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Short photoperiods alter cannabinoid receptor expression in hypothalamic nuclei related to energy balance

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ABSTRACT

This study examined the photoperiodic regulation of energy balance and cannabinoid receptor expression in the Siberian hamster (*Phodopus sungorus*) hypothalamus. Short day lengths, beginning at weaning, reduced food intake, body mass and fat pad masses and also decreased cannabinoid receptor immunostaining in the anterior and lateral hypothalamic nuclei of male hamsters. These data suggest a potential role for reduced cannabinoid drive in mediating short day-induced alterations in energy balance.

Most animals living outside of tropical climates experience continual, but predictable, fluctuations in environmental conditions. In the winter, dramatically reduced food availability temporally coincides with increased thermoregulatory requirements creating a potential energetic bottleneck. Many small mammals have evolved mechanisms to partition energetic resources differently at different times of the year. Energetically costly activities associated with reproduction are typically confined to the mild conditions of the spring and early summer [4]. During the harsh conditions associated with winter small animals living at high latitudes instead invest in processes that help them survive until the next breeding season. Because these phenotypic changes require significant time to develop, many small mammals respond to the annual cycle of changing photoperiod (day length) as a simple and relatively noise free cue for the changing seasons [2]. In the laboratory, the full suite of winter adaptations can be induced by housing animals in short day lengths (e.g., <10 h light/day) [28]. The photoperiodic cue is transduced from an environmental signal into a physiological one via the nighttime secretion of pineal melatonin [19]. Exogenous melatonin or exposure to short day lengths inhibits the reproductive system, alters several components of the immune system, modulates energy balance and food intake as well as affects body mass and composition [7,23].

In preparation for the winter, most small animals accumulate fat stores that can be used when food availability is low [12,15]. Other animals such as Siberian hamsters (*Phodopus sungorus*) employ a different strategy [7]. These small mammals lose body mass and reduce adiposity prior to the winter and are thus able to run their metabolic systems at a much lower rate [39]. The reduction in body fat and adiposity is mediated by both reduced energy intake and altered patterns of energy utilization. For instance, many studies have reported that Siberian hamsters eat less in short day lengths, although some studies have reported that the weight loss is independent of food intake or that weight loss precedes reductions in energy intake. We recently reported that short day lengths alter the ratio of proteobacteria: firmicutes bacteria in the hamster cecum, a pattern that has been associated with a reduced ability to liberate energy from foods and low adiposity in other species [1,18,36]. The reduction in food intake and altered metabolic factors effects are accompanied by alterations in hypothalamic neuropeptide systems that regulate energy balance; including leptin and agouti related peptide [21,22]. Additionally, short day lengths enhance adipocyte lipolysis via changes in sympathetic signaling in fat depots [3,6,40].

An additional system that is well-situated to coordinate metabolic function in a seasonal context is the endocannabinoid system. The endocannabinoid system consists of at least two receptors (CB1 and CB2 although CB1 is thought to play the dominant role in the nervous system), the endocannabinoids, and synthetic and degradative enzymes [8,11]. Activation of CB1

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receptors produces a net anabolic effect whereas cannabinoid receptor antagonists reduce food intake and body mass [37,38]. CB1 antagonists also improve glucose and lipid metabolism in obese humans through both central and peripheral mechanisms [37]. In the CNS, CB1 receptors are found largely at presynaptic membranes of GABA- and glutamatergic synapses and are present in hypothalamic regions associated with energy balance [9,33,35]. Further, the concentration of the cannabinoid ligand 2-AG levels increases with fasting and falls after refeeding [17]. Finally, a fully functioning and photoperiodically responsive endocannabinoid system has recently been described in the pituitary pars tuberalis with reduced 2-AG content during short day lengths [41]. In this study, we sought to determine if photoperiodic changes in food intake and adiposity were associated with changes in CB1 receptor expression in hypothalamic tissues that regulate energy balance. If endocannabinoid tone is important for photoperiodic regulation of metabolism, then the short day reductions in food intake and adiposity should be paralleled by reduced CB1 receptor expression.

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 guidelines. Male Siberian hamsters (*P. sungorus*) used in this study were bred in our colony at Ohio State University. All hamsters were bred in long day lengths (LD 16:8) and maintained in long days until weaning (21 days). At that point, hamsters were randomly assigned to either remain in long day lengths ($n = 8$) or were transferred to short days (SD 8:16; $n = 9$). All hamsters were housed individually after weaning.

After 9 weeks in the post weaning photoperiod, food intake was assessed daily for 6 consecutive days. On the last day of food measurement, hamsters were weighed, pelage score assessed (scored on a 4 point scale; 4 darker summer-like coats to 1 lighter winter-like coats). Hamsters were then deeply anesthetized and perfused transcardially with ice cold 0.1 M PBS followed by 4% paraformaldehyde. Reproductive tissues and fat depots were carefully dissected out and weighed to the nearest 0.1 mg. Brains were removed, post-fixed for 4 h, cryoprotected in 30% sucrose until they sunk, frozen on crushed dry ice, and then stored at −80 °C until they were sectioned at 14 μm on a cryostat and thaw-mounted on to Super Frost Plus slides (Fisher, Hampton, NH, USA). Sections were stored at −20 °C until further processing. Slides were air dried, rinsed in distilled water, and then blocked with rabbit serum and bovine serum albumin (BSA). Slides were then incubated for 24 h at room temperature with rabbit anti-CB1 antibody (Abcam, Cambridge, MA), washed repeatedly and then incubated with a biotinylated goat anti-rabbit secondary antibody and then visualized with ABC and DAB. Sections from both groups were processed side-by-side to minimize intergroup variation in staining intensity.

Photographs of multiple hypothalamic nuclei (lateral, anterior, paraventricular and arcuate) were taken with a Nikon E800 microscope at 20×. Images were digitized and proportional stained areas were assessed using Image J software (NIH). Briefly, images from each anatomical region were assessed by the software to determine the immunoreactive regions. Then fixed size rectangular 'scan boxes' were superimposed over the image and the percentage of stained area within the box was recorded. Proportional area was then expressed as the percentage of the tissue stained relative to the entire size of the scan box. All statistical comparisons were conducted with one way ANOVAs, except for body mass which was analyzed as a one-tailed t test, and p values were considered statistically significant if $p \leq 0.05$. Correlational analyses were conducted using Pearson product moment analysis.

Exposure to short photoperiods after weaning inhibited development of the reproductive tract in male Siberian hamsters. Hamsters housed in short day lengths had smaller testes ($F_{1,16} = 207.66$, $p < 0.000001$; Fig. 1A), smaller paired epididymides ($F_{1,16} = 48.80$, $p < 0.000001$; Fig. 1B) and smaller seminal vesicles ($F_{1,16} = 103.07$, $p < 0.000001$; Fig. 1C). Additionally hamsters housed in short day lengths had lighter winter-like pelage scores ($F_{1,16} = 69.18$, $p < 0.000001$; Fig. 1D). In addition to inhibiting reproductive development, hamsters housed in short day lengths exhibited attenuated somatic growth. Hamsters in short days weighed less overall ($F_{1,16} = 3.64$, $p < 0.05$ one tailed; Fig. 1E) and gonadal fat pad masses were significantly lower ($F_{1,16} = 24.36$, $p < 0.0001$; Fig. 1F), food intake was also reduced $F_{1,16} = 4.56$, $p < 0.05$; Fig. 1G).

Table 1
Short day lengths reduce CB1 receptor expression in the anterior and lateral nuclei of the hypothalamus. Mean (±SEM) relative optical density measurements of sections through the hypothalamus of long and short day male hamsters. ARC, arcuate nucleus; PVN, paraventricular hypothalamus; AH, anterior hypothalamus; LH, lateral hypothalamus.

	Long day	Short day
ARC	6.12 ± 0.86	4.93 ± 0.88
PVN	4.86 ± 0.78	4.19 ± 0.63
AH	5.98 ± 0.59	4.43 ± 0.48*
LH	6.38 ± 1.17	3.67 ± 0.33*

* $p < 0.05$.

Short day lengths reduced CB1 receptor staining in the anterior ($F_{1,15} = 4.59$, $p < 0.05$) and lateral hypothalamus ($F_{1,15} = 5.69$, $p < 0.05$), but did not alter CB1 expression in the arcuate ($F_{1,15} = 0.93$, $p > 0.05$) or paraventricular nuclei ($F_{1,15} = 0.46$, $p > 0.05$) of the hypothalamus (Table 1). Correlation analyses revealed a significant relationship between anterior hypothalamic CB1 expression and gonadal fat pad mass ($r = 0.56$, $p < 0.05$; Fig. 2) and between expression of the CB1 in the lateral hypothalamus and gonadal fat pad mass ($r = 0.61$, $p < 0.05$).

Short photoperiods induced a suite of physiological adjustments, including regression of the reproductive tract, reductions in food intake, and decreased fat depot masses. These photoperiodic adjustments were associated with reductions in CB1 receptor immunostaining in the lateral and anterior nuclei of the hypothalamus. There was a significant negative correlation between both anterior and lateral hypothalamic CB1 expression and gonadal fat pad masses. These data, in concert with a recent report linking endocannabinoid signaling to the photoperiodic-responsive pars tuberalis system in the Syrian hamster, suggest that the endocannabinoid system is both responsive to changing photoperiod and possibly acts as a downstream mediator of photoperiod-mediated adjustments in energy balance [41].

Feeding behavior is under the control of a remarkably complex neuronal and neuroendocrine system that is distributed across multiple brain regions and involves dozens of neurotransmitters, peptides and hormones [24,34]. Briefly, the hypothalamic energy balance network consists of a balance between the mutually antagonistic orexigenic peptides such as neuropeptide Y, orexin, and melanocortin concentrating hormone (MCH) and anorexigenic signals including corticotrophin releasing hormone (CRH), cocaine and amphetamine related transcript (CART) and melanocortins [34]. Although the system is complex and widely distributed, cannabinoid agonists have consistent and potent orexigenic effects, whereas CB1 antagonists decrease food intake [17,20]. CB1 receptors are located anatomically near cell groups that produce signals that regulate food intake; endocannabinoids acting on CB1 receptors modulate the activity of these cells [5]. Lateral hypothalamus cells contain the orexigenic peptides melanocortin concentrating hormone (MCH) and orexin, and also express the leptin receptor. Retrograde cannabinoid release from MCH neurons onto presynap-

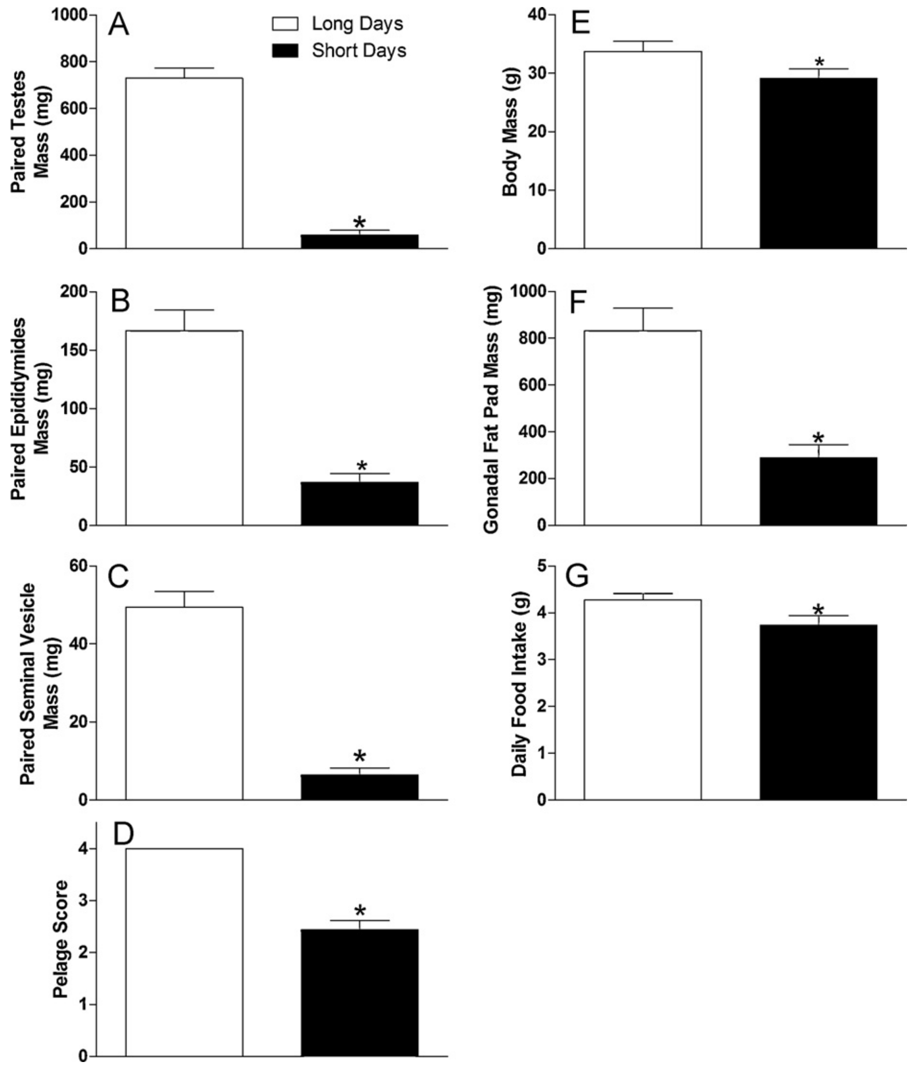

Fig. 1. Short day lengths induce reproductive regression, weight loss and reduction in food intake. All data are presented as mean (±SEM). (A) Paired testes mass, (B) paired epididymides mass, (C) paired seminal vesicle mass, (D) pelage score, (E) final body mass, (F) paired gonadal fat pad mass and (G) average daily food intake.

tic local GABA neurons inhibits GABA release and thus disinhibits the activity of MCH neurons [13,16].

Photoperiodic adjustments in energy balance-related genes and proteins have been reported in Siberian hamsters. Prolonged exposure to short day lengths reduces circulating leptin and also decreases the hypothalamic gene expression of the anorexigenic peptide proopiomelanocortin (POMC) and decreases the orexigenic agouti-related peptide, suggesting that neuropeptide systems exist that may serve to oppose the catabolic state that exists in short days [10,22]. Additionally, leptin receptor gene expression in the arcuate nucleus was also reduced [22]. This reduction in leptin signaling is important because the short day reduction in leptin and leptin receptor does not cause a compensatory rise in food intake, but rather seems to establish a new metabolic set point. Further studies are needed to investigate the interaction between leptin signaling and the endocannabinoid system.

Endocannabinoid signaling is by no means limited to the control of energy balance, as the system has been implicated in the regulation of reproductive neuroendocrine systems, immune responses, synaptic and structural plasticity, and the control of affective behaviors [14,25,31,32]. As photoperiod has been shown to modulate all of these systems, further examination of cannabinoid expression in photoperiodic mammals is warranted [27,29,30]. Indeed, the changes in CB1 receptor expression in the hypothalamus may be related to other functions in addition to energy balance. However, the localization of these receptors and the relationship between their expression and fat masses strongly suggests a connection.

Short photoperiod reduced CB1 expression in the lateral and anterior hypothalamus but did not alter CB1 receptor expression in either the arcuate or paraventricular nuclei of the hypothalamus despite the critical importance of these cell groups to energy balance. The lack of a change in CB1 receptor immunostaining does not at all preclude the possibility that endocannabinoid signaling overall is altered in these nuclei. However, the results reported here indicate that further examination of the orexin and MCH neurons in the lateral hypothalamus deserves additional study as a reduction in CB1 expression strongly and inversely mirrors the reduction in fat pad mass in these cells. These data suggest that the overall drop in circulating endocannabinoid signals in the anterior and lateral hypothalamus probably contribute to photoperiodic adjustments in energy balance. However, the inverse possibility cannot be ruled out; that is that changes in body mass, food intake and adiposity drive changes in CB1 expression rather than the other way around. Future studies will address the role of peripheral endocannabinoid signaling, as well photoperiodic adjustments in endocannabinoid ligand in the photoperiodic hypothalamus.

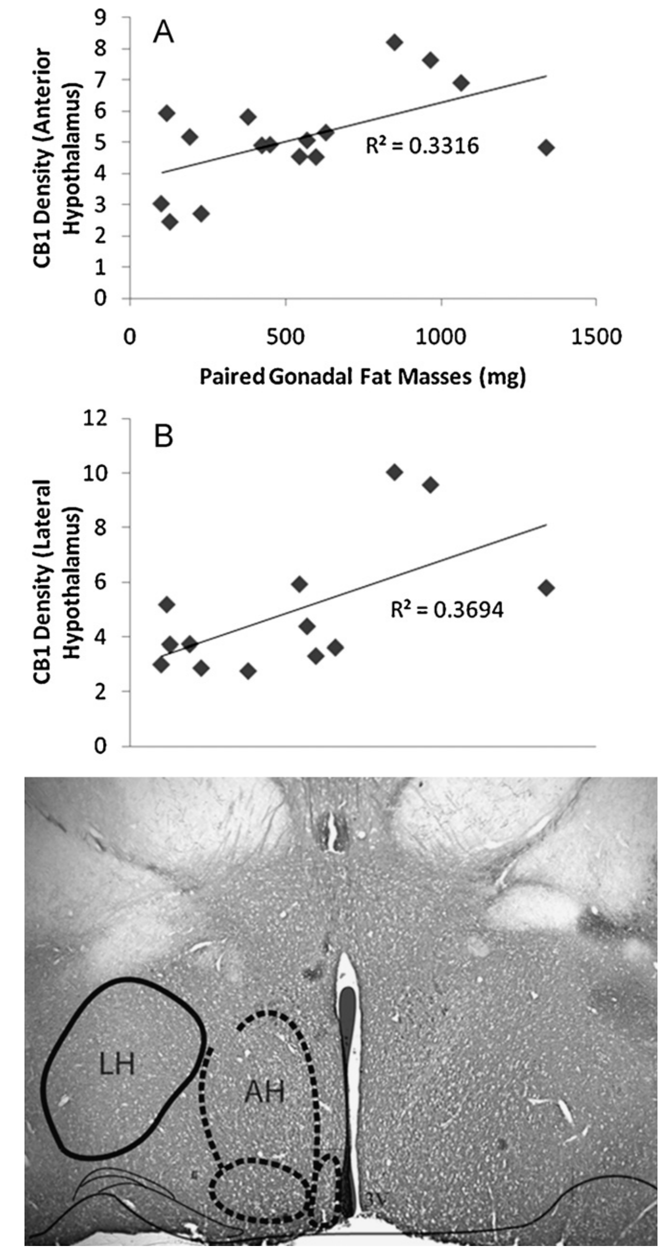

Fig. 2. Short photoperiod-induced reductions in CB1 expression correlate with reduced fat pad masses. Scatter plot of the relationship between CB1 receptor expression and fat pad mass in the (A) anterior and (B) lateral hypothalamus. Panel (C) indicates the anatomical location at which optical density measurements were taken [26].

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