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The Preovulatory Surge of Luteinizing Hormone Secretion in Mice: Variation in Magnitude Due to Ambient Light Intensity[1]

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ABSTRACT

In mice, an absence of ambient light during the 14 h subjective daytime of proestrus consistently yields a preovulatory release of luteinizing hormone (LH) that is 2–3 times greater than that observed under normal lighting in the laboratory (700–850 lux). Neither the timing of the LH surge in relation to the diurnal light cycle, nor the number of eggs ovulated in response to the LH surge, were different in light vs dark exposed females. Increasing the ambient light intensity by 10-fold on the day of proestrus yielded LH surges no different from those observed under normal animal-room lighting. Preovulatorylike surges in LH release produced by treatment of ovariectomized mice with exogenous steroids also were enhanced by dark exposure. One possible explanation for these observations is that normal animal-room lighting is far more intense than that ever experienced by wild house mice. Thus, the enhanced release of LH that is observed under dark conditions probably is the normal surge for this species under natural conditions. Correlatively, the suppression of the LH surge that is observed under animal-room lighting could be an abnormality that has not been eliminated by domestication because it does not affect productivity directly.

INTRODUCTION

Ovulation in the mammal is induced in part by a surge in the release of luteinizing hormone (LH) by the anterior pituitary. The temporal regulation of this surge and hence of ovulation itself is well defined in many nocturnal rodents. Neural signals are generated daily at a particular phase of the light cycle; when these signals are combined with an appropriate steroidal milieu, the preovulatory release of LH is affected (Everett and Sawyer, 1950; Schwartz, 1969; Legan et al., 1975). Whereas the timing of ovulation by the daily light cycle is a well studied phenomenon, the relationship between light intensity and ovulation, or indeed between light intensity and any other reproductive process, is not (Wurtman, 1975). Recently, we observed indications in mice that the preovulatory release of LH might be greatly enhanced by exposure to darkness during the subjective daytime of proestrus. If mice were maintained on a standard 14 h light:10 h dark cycle, with the LH surge expected at lights out, the LH surge was increased 2–3-fold in magnitude if the animal room remained dark throughout that 1 day. The experiments reported here both confirm and extend this observation. In particular, they demonstrate that the phenomenon noted above most probably is a reflection of light intensity *per se*.

MATERIALS AND METHODS

Several experiments will be reported, all dealing with the effect of light intensity either on ovulation or on the magnitude of the preovulatory release of LH. The methodological details of each experiment will be presented with its results. In general, however, all experiments utilized a basic design in which matched groups of adult CF-1 female mice were housed in 1 of 2 animal rooms. Light intensities were varied in these 2 rooms on the day that an LH surge was expected. Experimental females were killed throughout that evening to obtain blood for hormone assay, or they were killed the next morning for oviductal egg counts. Our initial observations were made with ovariectomized females in which preovulatorylike surges of serum LH were induced with steroids. All succeeding experiments utilized intact females in which proestrus was synchronized in each experimental group by social manipulation.

Animals and Animal Rooms

The CF-1 females used in these experiments were born in this laboratory and housed 6/cage in a male-free room during the time between weaning and experimentation at 60–70 days of age. Except on the day of a light adjustment, both of the experimental

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animal rooms were maintained on an automatic 14 h light:10 h dark cycle with lights on at 0600 h and off at 2000 h. Except on days of light adjustment, light intensities in these animal rooms averaged 800–850 lux inside the cages on the top of the cage racks and about 700 lux inside the bottom cages. Lighting in all experiments was fluorescent (Sylvania Cool White). The 2 animal rooms were also identical in size, temperature (21 ± 1°C), and air exchange rate (12 times/h). Finally, except where noted, no other animals were housed in these experimental rooms.

Synchrony of Proestrus

To avoid routine vaginal smearing in experiments with intact females, proestrus was synchronized within each experimental group by manipulating its social environment (Whitten, 1966; Bronson, 1971). The procedure of housing postweaning females in a male-free room results in a marked degree of suppression of their estrous cycles. Upon initiation of an experiment, all females were moved to 1 of the 2 experimental rooms. Each was immediately paired with a proven stud. The males' pheromones thus simultaneously initiated cycles in a large proportion of the females in an experimental group. This procedure routinely yields about 60% of the CF-1 females in proestrus on the third day of cohabitation in this laboratory. When all of the experiments with intact females reported herein are combined, 29% of the intact females mated during 1 of the first 2 days of cohabitation and were removed from the studies. The remaining females were killed for blood collection during the afternoon and/or evening of the third day of cohabitation. Vaginal smearing and uterine observation allowed detection of the 14% that were not in proestrus and such females also were removed from the study. Thus 57% of the females started in these studies actually were used for blood assay purposes.

Steroid Manipulation and Hormone Assay

One experiment utilized estradiol and progesterone administration. Estradiol-17β was mixed with Silastic adhesive (type A, No. 891, Dow Corning). The resulting suspension was packed in a 10 mm Silastic capsule (0.04 i.d., 0.85 o.d., Dow Corning) with each capsule containing 5 μg estradiol (Bronson, 1976). Progesterone was given by injection, 400 μg in 0.02 ml oil (s.c.). Serum LH concentrations were determined using the NIAMDD rat radioimmunoassay kit, previously verified for use in the mouse by Beamer et al. (1972). Results are expressed in terms of ng-equivalents of RP-1. LH assays were done on 100 μl of serum with a minimal detectable amount between 1–2.5 ng LH.

RESULTS

Our initial indications that the preovulatory release of LH could be enhanced by dark exposure on the day of proestrus stemmed from an experiment with ovariectomized females. In this experiment, 100 females were ovariectomized and immediately implanted with 5-μg estradiol capsules. Fifty females then were placed in each of the 2 experimental rooms. Seven days later, the lights were adjusted to remain off during the daytime in 1 room while the lights remained on for the normal 14 h period in the other room. An LH surge was induced in all females by injecting progesterone at 1000 of that day (under red light in the dark room; Bronson and vom Saal, unpublished data). All females were killed between 1600–2400 h of that evening to evaluate the magnitude of the LH surge. As shown in Fig. 1, the LH surge was 3-fold greater in magnitude following exposure to darkness during the subjective daytime than it was under the normal light:dark cycle. The 3-fold figure was calculated by comparing the combined averages for the 3 collection periods during the surge (1800, 2000 and 2200 h). Timing of the surge in relation to the daily light cycle was not altered by dark exposure for the 1 day. As expected, the progesterone induced surge was much smaller than that occurring in intact females (Bronson and vom Saal, unpublished data).

The next experiment was done with intact females to determine if their preovulatory surge in LH release would be also enhanced by exposure to darkness during the subjective daytime of proestrus. By use of the procedures for synchronizing proestrus that were outlined in the Materials and Methods section, 85 females were paired with males in each of the 2 experimental rooms. Blood from 50–55 females was collected in each room throughout the evening of Day 3 of cohabitation. The lights were adjusted in 1 room so that it would remain dark on Day 3; the other room was maintained on its normal light cycle. Red light was used again when necessary to check for vaginal plugs or for killing. The results of this study (Fig. 1) confirm the initial observations. The LH surge was 2.4 times greater in the intact females in the dark room than it was in the females exposed to normal animal-room lighting.

The third experiment queried whether a dark induced increase in the preovulatory LH surge would result in a greater number of ova being ovulated in response to the surge. Intact females used in this experiment were treated exactly as described for the previous experiment except that those females which mated on the third night (as shown by the presence of fresh vaginal plugs the next morning) were killed the next day and their oviducts searched

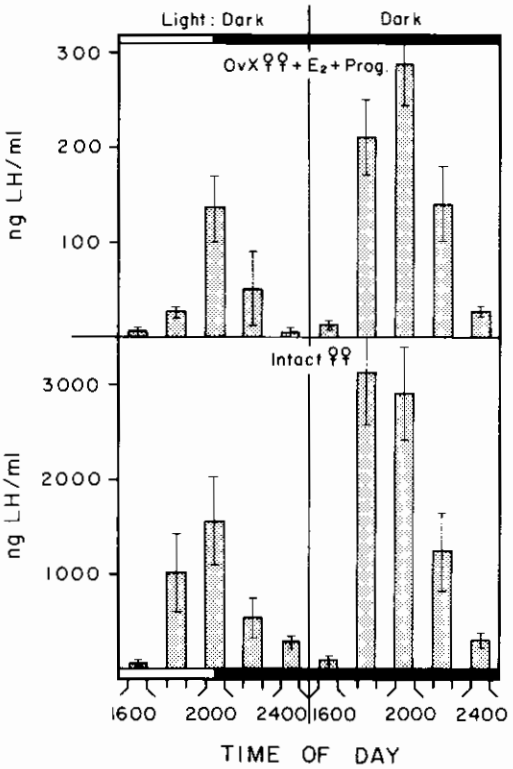

FIG. 1. Serum LH concentrations (ng/ml ± SEM) at different times of day in females exposed to light vs dark conditions during the subjective daytime of the day an LH surge was expected. Each mean is based upon 10 females in the experiment which involved steroid-induced surges in ovariectomized females. Nine to 12 females are represented by each mean in the experiment with intact females.

for ova. Eight females experiencing light on their day of proestrus averaged 13.9 ± 0.4 ova in their oviducts the next day. The comparable figure for dark exposed females was not significantly different (14.6 ± 0.9).

The fourth experiment examined the effect of light intensity *per se* on the preovulatory release of LH. Light in the 2 animal rooms was adjusted so that proestrus females were exposed to dark, 4–7 lux, 700–850 lux, or 3000–10,000 lux (all as measured inside the females' cages on different parts of the cage racks). The dark vs 3000–10,000 lux comparison was conducted first and it was followed immeditely by other 2 experimental treatments. Lighting was decreased from the normal 700–850 lux level by wrapping the existing fluorescent bulbs with aluminum foil except for small apertures. Lighting was increased to 3000–10,000 lux by installing a battery of the same brand of fluorescent bulbs beside a mouse rack. In all cases, 40 females were paired with males in each animal room at the beginning of an experiment. Lights were adjusted before 0600 h on the third day of cohabitation and 18–26 proestrus females from each treatment group were killed either at 1600 or 2000 h of that day. The resulting data strongly suggest a negative relationship between light intensity and surge magnitude (Fig. 2); regression analysis and trend analyses, however, both yielded nonsignificance ($P > 0.05$). The reason for the generally lower surge levels of LH in this experiment (Figs. 1, 2) is not known but is not traceable to assay variation.

DISCUSSION

The present results establish the fact that a lack of ambient light during the normal 14 h subjective daytime of proestrus enhances the preovulatory release of LH in mice. This enhancement does not seem to reflect simple phase-shifting of an underlying circadian rhythm since the lack of light for 1 day did not influence the expected timing of the LH surge, but only its magnitude (Fig. 1). Furthermore, the data suggest that light intensity *per se* is the relevant variable here as witnessed by the generally negative relationship between light intensity and surge magnitude (Fig. 2). An alternative explanation would be that the enhanced surge seen in dark exposed females

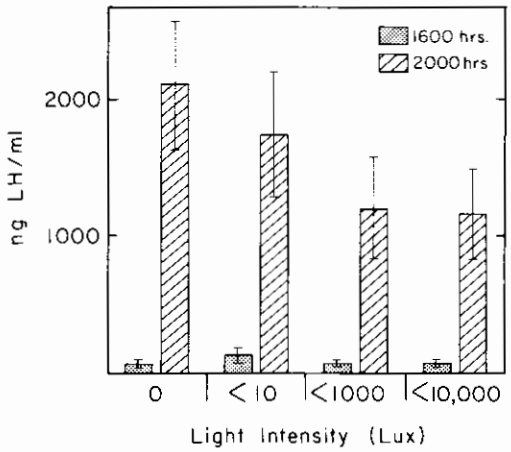

FIG. 2. Serum LH concentrations at 1600 or 2000 h on the day of proestrus in females exposed to 0, 4–7, 700–850, or 3000–10,000 lux during the 14 h subjective daytime of that day. Four to 8 females are represented by each 1600 h mean; 12–18 females were examined for each 2000 h mean.

actually was a response to novelty, i.e., a nonspecific reaction to a change of any kind (no light, acute exposure to red light). The lack of significance associated with the data on light intensity *per se* (Fig. 2) makes the novelty explanation viable even if improbable. If we assume that further work fully verifies the relationship between light intensity and the LH surge, several questions are immediately obvious. The question of physiological mediation is one example. Preliminary experiments suggest that the phenomenon is not related to pineal activity because pinealectomy did not alter the light vs dark difference in surge magnitude. Possibly a more interesting question, however, concerns the normalcy vs the sufficiency of the preovulatory LH surge in the domestic mouse maintained under laboratory conditions.

Wild *Mus musculus* undoubtedly never experience light intensities typical of most animal-room conditions. Wild house mice occur either as commensals of man, inhabiting his houses, barns and granaries, or, conversely, they occur as truly feral populations, living in grasslands, old fields, or cultivated areas. They are strictly nocturnal, spending their daytime hours inside the walls of buildings, in deep cracks or crevices in the ground, in convoluted burrows in earth, in stacked grain, or in chicken manure (Berry, 1970). In neither the commensal nor the feral situation then could one expect wild mice ever to encounter light intensities of over a few lux. On this basis one can speculate that the large, dark induced LH surge actually is the normal surge for this species and that the smaller surge seen at 700–850 lux is a laboratory abnormality.

It would be surprising if any reaction to a laboratory condition which interfered with productivity could have withstood the centuries of domestication behind the modern laboratory mouse (Staats, 1966). Thus, domestication and its consequent selection for productivity under laboratory conditions, should have promoted the ability of domestic mice to breed under the relatively intense light of the laboratory. If one assumes that the CF-1 is a typical stock of mice, the implication is that the magnitude of the LH surge, within the limits of these studies, is not related to productivity. The failure to see a reflection of dark vs light exposure on the number of ova shed supports this argument. Thus the present results may indicate the existence of a laboratory abnormality assumed previously to be "normal" and, correlatively, a natural consequence of the domestication of a nocturnal rodent.

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