreased the latency to respond to the thermal pain stimuli in furless SD hamsters compared with SD hamsters who retained their fur ($p < 0.001$). However, fur removal reduced pain thresholds in SD hamsters only, as the latency to respond did not differ between LD furless hamsters and LD hamsters that retained the fur on their paws ($p > 0.05$). There were no significant photoperiod differences among hamsters that had been treated with the depilatory cream ($p > 0.05$).

Discussion

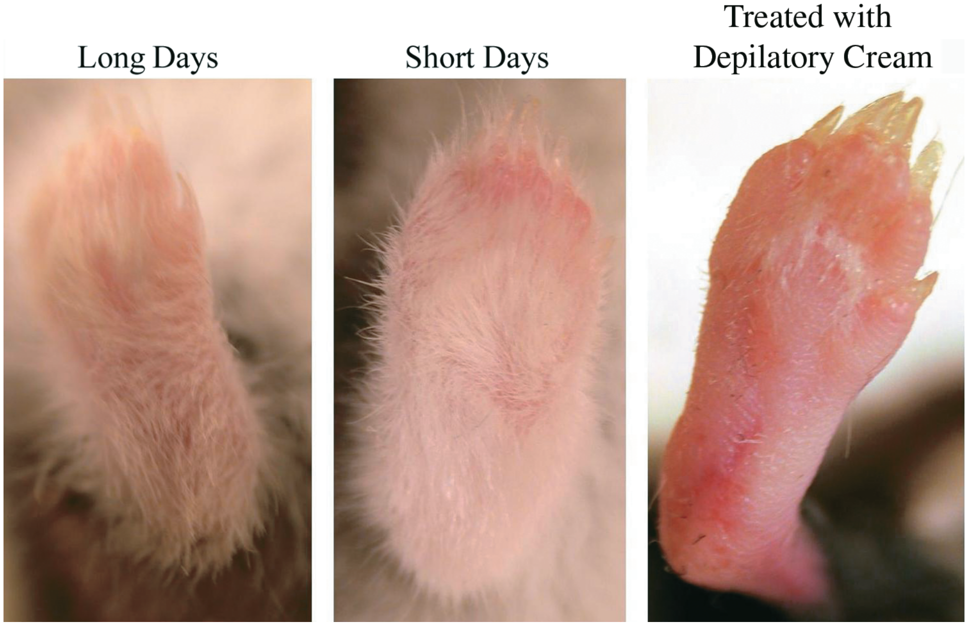
Short day lengths significantly increased nociceptive thresholds to the thermal stimulus. This effect was mediated by alterations in pelage characteristics on the hind paws, as hair removal completely eliminated the photoperiod difference in nociceptive response. Photographic analysis verified that hind-paw fur is much more dense in SD hamsters relative to those housed in long days (see Fig. 2). This increase in fur density in the extremities likely serves both to minimize conductive heat loss and to abrogate painful stimuli associated with foraging during the harsh winter months.

Fig. 1. Short day lengths increased thermal nociceptive thresholds in furred but not furless Siberian hamsters (*Phodopus sungorus*). Short-day (SD) nonresponsive hamsters did not increase pain threshold relative to long-day (LD) hamsters. Removal of fur with a depilatory cream completely eliminated photoperiod differences in pain responsivity. Asterisk indicates significantly different from LD-furred hamsters. Pound sign indicates significantly different from SD-furred hamsters.



Siberian hamsters are well adapted to harsh winter conditions. In the wild, these hamsters experience extremely low ambient temperatures during the winter with monthly above-ground averages around –24 °C, and some reports of individual days as cold as –45 °C (Weiner and Gorecki 1981; Weiner 1987). One relatively simple, but effective, strategy to cope with the reduced ambient temperatures and concomitant increase in thermoregulatory requirements is the winter acquisition of altered pelage characteristics (Kauffman et al. 2001*a*). Siberian hamsters in long day lengths have short gray–brown fur, but after transfer into short day lengths the pelage increases in length and density and becomes light in color (Duncan and Goldman 1985). These altered pelage characteristics have significant thermoregulatory benefits particularly in low ambient temperatures (Kauffman et al. 2001*a*, 2001*b*), as they can reduce conductive heat loss to the environment. It appears that the winter coats also serve to insulate tissues from painful temperature responses in the extremities (Kauffman et al. 2001*a*, 2001*b*). Pain systems putatively evolved to warn of the potential for tissue damage. Adaptations associated with protecting vulnerable tissues from damage would likely be more advantageous than those that simply increase the intensity at which stimuli become painful. The overall increase in fur length and density seems to have both insulative and thermoregulator effects. Neither Siberian hamsters nor deer mice (Kavaliers and Galea 1995) appear to display basal photoperiod differences (independent of fur modulation) in central pain processing, suggesting that if increased nociceptive thresholds are adaptive during the winter, then natural selection has favored a strategy of insulating the hamster paws rather than increasing anti-nociceptive mechanisms. It is not immediately apparent whether the altered pelage characteristics associated with chronic exposure to short day lengths evolved to increase nociceptive thresholds per se or whether changes in pain responsiveness are secondary to the thermoregulatory benefits gained by altering pelage properties. Additionally, selection pressure may also have led to the evolution of the spring molt back to the lighter coat. Reducing fur thickness and length may have served both to aid in thermoregulation during the relatively warm days of summer and also to prevent heat-induced tissue damage that might occur if the nociceptors were insulated by thick fur. Regardless of the origin of this photoperiodic difference, develop-

Fig. 2. Short day lengths alter pelage characteristics. Photographs of paws of representative Siberian hamsters (*Phodopus sungorus*) housed in (*a*) long days, (*b*) short days, or (*c*) following depilatory-cream treatment.



ment of reduced thermal sensitivity in winter-like conditions could potentially have significant fitness consequences for hamsters that must forage for food during the winter despite extremely cold temperatures and then also function in much warmer conditions in the summer.

Notably, fur removal only increased thermal sensitivity in SD animals. However, as the photographs indicate, it is obvious that the hind paws of LD hamsters are, nonetheless, relatively covered in fur. This suggests that an increase in fur density or length was necessary to get any protection from the thermal stimulus; i.e., the LD pattern of fur provided no additional insulation from the painful stimulus. Given the long latencies to respond in all groups (~2 min), it seems unlikely that this represents a floor effect. It is possible that the adaptive benefit of the LD fur pattern may be to minimize abrasive damage but is insufficient to insulate paw tissues from extreme temperatures. It is also possible, however, that SD hamsters utilize their fur behaviorally in a manner that allows them to maximize the insulation effects. Further experiments are needed to test that hypothesis. Finally, we did not assess whether the changes in pelage properties included length, density, or other aspects of the hair, issues that will be assessed in future studies.

In conclusion, photoperiod-mediated plasticity in the fur of Siberian hamsters appears to play a role both in thermoregulation and insulation from nociceptive responses, and thus has the added consequence of reducing responsiveness to painful stimuli. Although this pelage change may have evolved for thermoregulatory purposes, reductions in thermal sensitivity are nonetheless extant and may have important consequences for the survival of harsh winter conditions in Siberian hamsters.

Acknowledgements

We thank Jacqueline Barker and James Walton for technical assistance and Kate Karelina for helpful discussions of the data. Financial support was provided by National Institutes of Health grant MH57535 and National Science Foundation grant IBN 04-16987.

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