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Research report

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 (OBx) leads to a marked and sustained increase in gonadotrophin secretion which prevents normal testicular regression in short photoperiods. In contrast, among reproductively nonphotoperiodic 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.

1. 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].

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/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 responsive-

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ness 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 (*P. 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.

2. Materials and methods

2.1. Animals and housing conditions

Male Siberian hamsters (*P. 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 cm × 17 cm × 12 cm) with wood shaving beddings (Harlan Sani-Chips, Harlan Inc., Indianapolis, IN, USA) in a 15L:9D light–dark cycle (lights off at 18:00 h CST) until 4–5 months of age. Ambient temperature of the room was 20 ± 0.5 °C and relative humidity was maintained at 53 ± 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.

2.2. 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 nervous 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 an analgesic (Buprenex, 0.05 μg/g, s.c.) twice per day for two 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).

2.3. Photoperiod treatments

Two weeks after surgery (=week 0), hamsters were transferred either into short days (SD; 9 h light/day, lights on at 0900 h CST) or remained in long-days (LD; 15 h 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 1–4 (1 = dark "summer" fur, 4 = white "winter" fur; [11]) by a single trained observer who was blind to the treatment conditions.

2.4. 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 14:00 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].

2.5. Blood collection and determination of hormone concentrations

On week 8, blood samples (500 μl) 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 ELISA (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%.

2.6. 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.

2.7. Statistical analyses

Effects of photoperiod and bulbectomy on all dependent variables were assessed using 2 (LD, SD) × 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 $P < 0.05$.

3. Results

3.1. Semiquantitative assessment of bulbectomies

Necropsies revealed 7 complete and 10 incomplete (four LD, six SD) OB lesions. In the latter animals, the amount of tissue remain-