

# Depressed Prolactin Cell Activity in Long-Term Blind-Pinealectomized Female Hamsters Is Due to Loss of Estrous Cyclicity (43128)

JOHN S. MASSA AND DAVID E. BLASK

Department of Physiology and Biophysics, University of Iowa, Iowa City, Iowa 52242, and Arizona Health Sciences Center, University of Arizona, Tucson, Arizona 85726

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**Abstract.** Pinealectomy in the female golden Syrian hamster is not always completely effective in preventing the suppressive effects of long-term light deprivation due to blinding on pituitary prolactin (PRL) cell activity. We examined this curious phenomenon by measuring pituitary PRL mRNA levels, PRL synthesis, and radioimmunoassayable PRL, and correlating these changes with the status of estrous cyclicity. As expected, 12 weeks of light deprivation resulted in loss of estrous cyclicity and a >90% decline in all indices of pituitary PRL cell activity, compared with intact cycling controls. Pinealectomy prevented only 40–50% of this decline. However, if *noncycling* light-deprived pinealectomized animals were excluded, pinealectomy was completely effective, i.e., cycling intact control animals were no different than cycling blind-pinealectomized. We conclude that the inability of pinealectomy to completely prevent the decline in prolactin cell activity seen after blinding is due to the loss of estrous cyclicity in some blind-pinealectomized females, with the attendant loss of the prolactin-stimulating hormone estrogen.

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In both male and female golden Syrian hamsters, reproductive collapse and a decline in pituitary prolactin (PRL) cell activity takes place after several weeks of light restriction (1). Light restriction (defined as exposure to fewer than 12.5 hr of light per day) is effective in producing this decline in PRL cell activity regardless of whether it results from the naturally decreasing daylight of winter (2), a short photoperiod produced by artificial light (3), or optic nerve transection as a result of biorbital enucleation (i.e., blinding) (4, 5).

If the pineal gland is also removed at the time of the surgical blinding procedure, these declines in PRL cell activity are usually reported to be completely prevented in the male hamster, but often only partially prevented in the female hamster (1). This has been termed a "sexually dimorphic" response to blinding and pinealectomy (1).

The purpose of the study reported here is to examine this curious sexually dimorphic response of pituitary PRL cell activity in the female golden Syrian hamster. The specific question to be answered is: Why is pinealectomy not completely effective in preventing the reduction in PRL cell activity seen after long-term blinding in the female hamster?

## Materials and Methods

**Experimental Design.** In the present study, we measured pituitary PRL mRNA levels, PRL synthesis, radioimmunoassayable (RIA) PRL, and uterine weights in female hamsters after 12 weeks of being left intact (INT), blinded and sham pinealectomized (BS), or blinded and pinealectomized (BP). We also kept daily records of estrous cyclicity for each animal throughout the 12 weeks. Based on cyclic status after 12 weeks of treatment (see Results), the BP group was further subdivided for statistical comparison into BP cycling (BP-cyc) and BP acyclic (BP-acy).

**Animals and Surgery.** Young adult female (approximately 13 weeks old at the start of the experiment) golden Syrian hamsters (*Mesocricetus auratus*) were purchased from Charles River, Lakeview Hamster Col-

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ony, Newfield, NJ, maintained at 23°C with 14:10 light:dark cycle (lights on from 0600 until 2000 hr), and given standard laboratory chow and tap water *ad libitum*.

Details of the surgical procedures for pinealectomy, sham-pinealectomy, and blinding have been reported (4). Briefly, pinealectomies were performed by first drilling out a 4-mm bone disk, centered approximately on the confluence of sinuses. Fine forceps were then thrust just through the confluence of sinuses and the underlying pineal was grasped and withdrawn. The bone disk was replaced and the skin incision held together with metal wound clips. All groups (including INT, which received no surgery) received the same dose (8 mg/100 g of body wt) of sodium pentobarbital (Nembutal) anesthesia. The experimental protocols used in these studies received prior IACUC approval and all surgical and postoperative procedures were conducted in accordance with NIH approved guidelines.

**Estrous Cycle Determination.** The animals were permitted to acclimate to their surroundings for three weeks prior to surgery. Each hamster was then followed through three complete estrous cycles, and then for the 12 weeks of the experiment, by daily examinations using both the vaginal exudate (6) and vaginal smear (7) methods. During the course of the experiment, over 800 smears were dried on glass slides, stained, and examined with a microscope for the characteristic histology seen on each day of the cycle. All animals used in the study were cycling normally at the start of the experiment.

**PRL Cell Activity Assays.** Complete details of our methods for removing the pituitaries, measuring pituitary PRL mRNA levels, PRL synthesis, and RIA PRL have been reported (4). Briefly, PRL mRNA was measured via the cytodot method (8) using the rat PRL complementary DNA (cDNA) pPRL-1 (a gift from Richard A. Maurer, University of Iowa, Iowa City, Iowa), which we have validated for measuring PRL mRNA in the hamster (4). PRL synthesis was determined by measuring the *in vitro* incorporation of [<sup>3</sup>H] leucine into PRL during a 3-hr incubation, via tube gel electrophoresis of total <sup>3</sup>H proteins from the pituitary, cutting out the band corresponding to a known hamster PRL standard, and counting the radioactivity in that band (i.e., <sup>3</sup>H-PRL) in a scintillation counter (4). RIA PRL was measured with a homologous hamster RIA PRL kit (a gift from Frank Talamantes, University of California, Santa Cruz, CA) which was used according to published procedures (9).

**Statistics.** The data were subjected to a one-way analysis of variance followed by a Student-Newman-Keuls multiple range test. A *P* value < 0.05 was considered significant. Statistics were computed based on three groups (INT, BS, and BP) for comparison with previous work and on four groups (INT, BS, BP-cyc,

and BP-acy) to reflect that BP is actually composed of two subgroups based on cyclic status (see Results). The number of animals in each group was as follows: INT (*n* = 6), BS (*n* = 4), BP (*n* = 7), BP-acy (*n* = 4), and BP-cyc (*n* = 3).

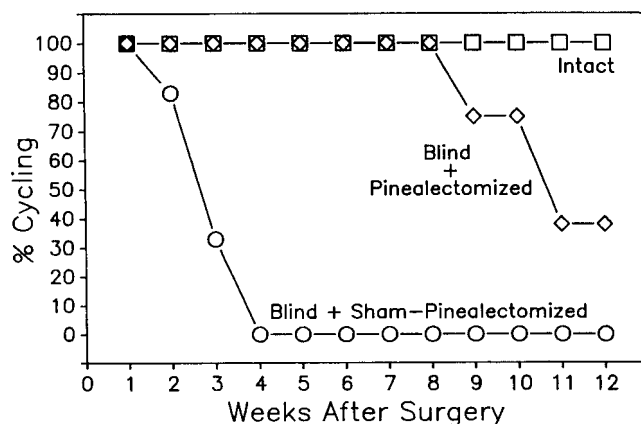
## Results

**Estrous Cyclicity.** All INT continued to cycle normally throughout the experiment, and all BS were acyclic by 4 weeks (Fig. 1). Until about 8 weeks after surgery, all BP continued to cycle; then some hamsters in the BP group stopped cycling so that, by 12 weeks, less than 50% of the animals in BP were cycling (Fig. 1). Thus, after 12 weeks, the BP group was composed of two subgroups: those that were acyclic (BP-acy) and those cycling (BP-cyc).

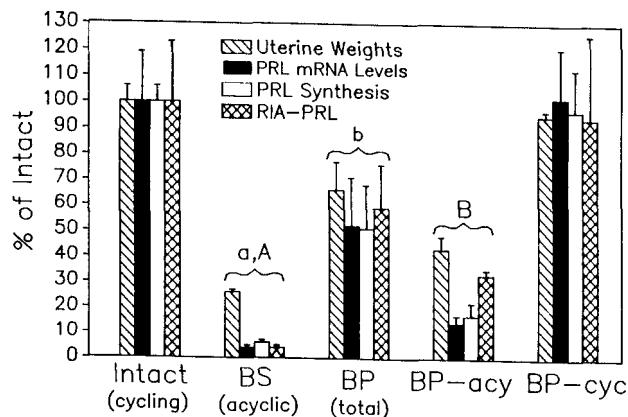
**Uterine Weights and Assays of PRL Cell Activity.** A common pattern was seen for changes in uterine weights, pituitary PRL mRNA levels, total pituitary PRL synthesis, and total pituitary RIA PRL (Fig. 2). In each case, BS was severely depressed (by 75–95%) compared with INT, whereas BP (total) was intermediate between INT and BS, restoring only 40–50% of INT levels. When BP-acy was grouped separately, however, BP-cyc was no different from INT, while BP-acy approached BS.

This pattern was the same after uterine weights were corrected for body weight, or if the indices of PRL cell activity (PRL mRNA levels, PRL synthesis, or RIA PRL) were expressed per milligram of pituitary (data not shown).

**Uterine Weights.** INT uterine weights ( $405 \pm 25$  mg) declined in the BS group after 12 weeks (Fig. 2) to levels seen in hamsters ovariectomized for the same period of time (Massa and Blask, unpublished observations). The differences in uterine weights (Fig. 2) correspond to the differences in cyclic status (Fig. 1).



**Figure 1.** Estrous cycle determinations in golden Syrian hamsters for 12 weeks following the indicated procedures. Note that, unlike the intact group (all cycling) or blind plus sham-pinealectomized group (all acyclic), the blind plus pinealectomized group is composed of both cycling and acyclic animals after about 8 weeks.



**Figure 2.** Evidence that pinealectomy plus blinding (BP total) does not prevent all of the effects of blinding alone (BS = blind plus sham-pinealectomy) because BP (total) is actually composed of two different groups based on cyclic status: BP-acy and BP-cyc. For the three-group comparisons based on cyclic status: BP-acy and BP-cyc. For the three-group comparisons: a,  $P < 0.05$  vs INT or BP; b,  $P < 0.05$  vs INT or BS. For the four-group (INT, BS, BP-acy, and BP-cyc) comparisons: A,  $P < 0.05$  vs INT, BP-acy, or BP-cyc; B,  $P < 0.05$  vs INT, BS, or BP-cyc.

**PRL mRNA Levels.** The relative PRL mRNA values (Fig. 2) were based on the counts per min (cpm) of the radiolabeled PRL cDNA probe,  $^{32}\text{P}$ -pPRL-1, which hybridized to an aliquot of pituitary cytotot sample, in cpm pituitary. For INT, this was  $183,733 \pm 34,133$  cpm pituitary.

**PRL Synthesis.** The relative "PRL synthesis" values (Fig. 2) were based on the disintegrations per min (dpm) of  $^3\text{H}$ leucine incorporated into PRL during a 3-hr incubation (in dpm pituitary). This is a measure of total PRL synthesis since it is the sum of  $^3\text{H}$ -PRL found after incubation both in the incubated pituitary and released into the incubation media. For INT, this was  $92,574 \pm 5,934$  dpm pituitary.

**RIA PRL.** The relative RIA PRL values (Fig. 2) were based on the micrograms of RIA PRL found after the 3-hr incubation (in  $\mu\text{g}$ /pituitary). Like PRL synthesis, it is a measure of total RIA PRL since it is the sum of RIA PRL found after the incubation both in the incubated pituitary and released into the incubation media. For INT, this was  $129.0 \pm 30.2$   $\mu\text{g}$ /pituitary.

**Body Weight and Pituitary Weight.** There were no differences in body weight as a result of treatment after 12 weeks (Table I). There were significant differences in pituitary weight due to treatment (Table I) but, as stated above, the pattern of changes in PRL cell activity was the same (and statistically significant), regardless of whether the data were expressed per pituitary or per milligram of pituitary.

## Discussion

The purpose of this experiment was to clarify the neuroendocrine basis of a previously reported (1) sexually dimorphic response of PRL cell activity to blinding combined with pinealectomy in the golden Syrian

hamster. We sought to answer the following question: Why, in the female hamster, is pinealectomy sometimes reported to be only partially effective in preventing the suppressive effects of blinding on PRL cell activity while, in the male, it is usually reported to be completely effective?

Our answer to this question is that, after about 8 weeks of blinding combined with pinealectomy, some female hamsters begin to lose estrous cyclicity, with an attendant loss of the PRL-stimulating hormone estrogen. [We have shown previously in the hamster that ovariectomy causes a decline in pituitary PRL mRNA levels (4) and that PRL cell activity fluctuates during the normal estrous cycle (10)]. Thus, PRL cell activity declines in these BP-acy animals after the loss of estrous cyclicity. If the various measurements of PRL cell activity in BP-acy are averaged in with those of BP-cyc animals, this value for BP (total) is intermediate between INT and BS, as has been reported previously (1).

Thus, in the present study, we have reproduced this phenomenon and have provided the first explanation of it, i.e., the BP group after long-term light deprivation due to blinding is composed of two groups based on cyclicity. This is also the first measurement of PRL mRNA levels in long-term (12 weeks) blinded and blind-pinealectomized female hamsters.

Before about 8 weeks, when INT and all BP are cycling normally (Fig. 1), pinealectomy can be said to be completely effective in preventing the suppressive effects of blinding on PRL cell activity. The evidence for this is that those still cycling after 12 weeks (BP-cyc) are no different than INT (Fig. 2). Also, previous work in our laboratory has shown that PRL cell activity in blinded females is no different than that in intact animals as long as estrous cyclicity is maintained (11). Why some animals become acyclic in the BP group after about 8 weeks, and whether the BP-cyc animals also would have eventually become acyclic, cannot be determined from the available data.

It is clear that, for about 8 weeks, pinealectomy prevents the reproductive collapse caused by blinding in all animals. After 8 weeks, blinding appears to cause (in some animals) a disruption of the estrous cycle through a mechanism that does not require the presence of the pineal gland. In the BS group, blinding causes a cessation of estrous cyclicity via a mechanism that does require an intact pineal.

Blinding alone (the BS group) caused a rapid cessation of estrous cyclicity (Fig. 1). We have shown previously (11) that the decline in PRL cell activity as a result of blinding in the female hamster is due to two factors: (i) blinding leads to the loss of estrous cyclicity with the attendant loss of the PRL-stimulating effects of estrogen and (ii) blinding can suppress PRL cell activity below that produced by ovariectomy alone (11). Various aspects of this ovary-independent effect of light

**Table I.** Body and Pituitary Weights in Golden Syrian Hamsters after the Indicated Surgery<sup>a</sup>

Treatment	Body weight (g)		Pituitary weight (mg)
	Start	12 weeks	
Intact (cycling)	163 ± 8	191 ± 14	4.54 ± 0.40
BS (acyclic 8–10 weeks)	159 ± 4	184 ± 13	2.50 ± 0.19 <sup>b,c</sup>
BP (total)	161 ± 4	196 ± 6	3.94 ± 0.44
BP-acy (acyclic 2–4 weeks)	165 ± 6	203 ± 9	3.15 ± 0.20 <sup>d</sup>
BP-cyc (cycling)	162 ± 2	186 ± 6	5.00 ± 0.53

<sup>a</sup> Values are the mean ± SE. The blind-pinealectomized group (BP) has been subdivided, based on estrous cyclicity, into acyclic (BP-acy) and cyclic (BP-cyc) subgroups.

<sup>b</sup>  $P < 0.05$  vs intact or BP (total), based on the three-group (intact, BS, and BP) comparison.

<sup>c</sup>  $P < 0.05$  vs intact or BP-cyc, based on the four-group (intact, BS, BP-acy, and BP-cyc) comparison.

<sup>d</sup>  $P < 0.05$  vs intact and BP-cyc, based on the four-group comparison.

deprivation on PRL cell activity have also been reported by others (12, 13).

A possible explanation for our observations is that the pinealectomy procedure did not result in the removal of the pineal gland, i.e., that our pineal removal surgery was flawed. We think this is an unlikely explanation because, at the time of sacrifice, the calvaria and general area of the confluence of sinuses of each animal were checked for the presence or absence of the pineal gland. This was done by someone who did not perform the surgery and did not know from which treatment group the animal came (i.e., blind sham pinealectomized, or blind pinealectomized). Based on this postmortem examination, and the actual visualization of the pineal during its removal at the time of surgery, we are confident that the animals reported to be pinealectomized in this study were, in fact, pinealectomized.

It is also logically possible that pinealectomy by itself may cause some disturbance of the central nervous system, resulting in acyclicity, that is independent of any effects of blinding. Although this possibility is not ruled out by our data, in a study by Reiter (14) pinealectomy alone for 24 weeks did not result in a loss of estrous cyclicity. Thus, we feel that some effect of blinding, that does not depend on the presence of the pineal gland, is the most likely explanation.

Finally, our data do not exclude the possibility that there exists among the female hamsters used for our experiments some genetic variation for a particular trait relevant to reproductive collapse. Theoretically, blinding and pinealectomy could have different effects depending upon the genotype of each animal.

The general significance of this work is as follows. Total lack of photic stimulation due to optic nerve transection can lead to reproductive collapse (acyclicity) and loss of PRL cell activity in the female hamster by two mechanisms; one that depends on the presence of an intact pineal gland (seen in the BS group) and one that does not (see in the BP-acy group).

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