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Reproductive Responses to Photoperiod Persist in Olfactory Bulbectomized Siberian Hamsters (*Phodopus sungorus*)

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Abstract

In reproductively photoperiodic Syrian hamsters, removal of the olfactory bulbs leads to a marked and sustained increase in gonadotrophin secretion which prevents normal testicular regression in short photoperiods. In contrast, among reproductively non-photoperiodic laboratory strains of rats and mice, bulbectomy unmasks reproductive responses to photoperiod. The role of the olfactory bulbs has been proposed to have opposite effects on responsiveness to photoperiod, depending on the photoperiodicity of the reproductive system; however, Syrian hamsters are the only reproductively photoperiodic rodent species for which the role of the olfactory bulb in reproductive endocrinology has been assessed. This experiment evaluated the role of the olfactory bulbs in the photoperiodic control of reproduction in Siberian hamsters (*Phodopus sungorus*), an established model species for the study of neural substrates mediating seasonality. Relative to control hamsters housed in long days (15 h light/day), exposure of adult male hamsters to short days (9 h light/day) for 8 weeks led to a temporal expansion of the pattern of nocturnal locomotor activity, testicular regression, decreases in testosterone (T) production, and undetectable levels of plasma follicle-stimulating hormone (FSH). Bilateral olfactory bulbectomy failed to affect any of these responses to short days. The patterns of entrainment to long and short days suggests that pre-pineal mechanisms involved in photoperiodic timekeeping are functioning normally in OBx hamsters. The absence of increases in FSH following bulbectomy in long days is incompatible with the hypothesis that the olfactory bulbs provide tonic inhibition of the HPG axis in this species. In marked contrast to Syrian hamsters, the olfactory bulbs of Siberian hamsters play essentially no role in the modulation of tonic gonadotrophin production or gonadotrophin responses to photoperiod.

Keywords

photoperiodism; biological rhythms; reproduction; brain lesion

Introduction

Changes in day length regulate reproductive physiology in many small rodents [32]. Seasonal changes in reproductive activity restrict breeding to the fraction of the year when food and ambient temperatures favor offspring survival [7].

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The neural pathways involved in the reproductive responses to photoperiod have been well-characterized. Light entrains a circadian pacemaker in the suprachiasmatic nucleus (SCN), which drives the onset and termination of nocturnal pineal melatonin secretion [38,40], the duration of which is necessary and sufficient for the generation of photoperiod-driven phenotypic transitions in the overwhelming majority of seasonal traits studied [8,16]. Longer-duration nightly melatonin signals act at the SCN and at thalamic targets to inhibit gonadotrophin secretion, reproductive physiology, and body mass, inducing the winter nonreproductive phenotype [2–4].

The olfactory bulbs have been prominently implicated in the indirect control of reproductive responses to photoperiod in rodents [28]. Similar to Siberian hamsters, Syrian hamsters exhibit reproductive regression in response to photoperiods shorter than 12.5 h of light per day [15]. Bilateral removal of the olfactory bulbs (OBx) abolishes [9,30], or substantially attenuates [5] short-day induced gonadal regression in Syrian hamsters. OBx triggers hypersecretion of follicle-stimulating hormone (FSH) in long days [9], which is accompanied by a decrease in sensitivity of FSH-secreting gonadotropes to gonadal steroid negative feedback [31]. Transfer of OBx hamsters with elevated FSH to inhibitory short photoperiods resolves this hypersecretion, but fails to drive FSH concentrations to lower, SD-typical levels, thus preventing gonadal regression [9,25].

Among rodents considered to be reproductively nonphotoperiodic (e.g., many laboratory strains of rats and mice), the olfactory bulbs exert a markedly different effect on reproductive responsiveness to photoperiod. Photoperiod manipulations that typically have no effect on reproductive physiology in rats (e.g., exposure to short days, blinding) yield a relatively modest inhibition of reproductive function, provided animals were subjected to OBx prior to puberty [33,34]. Indeed, photoperiodic timekeeping mechanisms analogous to those operant in Syrian hamsters are unmasked by OBx in rats [21,22] and mice [23].

The role of the olfactory bulbs in the regulation of reproductive physiology has been proposed to vary, in a species-specific manner, as a function of whether the species is reproductively photoperiodic [28]. In reproductively nonphotoperiodic species (rats, mice), the olfactory bulbs provide a tonic stimulatory influence on gonadotrophin secretion; OBx eliminates this stimulation, and unmasks weak reproductive responses to photoperiod and melatonin. Among Syrian hamsters, in contrast, the olfactory bulbs exert a tonic inhibitory effect on the HPG axis; their removal disinhibits gonadotrophin secretion, and abolishes gonadal responses to short days. To date, however, Syrian hamsters are the only reproductively photoperiodic rodent species for which the role of the olfactory bulb in reproductive endocrinology has been assessed. In this regard, Syrian hamsters may be representative of reproductively photoperiodic rodents in general, or they may be idiosyncratic. In light of the absence of comparative data on the effects of OBx in photoperiodic rodents, the goal of this study was to evaluate the role of the olfactory bulbs on photoperiodic control of the reproductive system in Siberian hamsters (*Phodopus sungorus*), a well-established model for the study of neurobiological mechanisms mediating seasonality. Following exposure to short, winter-like photoperiods (<13 h of light/day), Siberian hamsters exhibit decreases in body mass, food intake, gonad size, gonadotrophin secretion, and gonadal androgen production [16]. In the present study, male hamsters reared in a long-day photoperiod were subjected to OBx or sham-OBx procedures, and circadian, behavioral, neuroendocrine, and reproductive responses to short photoperiod treatments were assessed over the next 8 weeks.

Materials and Methods

Animals and housing conditions

Male Siberian hamsters (*Phodopus sungorus*) were obtained from a breeding colony maintained at the University of Chicago. Hamster pups were weaned at 18–21 days of age and housed 2–4 per cage with same-sex siblings in polypropylene cages (28 × 17 × 12 cm) with wood shaving beddings (Harlan Sani-Chips, Harlan Inc., Indianapolis, IN, USA) in a 15L:9D light-dark cycle (lights off at 1800 h CST) until 4–5 months of age. Ambient temperature of the room was $20 \pm 0.5^{\circ}\text{C}$ and relative humidity was maintained at $53 \pm 2\%$. Food (Teklad Rodent Diet 8604, Harlan Inc.) and filtered tap water were provided *ad libitum*. All procedures conformed to the “Principles of laboratory animal care” (NIH publication No. 86–23, revised 1985) and the USDA Guidelines for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Chicago.

Surgical procedures

Hamsters were subjected to surgical olfactory bulbectomy (OBx; $n = 25$) or a sham-OBx procedure ($n = 24$) under sodium pentobarbital anesthesia (Nembutal, 0.05 mg/g, i.p.; [30]). Hamsters were immobilized in a stereotaxic apparatus and a small (~2 mm) hole was drilled in the frontal bone near the caudal extent of the nasal bone. Bilateral bulbectomies (OBx) were performed by bilateral aspiration of the olfactory bulbs (using a modified 200 μl pipette tip) from the anterior border of the olfactory bulbs to the frontal poles, without disturbing the superior sagittal sinus. This procedure removes all neural pathways from the olfactory bulbs to the brain, including the main olfactory bulb, the accessory olfactory bulb, and the nervus terminalis [27]. The sham-OBx procedure entailed drilling of the skull and a comparable amount of blood loss, without insertion of the aspiration pipette. After surgery, hamsters received analgesia (Buprenex, 0.05 $\mu\text{g/g}$, s.c.) twice per day for 2 successive days.

A total of 24 sham and 17 OBx hamsters survived >2 weeks. Hamsters were then randomly assigned to long and short day photoperiods (sham: $n=12$ /photoperiod; OBx: $n=8$ LD, $n=9$ SD).

Photoperiod treatments

Two weeks after surgery (= week 0), hamsters were transferred either into short days (SD; 9h light/day, lights on at 0900 h CST) or remained in long-days (LD; 15h light/day). Hamsters were weighed (± 0.1 g) weekly, and estimated testis volumes (ETVs) were determined at two-week intervals. ETVs were obtained by measuring the length and width of the left testis through the abdominal skin with analog calipers while under light isoflurane anesthesia. In hamsters, ETV is positively correlated with testis weight, circulating testosterone and spermatogenesis [17,35]. On week 8 stage in the seasonal pelage color cycle was assessed in each hamster using an integer scale of 1 to 4 (1= dark “summer” fur, 4= white “winter” fur; [11]) by a single trained observer who was blind to the treatment conditions.

Locomotor activity measures

Home cage activity data were collected using passive infrared motion detectors (Coral Plus, Visonic, Bloomfield, CT) positioned 22 cm above the cage floor. Motion detectors registered activity whenever 3 of 27 zones were crossed. Activity triggered closure of an electronic relay, which was recorded by a PC running ClockLab software (Actimetrics, Evanston, IL).

The timing of activity was analyzed using ClockLab software according to methods described by Evans et al. [13]. Briefly, a 24 h histogram was produced for each hamster by averaging activity counts in 5-min bins over a 7–10 day window between weeks 6 and 8. For each

histogram, activity onset was defined as the first 5-min time bin after 1400 h with average counts exceeding the daily overall mean level; activity offset was defined as the last time point exceeding this threshold. The duration of daily activity, α , was calculated as the interval between activity onset and activity offset [13].

Blood collection and determination of hormone concentrations

On week 8, blood samples (500 μ l) were collected 5 h before lights-off under light anesthesia from the right retro-orbital sinus using heparinized Natelson collection tubes. Blood collections were performed in a room separate from the general animal colonies, and following the procedure, hamsters were separated from the colony until all blood collections for the day were completed. Animal handling during the blood collection was kept to a minimum (<1 min). Following collection, blood samples remained on ice <1 h and were centrifuged at 300 g for 30 min at 4 °C. Plasma was stored at -80 °C until assayed for hormone concentrations.

Testosterone was measured in a single ELISAs (Correlate-EIA; Assay Designs, Ann Arbor, MI, USA) according to the manufacturer's instructions. The testosterone ELISA had a sensitivity of 5.7 pg/ml, an intra-assay coefficient of variation (CV) of 10.8% and an inter-assay CV of 9.3%. Serum FSH concentrations were determined in a single RIA (IRMA coated tube assay; MP Biomedicals) at the Northwestern University Radioimmunoassay Core. The use of the heterologous (rat) reagents to measure FSH in Siberian hamster has been validated previously by this lab [19,39]; the regression lines for the standard curve and serial dilutions of pooled hamster serum are parallel. The lower limit of detection was 2.65 ng/ml and the intra-assay coefficient of variation was 2.96%.

Verification of OB Integrity

Examination of the olfactory cavity was performed at necropsy. OB remnants that were not aspirated during surgery were dissected and weighed (± 0.1 mg). Mean OB mass was calculated for all sham-OBx hamsters, and the completeness of the OBx procedure was calculated by dividing the amount of remaining OB tissue by the grand mean OB mass.

Statistical analyses

Effects of photoperiod and bulbectomy on all dependent variables were assessed using 2 (LD, SD) X 2 (OBx, Sham) factorial ANOVAs, with the exception of pelage scores which were compared using a Mann-Whitney U test. The incidences of responsiveness to SD were compared between OBx and intact hamsters using a χ^2 test. Half of the FSH samples fell below the lower limit of detectability of the FSH assay; these values were assigned the value of the lower limit (2.65 ng/ml) and were included in all statistical calculations [39]. χ^2 statistics were used to compare the proportion of hamsters with detectable vs. undetectable FSH concentrations.

All statistical calculations were conducted using Statview 5.0 (SAS Institute, Cary, NC). Where permitted by significant F statistics, pairwise comparisons were conducted using Fisher's PLSD tests. Differences were considered statistically significant if $P < 0.05$.

Results

Semiquantitative Assessment of Bulbectomies

Necropsies revealed 7 complete and 10 incomplete (4 LD, 6 SD) OB lesions. In the latter animals, the amount of tissue remaining ranged from 1.3 to 7.2 mg (MEAN $\pm$ SD: 4.1 ± 2.1 mg). For all OBx hamsters, this exceeded the 'completeness' threshold of 80% removal as described by Bittman et al. [5]. Among OBx hamsters, the completeness of the bulbectomy

procedure did not affect the any of the somatic or reproductive responses to photoperiod reported below ($F < 1.39$; $P > 0.1$, all comparisons).

Effects of Bulbectomy on Locomotor Activity Rhythms

Entrainment—Hamsters housed in LD exhibited earlier activity onsets relative to those housed in SD ($F_{1,36} = 8.6$, $P < 0.01$), but entrainment of activity onsets was not affected by surgical condition ($F < 0.1$, $P > 0.9$; Fig. 1). Activity offsets were likewise influenced by photoperiod ($F = 6.5$, $P < 0.05$), but were not affected by the OBx procedure ($F = 1.6$, $P > 0.2$); hamsters housed in LD terminated activity closer in time to lights off relative to hamsters housed in SD ($P < 0.05$, all comparisons).

Circadian Alpha—Alpha was approximately 4.3 h longer among SD (MEAN $\pm$ SD: 13.0 ± 1.6 h) relative to LD (8.7 ± 0.5 h) hamsters ($F = 111.1$, $P < 0.0001$), but was unaffected by surgical condition ($F_{1,36} = 0.4$, $P > 0.5$; Fig. 1).

Effects of Bulbectomy on Somatic, Reproductive, Neuroendocrine, and Behavioral Responses

Body mass—Both photoperiod ($F_{1,36} = 20.6$, $P < 0.0001$) and surgical condition ($F = 18.6$, $P < 0.0001$) affected body mass responses to SD, but the interaction between the two was not significant ($F < 0.1$, $P > 0.9$; Fig. 2A). Among intact hamsters, maintenance in LD for 8 weeks was associated with a slight gain in body mass, whereas exposure to SD caused a decrease in body mass. In contrast, OBx hamsters did not lose body mass in SD, and OBx hamsters in LD exhibited significant gains in body mass.

Testis size—Photoperiod treatments significantly affected testicular responses between weeks 0 and 8 ($F_{1,36} = 19.6$, $P < 0.0001$), but surgical condition was without effect on testis sizes ($F = 0.6$, $P > 0.4$; Fig. 2B). Among both intact and OBx hamsters, exposure to SD precipitated gonadal regression, whereas maintenance in LD was compatible with maintenance of fully-developed testis sizes. Decreases in testis sizes in SD hamsters were first evident on week 4 ($P < 0.05$, both comparisons); at no point between weeks 0 through 8 did testis sizes differ between intact and OBx hamsters within a given photoperiod condition ($P > 0.1$, all comparisons).

Incidence of reproductive nonresponsiveness—The proportion of individual animals that were reproductively nonresponsive to SD was unaffected by olfactory bulbectomy. One of 12 olfactory bulb-intact hamsters, and one of 9 OBx hamsters failed to exhibit gonadal responses to SD ($\chi^2 = 0.05$, $P > 0.8$).

Pelage moult—By week 8, initiation of winter fur moult (fur score > 2) was evident in 25% (3 of 12) of SD-intact hamsters and in 11% (1 of 9) of SD-OBx hamsters ($\chi^2 = 0.64$, $P > 0.4$); winter fur moults were not observed in any LD-housed hamsters (data not shown).

Plasma follicle-stimulating hormone—Two hamsters provided plasma samples that were of inadequate volume for FSH assay. FSH concentrations were below the lower limit of detectability in 10 of 11 SD-sham hamsters, whereas FSH was detectable in the plasma of 8 of 12 LD-sham samples ($\chi^2 = 7.99$, $P < 0.005$; Fig. 3A). Among OBx hamsters, FSH concentrations were undetectable in 6 of 9 SD hamsters, but were detectable in all LD hamsters ($\chi^2 = 7.47$, $P < 0.01$).

Plasma testosterone—Photoperiod ($F_{1,36} = 17.6$, $P < 0.0005$) but not surgical condition ($F = 4.0$, $P > 0.05$) affected plasma testosterone concentrations (Fig. 3B). Among both intact and

OBx hamsters, testosterone concentrations were significantly lower in SD relative to LD photoperiods ($P < 0.01$, both comparisons).

Food intake—Photoperiod ($F_{1,37}=13.9$, $P < 0.001$) but not surgical condition ($F_{1,37}=1.6$, $P > 0.2$) affected food intake as measured between week 5 and week 7 (Fig. 4A). This effect was largely driven by an increase in food intake among SD-OBx hamsters. Mass-specific food intake during this interval was likewise increased by exposure to SD ($F=34.3$, $P < 0.0001$) but the effect of bulbectomy was not significant ($F=2.33$, $P > 0.1$); per gram body mass, both intact and OBx hamsters in SD consumed more food relative to all other groups (Fig. 4B).

Discussion

Following exposure to short days, neurologically-intact male Siberian hamsters exhibited decreases in plasma FSH and T concentrations, and regression of the testes, relative to intact males housed in long days. OBx hamsters exposed to short days likewise exhibited low gonadotrophin concentrations, decreases in gonadal androgen secretion, and testicular regression. The data indicate that the olfactory bulb is not a necessary component of the mechanism by which seasonal changes in photoperiod affect multiple aspects of reproductive function in this species. The role of the olfactory bulb in the tonic regulation of the HPG axis of Siberian hamsters stands in marked contrast to that of Syrian hamsters. In the latter species, the olfactory bulb provides tonic inhibition of the hypothalamic gonadotrophin neurosecretory system [28,30], whereas in the former species, this structure appears to provide little functional input to the HPG axis. Thus, across the two most commonly used mammalian models of reproductive photoperiodism, the olfactory bulb plays categorically different roles in the genesis of reproductive photoperiodism and the regulation of the reproductive axis.

The absence of a substantial effect of OBx on all measures of entrainment to short days is consistent with the absence of an effect of OBx on reproductive responses to short photoperiod. Expansion of circadian alpha and the consequent increase in the duration of nocturnal melatonin secretion [10,40] are necessary for reproductive inhibition in short days [14]. In light of the strong temporal relation between the circadian pattern of locomotor activity and melatonin secretion [12] and the absence of evidence that OBx markedly affects entrainment to short days [5] or pineal melatonin production [29], we propose that pre-pineal events necessary for reproductive responses to short days are not affected by OBx in Siberian hamsters.

FSH concentrations were low or undetectable in the plasma of most hamsters (Fig. 3). Photoperiod did not affect mean values of FSH in any treatment groups; however, FSH was undetectable in the majority of samples from SD hamsters, whereas it was quantifiable in a significantly higher proportion of LD hamsters. Photoperiod effects on FSH are typically robust in juvenile Siberian hamsters undergoing LD-induced puberty [37,39], whereas in adults that have already completed reproductive development, photoperiod differences in FSH tend to be smaller or absent [1,24]. The main inference we take from the present data is that OBx failed to trigger increases in FSH production in either photoperiod.

In Syrian hamsters, OBx induces hypersecretion of FSH in long days [9], and transient increases in luteinizing hormone, prolactin, and T production [9,25]. Transfer of OBx hamsters to inhibitory photoperiods resolves FSH hypersecretion, but fails to drive FSH concentrations to normal SD-like levels, thereby preventing gonadal regression [9,25]. None of these effects of OBx on the HPG axis was observed in the present study. FSH concentrations were relatively low in both photoperiods, consistent with previous reports in post-pubertal Siberian hamsters [14,36,39], but there was no effect of OBx on the proportion of individuals with undetectably low FSH concentrations (Fig. 3). The mechanism(s) by which the olfactory bulbs

normally provide tonic inhibition of gonadotrophin secretion in Syrian hamsters have not been fully identified. OBx increases the threshold necessary for exogenous testosterone to inhibit FSH secretion, suggesting that the olfactory bulbs may regulate gonadotrophin secretion indirectly, via changes in feedback sensitivity to gonadal steroids [31]. The absence of OBx-induced increases in FSH in the present study suggests that an analogous influence of the olfactory bulbs on feedback sensitivity to steroids is unlikely to be operant in Siberian hamsters.

There is a graded effect of OBx on inhibition of gonadal responses to short days in Syrian hamsters: partial bulbectomy permits complete gonadal regression, whereas complete OBx lesions markedly attenuate gonadal responses [5]. In the present study, several hamsters in both photoperiods failed to receive complete lesions of the olfactory bulb (range= 80%–99% bulb removal); however, hamsters sustaining complete (100%) lesions of the olfactory bulb exhibited somatic, gonadal, and hormonal responses to long and short days that did not differ from those of hamsters sustaining incomplete lesions (Fig. 2). These data suggest that the lack of an effect of OBx on reproductive responses in the present study was not driven by the subset of hamsters that sustained incomplete lesions.

In the present study, short days failed to induce the typical decrease in food intake even in sham-OBx hamsters (Fig. 4). The reasons for this outcome are not clear, as photoperiod was clearly altering circadian entrainment, and inhibiting body mass and reproductive physiology in sham-OBx hamsters. Others have reported a similar absence of food intake responses to short photoperiod following sham neurological lesions [4,6]. OBx induced substantial increases in body mass among LD hamsters, and prevented the SD-induced decrease in body mass in SD hamsters (Fig. 2). Food intake was also greater following OBx, but only in SD hamsters (Fig. 4). This results are consistent with somatic and ingestive responses to OBx seen in other photoperiodic and seasonally-breeding rodents. Syrian hamsters exhibit pronounced increases in food intake and body mass following OBx [26], and European hamsters (*Cricetus cricetus*) increase their food intake and exhibit weight gains following OBx, even when performed during the weight-loss phase of the circannual cycle [20]. Together with the present report, convergent data from multiple species are consistent with the hypothesis that the olfactory bulb exerts a tonic inhibitory effect on food intake and body mass throughout the year.

The present results indicate that brain lesions which abolish reproductive responses to short photoperiods in Syrian hamsters fail to achieve the same effect in reproductively photoperiodic Siberian hamsters. Although these species share a substantial degree of similarity in the neural substrates that participate in the generation of seasonally-appropriate melatonin signals [32], there is precedent for species differences in the post-pineal melatonin targets necessary for reproductive responses to melatonin; e.g., destruction of the SCN abolishes gonadal responses to melatonin in Siberian hamsters [3], but is largely without effect in Syrian hamsters [18]. The present report indicates that the olfactory bulb of Siberian hamsters plays essentially no role in the modulation of tonic gonadotrophin production or gonadotrophin responses to photoperiod, in marked contrast to Syrian hamsters.

Taken together, the data are consistent with the conclusion that bulbectomy is without effect on mechanisms that participate in photoperiodic timekeeping at both pre-pineal (entrainment to photoperiod) and post-pineal (regulation of gonadotrophin responses) levels in Siberian hamsters. The present results extend the comparative investigation of the role of the olfactory bulb in reproductive photoperiodism by increasing the number of species to two, each with markedly dissimilar outcomes. Additional comparative data may permit insights into the ecological or evolutionary significance of tonic olfactory bulb inhibition/excitation of gonadotrophin secretion in mammals.

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Figure 1.

Mean $\pm$ SEM activity onsets and offsets of OBx and sham-operated male Siberian hamsters maintained in LD or SD for 8 weeks. Shading indicates the time of the 9 h and 15 h scotophases in LD and SD, respectively. Surgical and photoperiod treatments as described in Figure 1. # $P < 0.05$, LD vs. SD.

Figure 2.

Mean $\pm$ SEM (A) body masses and (B) estimated testis volumes (ETV) of male Siberian hamsters maintained in long days (LD; 15L:9D) or short days (SD; 9L:15D) for 8 weeks. Two weeks before photoperiod manipulations began, hamsters were subjected to aspiration removal of the olfactory bulbs (OBx) or a sham surgical procedure. Bar plots to the right depict data from OBx hamsters that sustained removal of the major extent of the bulbs (80–99% of tissue absent at necropsy; grey bars) and complete removal of the bulbs (100% of tissue absent at necropsy; black bars). * $P < 0.05$ vs. all other groups, # $P < 0.05$ vs. LD, within surgical condition.

Figure 3. Mean $\pm$ SEM plasma concentrations of (A) follicle-stimulating hormone (FSH) and (B) testosterone in OBx and sham-operated male Siberian hamsters maintained in LD or SD for 8 weeks. Surgical and photoperiod treatments as described in Figure 1. Horizontal line in Panel A indicates the lower limit of detectability of the FSH radioimmunoassay (2.65 ng/ml); the proportion of samples with detectable concentrations of FSH is indicated along the abscissa. # $P < 0.05$ vs. LD, within surgical condition.

Figure 4.

Mean $\pm$ SEM 24 h food intake of OBx and sham-operated male Siberian hamsters maintained in LD or SD for 8 weeks. Surgical and photoperiod treatments as described in Figure 1. * $P < 0.05$ vs. all other groups. ## $P < 0.05$ vs. LD-sham and LD-OBx values.