

# Photoperiodic Control of Oestrous Cycles in Syrian Hamsters: Mediation by the Mediobasal Hypothalamus

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## Abstract

To assess whether the mediobasal hypothalamus (MBH) is necessary for photoperiodic control of oestrous cycles and prolactin secretion, we tested intact female Syrian hamsters (controls) and those that had sustained unilateral or bilateral lesions of the MBH. All hamsters displayed 4-day oestrous cycles postoperatively in the long-day photoperiod (14 h light/day); control females and those with unilateral MBH damage ceased to undergo oestrous cycles approximately 8 weeks after transfer to a short-day photoperiod (10 h light/day), whereas 12 of 15 females with bilateral MBH lesions continued to generate 4-day oestrous cycles throughout 22 weeks in short days. Serum prolactin concentrations were either undetectable or low in all hamsters 8 or 14 weeks after the transfer to short-day lengths, but increased above long-day baseline values by week 22. We conclude that melatonin-binding sites in the MBH mediate suppression of oestrous cycles but not prolactin secretion by short-day lengths; recovery of prolactin secretion in females during prolonged exposure to short-day lengths reflects development of refractoriness to melatonin in a substrate distinct from the MBH. These findings suggest that separate neural pathways mediate photoperiodic control of gonadotropin and prolactin secretion in female hamsters.

Synchronization of seasonal reproductive rhythms by day length is well documented for male and female mammals (1). Day length information is transduced in the nervous system by melatonin acting on several localized target tissues (2). The duration of nocturnal melatonin secretion is highly correlated with the length of the night (3, 4), and determines whether animals adopt the winter or summer phenotype. In Syrian hamsters, elevated melatonin secretion for >8 h per night promotes gonadal regression, whereas melatonin durations <6 h sustain gonadal growth (4). Blood prolactin concentrations are relatively high in long-day lengths, but decline to low or undetectable values in short photoperiods (5). With prolonged exposure to short-day lengths, many species become refractory to long nightly melatonin signals and undergo spontaneous recovery of gonadotropic, lactotropic and steroid hormone secretion to generate blood concentrations equal to or greater than those in long days (6–9).

Maywood and colleagues (10, 11) eliminated high-density melatonin binding sites in mediobasal hypothalamic (MBH)

tissue of male hamsters; animals with bilateral lesions of this brain region (MBHx) failed to undergo testicular involution when challenged with short-day lengths or long-duration melatonin infusions, each of which induced testicular regression in neurologically intact controls. Both treatments, however, did depress prolactin concentrations in MBHx males. The authors concluded that 'an intact MBH is essential for melatonin to exert its photoperiodic control over gonadotropic but not lactotropic function in the Syrian hamster' (10). Similar conclusions emerged from studies of sheep (12, 13).

The melatonin target tissues implicated in photoperiodic control of reproduction of female rodents are much less well understood. In female Syrian hamsters, lateral projections from the paraventricular nucleus (PVN) of the hypothalamus are critical for the control of pineal-dependent ovarian function (14, 15). Knife cuts ventral to the PVN or ablation of this nucleus preserve normal oestrous cycles in short-day female hamsters (15, 16). Interruption of projections from the

PVN most likely interfere with photoperiodic control of female reproduction by eliminating or degrading pineal secretory activity (17) rather than by influencing target tissues that decode the duration of nightly melatonin signals.

The aim of the present study was to determine whether the MBH structures that mediate photoperiodic control of reproduction in male hamsters perform a similar role in females. Specifically, we tested the hypothesis that the suppression of oestrous cycles and prolactin secretion by short-day lengths would not occur in females lacking melatonin target tissues in the MBH.

## Methods

Young adult female Syrian hamsters (*Mesocricetus auratus*; Hsd:Han:AURA) were obtained from Harlan Sprague-Dawley (Madison, WI, USA) and maintained in our laboratory in a 14 : 10 light : dark cycle (14 h light/day; lights on at 03.30 h). Food and water were available *ad libitum* and room temperature was maintained at  $22 \pm 2$  °C. Animals were housed individually in polypropylene cages on pine shavings. After an acclimation interval of 24 days, oestrous cycles were monitored for each female by daily visual inspection of the vagina. The interval between successive appearances of stringy postestrous vaginal discharge (18) was used to determine oestrous cycle length. After hamsters had displayed several consecutive 4-day oestrous cycles, they were assigned to one of the surgical treatment conditions described below. After approximately 3 weeks of postoperative testing in long days, they were transferred to an 8 : 16 light : dark cycle (lights on at 09.30 h) for the remainder of the study.

### Blood sampling

1.0 ml of blood was withdrawn between 13.00 h and 15.00 h from the retro-orbital sinus of each hamster after 8, 14 and 22 weeks of short-day treatment, respectively, and assayed for serum concentrations of prolactin. In each case, the hamster was anaesthetized with isoflurane vapors and blood was left to clot overnight at 4 °C, centrifuged at 3500 r.p.m. for 20 min and serum samples stored at -80 °C for subsequent radioimmunoassay. Baseline long-day prolactin concentrations were determined for a subset of six females bled between 11.30 h and 12.00 h, 18 days prior to transfer to the short-day photoperiod. At all sampling intervals, animals were randomly sampled with regard to stage of the 4-day oestrous cycle; thus, the long-day control group should represent the range of mid-day prolactin values seen over a 4-day cycle in long-day lengths.

### Radioimmunoassays

Serum prolactin concentrations were determined in a homologous Syrian hamster assay (Dr A. F. Parlow, Pituitary Hormones and Antisera Center, Harbor-UCLA Medical Center, Torrance, CA, USA) that is in routine use (19–22). Hamster prolactin (PRL) (AFP-10302 E; 4 µg) was iodinated (Amersham Pharmacia IMS 30, Oakville, ON, Canada) to low specific activity (5%) using Chloramine T (BDH Inc., Toronto, ON, Canada) and separated (Sephadex G100, Sigma, St Louis, MO, USA). An average of 13 000 c.p.m. of iodinated haPRL in 100 µl was added to each tube and reacted against the primary antibody (rat-anti-haPRL, 100 µl of 1 : 16 000 working dilution; #AFP-7472988) and reference standard (40 pg/tube through 20 ng/tube; #AFP-10302-E; 100 µl) to yield a reaction volume of 300 µl, a total binding of 25% and nonspecific binding of 5%. Twenty-four hours later, second antibody (goat-anti-rat gamma globulin, 100 µl of 1 : 16 working dilution; titre P4 lot #9TA814; Antibodies Inc., Davis, CA, USA) and 30% polyethylene glycol (100 µl; 8000 MW, Fisher Scientific, Fair Lawn, NJ, USA) were used to centrifuge antibody-bound prolactin into a pellet. Supernatant was aspirated and discarded. Four assays from a single iodination were used for sample determinations. Unknown serum samples were assayed at 50 µl (diluted to 100 µl) against a triplicate standard curve. In 147 of 247 unknown samples, volume permitted determinations in duplicate. Six replicates from two pools of stored serum from male Siberian hamsters, *Phodopus sungorus*, were used as internal controls in each of the four assays. The pool at 36% binding averaged  $21.7 \pm 1.36$  ng/ml with an intra-assay variance of 21.4% and an inter-assay variance of 20.8%. The pool at 13% binding averaged  $92.0 \pm 7.2$  ng/ml with an intra-assay variance of 26.3% and an inter-assay

variance of 27.0%. All determinations falling below the lowest standard were rounded up to that limiting concentration of 0.80 ng/ml before analyses. The two determinations which fell above the highest standard were rounded down to a concentration of 400 ng/ml. Duplicate determinations were averaged to yield a sample prolactin concentration in ng/ml.

### Surgical procedures

#### Brain lesions

Surgery was performed under deep anaesthesia induced by a ketamine cocktail (21 mg ketamine, 2.4 mg xylazine and 0.3 mg acepromazine/ml injected ip in a dose of 0.34 ml per 100 g body mass) and supplemented as needed with isoflurane vapors. Lesions aimed at the MBH were made using a Radionics Model RFG-4 A Research RF Lesion Generator system (coordinates: 1.4 mm anterior to bregma, 0.4 mm lateral to midline and 7.4 mm below dura, with incisor bar set 5 mm above the interaural line) (Radionics, Burlington, MA, USA). Current was delivered with an electrode tip temperature of 80 °C for 15 s per lesion. In one group of animals, the lesions were placed bilaterally and in another unilaterally. During sham operations, the electrode was lowered to a depth of 0.3 mm above the MBH and no current was passed.

At the end of the experiment, hamsters were administered a lethal dose of pentobarbital sodium and perfused transcardially with 0.9% NaCl (950 ml) followed by 10% formalin in phosphate-buffered saline (50 ml). Brains were removed and transferred to 50 ml of a 15% sucrose/10% formalin/phosphate-buffered saline solution overnight, sliced on a freezing microtome at 40 µm, stained with cresyl violet and examined microscopically by two individuals who were unaware of the oestrous cycle or prolactin data. All procedures were approved by the Animal Care and Use Committee of the University of California at Berkeley.

### Statistical analysis

The proportion of animals that continued to undergo cycles in the 10 h light cycle was compared by Fisher's exact probability test or chi-square test where appropriate. Changes in serum prolactin concentrations within groups over time were analysed by paired *t*-tests (two-tailed) and serum prolactin concentrations between groups at the different time points were analysed by *t*-tests for independent samples. Oestrous cycle measures were analysed by one-way ANOVA using Statview 5 (SAS Institute Inc., Cary, NC, USA). Serum prolactin concentrations as a function of stage of the oestrous cycle were analysed via regression analysis.  $P < 0.05$  was considered statistically significant.

## Results

### Histological analysis of brain lesions

Representative bilateral and unilateral brain lesions are illustrated in Fig. 1. In addition to damage at the juncture of the dorsomedial and ventromedial hypothalamic nuclei, the lesions typically also encompassed varying amounts of the dorsomedial area of the hypothalamus just above the third ventricle anterior and dorsal to the dorsomedial nucleus of the hypothalamus (DMH), and the tuber cinereum.

### Oestrous cycles

#### Postoperatively in long days

Each of the hamsters retained for subsequent testing manifested at least three 4-day oestrous cycles postoperatively.

#### After transfer to short days

Unoperated ( $n = 6$ ) and sham-operated females ( $n = 8$ ) did not differ from each other on any measure and were combined to form a single control group for purposes of statistical analysis. Additional groups, formed post hoc on the basis of extent and location of brain damage, consisted of hamsters with bilateral MBH lesions ( $n = 15$ ) or unilateral damage to the MBH ( $n = 9$ ).

Oestrous cycle data are summarized in Table 1. Control females generated approximately fifteen 4-day cycles prior to the onset of acyclicity, characterized by the absence of postovulatory vaginal discharge in each animal. They remained reproductively quiescent for approximately 12 weeks before resuming oestrous cycles and then generated an average of eleven 4-day cycles before the experiment was terminated. A similar pattern was recorded in unilateral MBHx females (Table 1), the only differences being that onset of acyclicity and resumption of oestrous cycles occurred a few weeks earlier

in the unilateral MBHx than control females ( $P < 0.05$  in each case).

Twelve of 15 females with bilateral MBH damage continued to undergo oestrous cycles throughout 22 weeks of short-day treatment; in this respect they differed from control and unilateral MBHx females, all of which became reproductively quiescent (bilateral MBHx females versus control and unilateral MBHx females,  $P < 0.001$  in each case, Fisher's exact probability test). Latency to onset of acyclicity in the three MBHx animals whose cycles ceased after transfer to short-day treatment did not differ from that of either the control or unilateral MBHx hamsters (Table 1) ( $P > 0.50$ ), but duration of reproductive quiescence was shorter in these MBHx females than for the latter two groups (Table 1) ( $P < 0.02$  in each case).

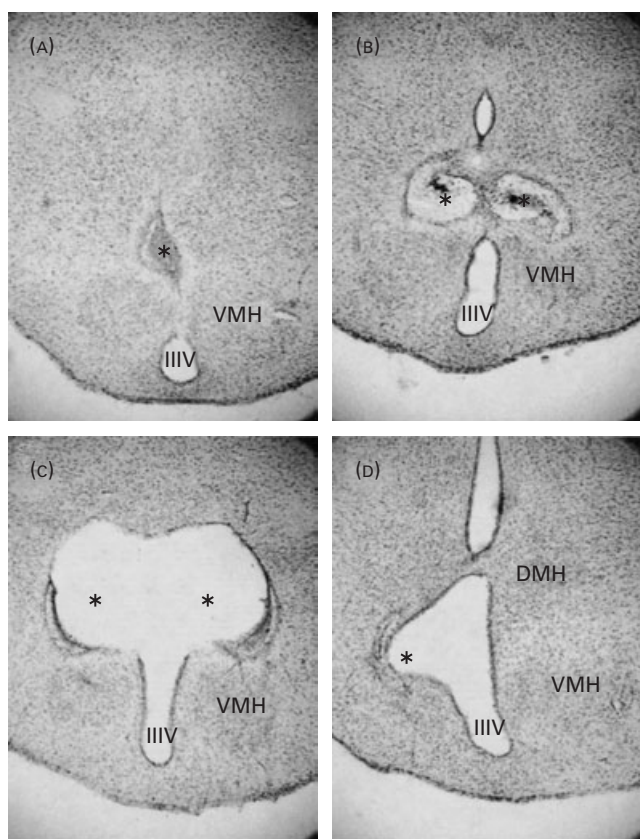


FIG. 1. Photomicrographs of coronal sections illustrating small (A), medium (B) and large (C) bilateral and unilateral (D) lesions of the mediobasal hypothalamus. Asterisks indicate the approximate centre of the lesion. IIIIV, Third ventricle; VMH, ventromedial nucleus; DMH, dorsomedial nucleus.

### Prolactin

Prolactin was detectable in each hamster sampled during maintenance in the LD photoperiod ( $13.8 \pm 3.7$  ng/ml; range of 4.9–27 ng/ml;  $n = 5$ ) but was undetectable in each of these females after 14 weeks in the SD photocycle ( $< 0.8$  ng/ml;  $P < 0.03$ ). By week 22, prolactin concentrations were elevated above baseline LD values ( $P < 0.03$ ; Fig. 2). Data from a sixth female were excluded from this analysis because it was still undergoing oestrous cycles at the time of week 14 blood sampling.

Control animals, and those that sustained unilateral MBH lesions, had low or undetectable prolactin concentrations after 8 and 14 weeks of short-day treatment (Fig. 3). In the bilateral MBHx group, prolactin concentrations decreased significantly between weeks 8 and 14 of short-day treatment (paired  $t$ -test;  $P < 0.04$ ); prolactin concentrations were significantly higher in this than the other groups at week 8 ( $t$ -test;  $P < 0.005$  for each comparison) but not at weeks 14 ( $t$ -test;  $P > 0.06$  versus control;  $P > 0.1$  versus unilateral MBHx) or 22 ( $t$ -test;  $P > 0.4$  for each comparison; Fig. 3). Prolactin concentrations were markedly elevated on week 22 for all three groups compared to week 8 or 14 values ( $P < 0.001$ , paired  $t$ -test).

Regression of prolactin concentration against stage of the oestrous cycle yielded a significant correlation only at week 14 ( $P < 0.02$ ; data not shown); this analysis was restricted to females that continued to undergo oestrous cycles in the 8 h light cycle. Regression analyses of prolactin and stage of cycle at weeks 0, 8 and 22 failed to yield values that approached significance ( $P > 0.4$  in each case; data not shown).

TABLE 1. Oestrous Cycle Characteristics for Control Animals and Those with Mediobasal Hypothalamus (MBH) Lesions.

Group	No. of cycles to acyclicity	Days acyclic	Day of last 4-day cycle (day 1 = January 1)	No. of cycles post recrudescence	Proportion of animals continuing to cycle in short day lengths
Control (n = 14)	14.6 ( $\pm 1.5$ ) <sup>a*</sup>	83.7 ( $\pm 5.5$ ) <sup>a</sup>	226.1 ( $\pm 5.9$ ) <sup>a</sup>	11.1 ( $\pm 0.9$ ) <sup>a</sup>	0/14 <sup>a</sup>
Bilateral MBHx (n = 15)	13.7 ( $\pm 1.3$ ) <sup>a,b†</sup>	50.0 ( $\pm 8.2$ ) <sup>b†</sup>	220.7 ( $\pm 9.7$ ) <sup>a,b†</sup>	15.7 ( $\pm 1.3$ ) <sup>b†</sup>	12/15 <sup>b</sup>
Unilateral MBHx (n = 9)	10.6 ( $\pm 0.9$ ) <sup>b</sup>	85.6 ( $\pm 5.0$ ) <sup>a</sup>	205.9 ( $\pm 4.2$ ) <sup>b</sup>	12.7 ( $\pm 0.7$ ) <sup>a,b</sup>	0/9 <sup>a</sup>

\*Values with different superscript letters differ significantly ( $P \leq 0.05$ ). †Only the three bilateral MBHx animals that became acyclic in short day lengths were included in these analyses.

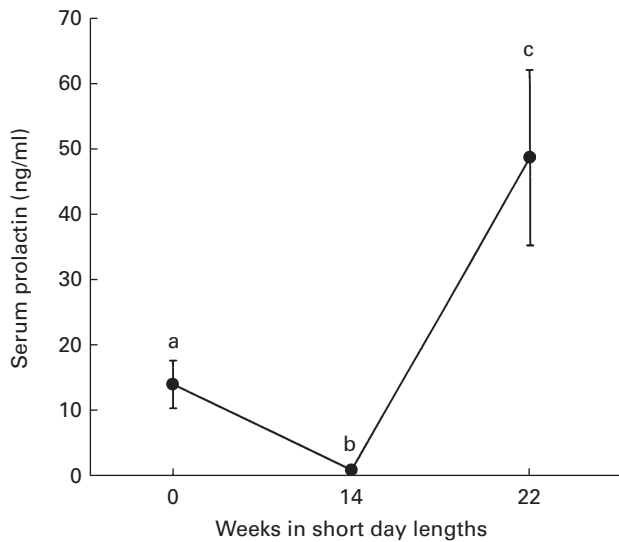


FIG. 2. Mean ( $\pm$ SEM) serum prolactin concentrations for five control females during testing in the long-day photoperiod (week 0) and after 14 and 22 weeks in short day lengths. Time points with different letters differ significantly ( $P < 0.05$ ).

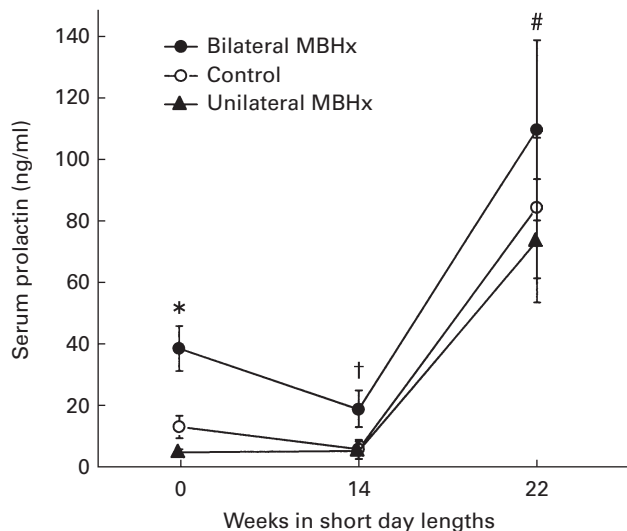


FIG. 3. Mean ( $\pm$ SEM) serum prolactin concentrations for control hamsters (sham- and unoperated) and all hamsters bearing bilateral or unilateral damage to the mediobasal hypothalamus (MBH). \*Bilateral MBHx group differed significantly from the other groups at week 8 ( $P < 0.005$ ). #Week 22 values were significantly elevated in all groups compared to week 8 and 14 values ( $P < 0.001$ ). †Week 14 value was significantly lower than the week 8-value in the bilateral MBHx group ( $P < 0.04$ ).

## Discussion

Syrian hamsters with bilateral lesions centred at the junction of the ventromedial and dorsomedial nuclei of the hypothalamus (MBHx hamsters), unlike neurologically intact controls, continued to generate 4-day oestrous cycles during maintenance in short day lengths. Plasma prolactin concentrations were, however, suppressed in both MBHx and control

females housed in the short photoperiod. These results suggest that photoperiodic control of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secretion on the one hand and prolactin on the other, are mediated by separate neuroendocrine substrates. This confirms and extends findings on male Syrian hamsters (10, 11) and also supports the conclusion of Lincoln and Richardson (12), based on studies of sheep, that the melatonin signal that encodes day length acts in the hypothalamus to regulate the gonadotropin-gonadal axis and to influence prolactin secretion within the pars tuberalis of the pituitary gland. In rodents, the hypothalamus also has been implicated in photoperiodic control of prolactin secretion; microdialysis of melatonin in a short-day pattern to the suprachiasmatic nucleus (SCN) suppressed prolactin concentrations of juvenile Siberian hamsters (2). In Siberian hamsters, SCN is both a termination point of the retinohypothalamic tract that communicates day length information to the endocrine system (23, 24) and contains high concentrations of melatonin-binding sites (25), but see also (26).

The persistence of oestrous cycles in short-day hamsters with MBH lesions may be due to the elimination of melatonin binding sites in the DMH. In male Syrian hamsters, melatonin binding sites are prominently distributed throughout the rostral extent of this nucleus and absent in the adjacent ventromedial nucleus of the hypothalamus (VMH) and from the median eminence (11). Because the distribution of melatonin binding sites in adult female Syrian hamsters is unknown and sex differences in photoperiodic control of hypothalamic function exist in this species (27), the possibility remains that the effects reported in this study are unrelated to elimination of melatonin receptors in the MBH. This view is suggested by the finding that very small lesions that spared substantial portions of the MBH nevertheless preserved oestrous cycles in short day lengths.

It is unlikely that the persistence of oestrous cycles in MBHx females is due to disruption of melatonin secretory activity. The dorsomedial hypothalamus does not participate in neural control of the pineal gland; ablation of this structure neither affects the circadian rhythm of melatonin production nor the inhibition of pineal melatonin metabolism by light (28, 29). Furthermore, male MBHx hamsters infused with short-day melatonin signals fail to undergo gonadal involution, thereby supporting the notion that the absence of an adequate short-day melatonin signal is not responsible for the continuation of reproduction in MBHx hamsters (10). The decline in prolactin concentrations in short day lengths in female MBHx hamsters also suggests that these animals are generating normal short-day melatonin signals. Higher prolactin concentrations in MBHx compared to control females may in part reflect persistence of oestrous cycles and consequently higher oestradiol concentrations in the former animals; oestradiol stimulates prolactin secretion in both long- and short-day female Syrian hamsters (5).

MBHx lesions may possibly sustain oestrous cycles in short day lengths by impairing mechanisms that increase feedback sensitivity of gonadotropins to steroid hormones (11). In male Syrian hamsters, some neural interventions prevent involution of the reproductive apparatus in short day lengths by inducing chronic increases in blood FSH

concentrations (30), which override the effects of melatonin on gonadotropin secretion. Such a mechanism is unlikely to account for continuation of reproduction in female MBHx hamsters; oestrous cycles are contingent on precise timing mechanisms and an LH surge every fourth day, and are not compatible with chronically elevated gonadotropin concentrations.

The mediobasal hypothalamus is extensively connected to other medial hypothalamic areas with high densities of melatonin-binding sites (e.g. SCN) (10). The possibility that day length controls gonadotropin secretion via projections from the DMH to the SCN is not supported by the observation that male SCNx Syrian hamsters undergo gonadal involution during treatments with melatonin (17, 31), whereas their MBHx counterparts do not (10, 11). In females, however, the issue remains unresolved and difficult to test because ablation of the SCN induces persistent vaginal and behavioural oestrus (32).

The respective contributions of MBH cell bodies versus fibres of passage to photoperiodic control of oestrus also remains unknown. The importance of cells bodies is suggested by the observation that microimplants of melatonin in the MBH of ewes lead to increases in LH secretion, whereas implants in other brain regions are ineffective (13).

Serum prolactin concentrations of control females were approximately 14 ng/ml in long-day lengths, declined to undetectable values (<0.8 ng/ml) after 14 weeks in short days, only to rebound to approximately 50 ng/ml after 8 weeks. The recovery of prolactin secretory activity in female hamsters with prolonged exposure to short days is correlated with gonadal recrudescence (33) and attributed to a loss of responsiveness of melatonin target tissues to short day melatonin signals (refractoriness). Because our experimental design did not include a second control group maintained in long days for 22 weeks, we cannot determine whether the elevated prolactin concentrations at week 22 relative to week 0 long-day values, represent a postrecrudescence hyperprolactinemia or an unrelated effect of ageing or experimental procedures. There is precedent for hypersecretion of gonadal steroids in photorefractory male Syrian hamsters (7). It is unlikely that the increase in prolactin secretion at week 22 is a consequence of sampling at a particular stage of the oestrous cycle; prolactin concentration and oestrous cycle stage were not correlated at this time point. Nor is it likely that the increase in prolactin secretion at week 22 is related to resumption of oestrous cycles because prolactin secretion also increased at this time point in MBHx females that had continued to undergo cycles throughout the course of short-day exposure.

In an earlier study of male Syrian hamsters (8), we reported that unilateral damage to the VMH, when combined with contralateral destruction of other hypothalamic tissue, was as effective as bilateral VMH damage in eliminating photoperiodic control of testicular function. In the present experiment, photoperiodic control of oestrous cycles did not differ between females with unilateral MBH damage and control females. Unilateral neuronal integrity of the MBH is sufficient to mediate effects of short day lengths on reproduction.

Three of 15 females that sustained bilateral damage to the MBH remained responsive to short days postoperatively, but

their oestrous cycles were less severely disrupted than those of intact females. The location and extent of tissue damage in these MBHx females did not differ in any obvious manner from that sustained by hamsters which continued to display oestrous cycles in short days. Variable outcomes after seemingly comparable neural insults are commonplace (34), and not presently understood.

In summary, the MBH is an essential component of the neural system by which day length controls gonadotropin secretion necessary for generation of oestrous cycles, but is not implicated in photoperiodic control of prolactin secretion.

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