Rate of reproductive involution following either exposure to short days or daily administration of melatonin is faster in inbred than in random-bred female Syrian hamsters

R. J. Reiter, I. Sabry, M. Nordio, M. K. Vaughan and S. Migliaccio

Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, Texas 78284-7762, U.S.A.

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ABSTRACT

The onset of cessation of oestrous cyclicity and associated organ and hormonal changes were compared in random-bred (RB) and inbred (IB) female Syrian hamsters kept either under short days (8 h light:16 h darkness; 8L:16D) or long days (14L:10D) and given daily afternoon injections of 25 µg melatonin. In response to short-day treatment, 100% of the IB hamsters exhibited vaginal acyclicity within 35 days; by comparison, none of the RB animals were acyclic at this time. The IB hamsters also exhibited other changes associated with exposure to short days, including increased body weight, enlarged ovaries, regressed uteri, elevated pituitary concentrations of FSH, and depressed pituitary and plasma concentrations of prolactin. At this time, only the pituitary FSH levels were increased in the RB animals kept under the same short-day conditions.

In a second experiment, RB and IB female Syrian hamsters were maintained under long days (14L:10D) and the rate of reproductive regression in response to daily afternoon injections of melatonin was compared. After 8 weeks of melatonin injections, 80% of the IB females were anoestrous, while all RB hamsters were still exhibiting 4-day oestrous cycles. Other changes associated with melatonin administration in the IB females included a marked drop in uterine weight and a depression in pituitary and plasma prolactin levels. The RB hamsters, although they were all still cyclic after 8 weeks, had increased body and ovarian weights, increased pituitary concentrations of FSH, and lower pituitary and plasma prolactin levels.

The results show that the IB strain of Syrian hamster, compared with the RB strain, responded more quickly with vaginal acyclicity to both exposure to short days and daily injections of melatonin. On the other hand, the IB hamsters responded more slowly in terms of oestrous acyclicity to melatonin injections than to treatment with short photoperiod.

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INTRODUCTION

The reproductive systems of Syrian hamsters (Mesocricetus auratus) are very sensitive to photoperiodic manipulation, such that when either males (Hoffman & Reiter, 1965; Gaston & Menaker, 1967) or females (Hoffman & Reiter, 1966; Reiter & Hester, 1966) are placed under short-day conditions that provide less than 12.5 h of light per day their peripheral reproductive organs undergo morphological (Reiter, 1968a,b) and functional (Sorrentino & Reiter, 1970; Reiter, 1973, 1974; Berndston & Desjardins, 1974; Bridges & Goldman, 1975) involution. Furthermore, hypothalamic metabolism related to the function of the peripheral sexual organs is altered accordingly (Kumar, Chen, Besch et al. 1982; Kumar, Besch, Millard et al. 1984; Steger, Reiter & Siler-Khodr, 1984; Hastings, Herbert, Martensz & Roberts, 1985a; Roberts, Martensz, Hastings & Herbert, 1985). If hamsters kept under long days are given daily afternoon injections of melatonin their neuroendocrine-reproductive system atrophies in a manner reminiscent of that seen in hamsters kept under short-day conditions (Reiter, Blask, Johnson et al. 1976; Tamarkin, Westrom, Hamill & Goldman, 1976).

In the bulk of the studies where these observations have been made, the interval required to achieve the sexual changes reported was approximately 55–70 days (Reiter, 1980). These studies used what are generally referred to as random-bred (RB) Syrian hamsters. Recently, a series of papers appeared in which it was reported that the reproductive system of...
an inbred (IB) strain of Syrian hamster responded with involution within 15–20 days after exposure to short day (Hauser & Benson, 1986, 1987a,b). This is a much briefer interval than that required to induce sexual involution in the RB strain of hamsters, although in the studies of Hauser & Benson (1986, 1987a,b) no comparisons were made between the RB and IB strains. Thus the possibility remained that there was something unique about the environment, rather than a genetic predisposition, which resulted in the IB hamsters responding very quickly to exposure to short days. To attempt to resolve this question, we examined the reproductive involutional changes in IB Syrian hamsters kept under short daily photoperiods and compared these alterations with the reproductive physiology of RB hamsters kept under the same conditions. Additionally, we compared the reproductive responses of IB and RB hamsters exposed to long days and given daily afternoon injections of melatonin.

MATERIALS AND METHODS

Female hamsters were selected for these studies so that the functional status of their reproductive system could be monitored daily by examining vaginal cyclicity. Syrian hamsters are known to have highly regular 4-day oestrous cycles; the cycles can be followed easily by examining the discharge from the vaginal introitus on a daily basis. With this method it has been shown that female Syrian hamsters have a post-oestrous discharge on the morning of every fourth day when they are normally cyclic (Orsini, 1961). Interruption of these 4-day discharges is indicative of suppression of reproduction (Sorrentino & Reiter, 1970).

The North American derived stock of hamsters originated from a single mother and her three pups captured near Aleppo, Syria in 1930 (Hoffman, 1982). Charles River (Cambridge, MA, U.S.A.) obtained the F31 generation of hamsters in 1965 from the University of Pennsylvania from which is derived the inbred strain (LSH/Ss Lak) used in these studies. All animals were bred and maintained in long photoperiodic conditions before arrival in San Antonio. Young adult RB (50–60 days of age) were purchased from Sasco; similarly aged IB (LSH/Ss Lak) females were purchased from Charles River. Upon arrival, they were placed four or five per polycarbonate cage and food (Purina Lab-Blox) and tap water were available ad libitum. The animals were housed in an environmentally controlled room, illuminated (fluorescent Super Saver, 300–500 lux) daily from 0.600–20.00 h (14 h light:10 h darkness; 14L:10D) unless otherwise specified. Animals were used for experiments after 1 week of acclimatization to the animal quarters. The mean body weight of the RB females at the onset of the experiments was 77 ± 4 g (mean ± S.E.M.) while the mean weight of the IB females was 71 ± 2 g.

Experiment 1

Two groups of RB (eight to ten each) and two groups of IB (eight to ten each) female hamsters were used in this study. One group of RB and one group of IB hamsters were placed under a short-day photoperiod regimen of 8L:16D (lights on 10.00 h). The remaining two groups of hamsters were kept under long daily photoperiods (14L:10D; lights on 06.00 h). Temperature in the animal rooms was held constant at 22 ± 2 °C. On each morning during the study the vaginal introitus of each animal was examined according to the method of Orsini (1961) to determine the presence or absence of the post-oestrous discharge.

The experiment was continued for 35 days until the bulk of the IB hamsters exhibited vaginal acyclicity. Hamsters were considered acyclic when they had missed two consecutive post-oestrous discharges. At this point, all animals were killed by decapitation between 13.00–15.00 h. Trunk blood samples were collected in heparinized centrifuge tubes, and body and organ (ovaries, uterus, and anterior pituitary glands) weights were recorded. Plasma samples were stored frozen for later hormone analyses. Likewise, anterior pituitary glands were disrupted by sonication and frozen for hormone determinations.

Experiment 2

This experiment was conducted simultaneously with the first study. Four groups (eight to ten hamsters each of RB and IB) were kept under 14L:10D (lights on at 06.00 h). Their reproductive status was monitored by examining vaginal discharge daily. One group each of the RB and IB hamsters received a daily s.c. injection of 25 μg melatonin (sigma Chemical Co., St Louis, MO, U.S.A.) in 0·1 ml alcoholic saline. Control hamsters received the diluent only. All injections were given between 16.00 and 17.00 h.

The experiment was continued for 8 weeks until the majority of the IB hamsters exhibited vaginal acyclicity. All animals were decapitated at this time between 13.00 and 15.00 h. Body and organ weights were recorded and blood and tissue samples treated as in experiment 1.

Hormone determinations

Plasma and pituitary concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were estimated using kits provided by NIADDK as previously described (Vaughan, Richardson, Petterborg et al. 1986). Pituitary and
plasma concentrations of prolactin were determined using an homologous assay (Soares, Colosi & Talamantes, 1983).

Statistical analysis

Data were statistically analysed using two-way analysis of variance followed by Student–Newman–Keuls test.

RESULTS

Experiment 1

Both the RB and IB hamsters exhibited regular 4-day oestrous cycles for the first 3 weeks of the study (Fig. 1). From the middle of week 3, however, the IB hamsters kept under short days rapidly lost vaginal cyclicity as evidenced by the lack of post-oestrous discharges; thus, by the end of week 5 all ten IB females were acyclic. At this point, the RB females maintained under 8L:16D continued to exhibit their normal 4-day oestrous cycles. At the end of week 5, all hamsters were killed as described above.

The mean body weight of all of the IB females was significantly \( P < 0.001 \) less than the mean body weight of the RB animals (Fig. 2a); the body weights of the IB hamsters averaged roughly 70 g less per animal. Compared with the IB hamsters on long days, those on short days were slightly larger \( P < 0.01 \). Likewise, ovarian weights in the IB hamsters on short days increased \( P < 0.001 \) compared with those in 14L:10D), typical of hamsters kept under restricted light conditions. The IB hamsters kept under short days had much smaller uterine weights \( P < 0.001 \) than those under long days (Fig. 2a). Among the RB hamsters, body, ovarian, and uterine weights were not affected by exposure to short days for 5 weeks (Fig. 2a).

Pituitary concentrations of LH, FSH, and prolactin are shown in Fig. 2b. In the IB hamsters, pituitary LH levels were not influenced by 5 weeks of exposure to short days although FSH concentrations were increased significantly \( P < 0.01 \) and prolactin concentrations diminished \( P < 0.001 \). In the RB group of hamsters, exposure to short days increased \( P < 0.001 \) pituitary FSH levels while pituitary concentrations of LH and prolactin remained unchanged.

Plasma prolactin levels in IB hamsters were suppressed \( P < 0.01 \) by 5 weeks exposure to short days (Fig. 3a); this was not the case in the RB hamsters. Circulating concentrations of LH and FSH did not vary among the treatment groups in either the IB or RB hamsters (data not shown).

Experiment 2

In this study, groups of RB and IB hamsters were kept under 14L:10D and treated with either diluent or melatonin every afternoon for 8 weeks. For the first 5 weeks of the study, all females exhibited the normal 4-day post-oestrous vaginal discharge (Fig. 1b). Thereafter, the IB hamsters receiving melatonin began to exhibit oestrous acyclicity and by the end of week 8, when the study was terminated, eight out of ten were no longer cycling. All animals receiving daily injections of diluent, as well as the RB hamsters that received melatonin, continued to cycle after 8 weeks of treatment.

As in experiment 1, the final body weights of the IB females were much less \( P < 0.001 \) than those of the
RB animals (Fig. 4a). Melatonin treatment in the RB groups caused a significant \( (P < 0.01) \) increase in mean body weight. Mean ovarian weights of IB females were unaffected by daily injections of melatonin although the ovaries of the melatonin-injected RB hamsters were larger \( (P < 0.01) \) than those given diluent (Fig. 4a). Melatonin treatment in the IB animals markedly reduced uterine weights \( (P < 0.001) \) relative to those in the diluent-treated controls, but a similar reduction was not apparent in the RB hamsters given daily injections of melatonin.

Pituitary LH levels were similar in all groups of RB and IB hamsters (Fig. 4b). Mean pituitary FSH levels increased \( (P < 0.05) \) in RB females after melatonin treatment, but not in IB animals. Melatonin administration daily for 8 weeks significantly depressed pituitary prolactin concentrations in both the IB \( (P < 0.05) \) and RB \( (P < 0.001) \) female hamsters compared with their respective diluent-treated controls.

Circulating concentrations of prolactin were diminished by melatonin treatment in both the IB and RB animals (Fig. 3b). Neither plasma LH nor FSH levels were significantly changed by melatonin administration to either IB or RB female hamsters (data not shown).

**DISCUSSION**

Typically, when adult female Syrian hamsters are kept under short-day conditions vaginal cyclicity is
interrupted (Sorrentino & Reiter, 1970), the ovaries become enlarged due to the proliferation of the interstitial tissue (Reiter, 1968b) while vesicular follicles and corpora lutea disappear (Reiter, 1968b), the uterus becomes grossly and microscopically infantile (Reiter & Hester, 1966), serum levels of LH and FSH exhibit a daily afternoon surge (as opposed to once every 4 days in cyclic females (Seegal & Goldman, 1975; Bittman & Goldman, 1979; Goldman & Brown, 1979), prolactin levels in the blood are diminished (Reiter, 1980), pituitary concentrations of LH and FSH increase (Reiter & Johnson, 1974a,b; Reiter, 1980) while prolactin levels in the anterior pituitary fall (Reiter & Johnson, 1974a,b; Blask, Leadem, Orstad & Larsen, 1986), and a variety of hypothalamic changes occur which may relate to the cessation of reproductive capability in these animals (Kumar et al. 1982, 1984; Steger et al. 1984; Hastings et al. 1985a; Hastings, Herbert, Martensz & Roberts, 1985b; Roberts et al. 1985).

The changes reported in the present study for the IB strain of Syrian hamster were, in general, not as severe as in some of the reports cited above; this is probably due to the fact that, in the current study, the animals were killed shortly after the onset of oestrous acyclicity. Had the experiment been prolonged, as in many of the previous studies, presumably the reproductive changes would have become even more obvious. It is apparent from the current findings that the alterations that accompany reproductive regression induced by short days or melatonin treatment are similar in IB hamsters to the known pattern of reproductive and hormonal changes observed previously in similarly treated RB animals with atrophic gonads. This is in agreement with the results obtained by Hauser & Benson (1986, 1987a,b), who also observed hormonal responses as well as changes in hypothalamic sensitivity to gonadal steroids in the IB strain of hamsters similar to those reported in the RB hamster strain (Turek, 1977; Jorgeson & Schwartz, 1984, 1985). Thus, it is very likely that the mechanism governing the induction of reproductive regression by short photoperiods in these two strains of hamsters are identical.

The current study shows, for the first time, that indeed the rate of sexual involution due to either short days or daily afternoon melatonin injections is more rapid in IB hamsters. While this could certainly be inferred from the results obtained by Hauser & Benson (1986), they never made a comparison of IB and RB hamsters and the possibility existed that there was something unique to their experimental conditions which resulted in what appeared to be a quicker than usual regressive response in IB hamsters. In the present experiment, the IB hamsters exposed to short photoperiods all experienced vaginal acyclicity after 5 weeks of treatment; at this time all of the RB hamsters were still exhibiting normal 4-day oestrous

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Plasma prolactin concentrations in random bred (RB) and inbred (IB) female Syrian hamsters maintained under either long (LD; 14L:10D) or short day (SD; 8L:16D) conditions for 5 weeks. (b) Plasma prolactin concentrations in RB and IB hamsters maintained under 14L:10D photoperiods and given either daily afternoon melatonin (Mel) injections or diluent (Dil) for 8 weeks. Means ± S.E.M. are indicated. *P<0.05, **P<0.001 compared with respective LD or diluent-treated groups (two-way ANOVA followed by Student–Newman–Keuls test).
cycles. Similar results were obtained in the study in which daily afternoon injections of melatonin were used to suppress reproductive function. In this case, 80% of the IB hamsters were vaginally acyclic after 8 weeks of melatonin administration while RB hamsters were normally cyclic.

There are, however, some minor differences between the findings obtained by Hauser & Benson (1986, 1987a,b) and those reported herein. Thus, they report that short-day treatment resulted in anoestrus within 20 days; this precedes the onset of acyclicity in the current study by roughly 15 days, suggesting that the conditions of the studies may have been slightly different. There are a number of factors, e.g. ambient temperature (Li, Reiter, Vaughan et al. 1987) and nutritional conditions (Hoffman, Johnson, Vaughan & Reiter, 1987), that predispose or otherwise modify the reproductive responses of the female hamster to restricted photoperiods or injections of melatonin. The difference in the time of onset of vaginal anoestrus in the studies in question may relate to one of these factors or other unknown conditions. It is certain, however, that under identical conditions the neuroendocrine-reproductive axis shuts down more quickly in short-day- or melatonin-treated IB hamsters than in RB animals. These presumably genetically based differences are noteworthy because they may help to explain the gradually increasing duration of time required for short days to induce gonadal atrophy in RB hamsters. It has been proposed that

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this increasing interval may relate to the genetic pool from which the parent stock is selected (Reiter, 1980).

There is another interesting feature concerning the current observations. Typically, in earlier studies it was observed that the reproductive responses of RB hamsters to daily 25 μg melatonin injections occurred more rapidly than these responses in short-day-exposed RB hamsters (Reiter, 1980). In the case of the IB strain, however, this was clearly not the case. Whereas all of the short-day-exposed females became acyclic within 35 days, even after 56 days only eight of ten melatonin-injected animals were anestrous. These two studies were conducted simultaneously using the same facilities; thus, the differences are not explicable in terms of seasonal or other environmentally related conditions. These findings seemingly support the conclusion that the reproductive system of the IB Syrian hamsters may be more susceptible to inhibition by short days than to daily melatonin injections, in contrast to the situation in RB hamsters.

It is possible, however, that the neuroendocrine-reproductive axis of the IB strain of hamsters may actually be more sensitive to melatonin than to short-day exposure but their reproductive organs degenerated more slowly for the following reason. In Syrian hamsters, an injection of a large quantity of melatonin daily can apparently down-regulate the melatonin receptors and thereby render their reproductive system insensitive to inhibition by the pineal gland (Chen, Brainard & Reiter, 1980). If, in fact, the IB hamster strain exhibits an increased sensitivity to melatonin then the 25 μg daily melatonin injection may have been sufficient to partially down-regulate the melatonin receptors, thereby delaying the atrophic response of the reproductive system. This explanation seems plausible, considering the fact that the neuroendocrine-reproductive axis of the IB hamsters exhibit a more rapid involvolvment in response to short-day treatment.

Not uncommonly, the body weights of Syrian hamsters increase after short-day exposure (Hoffman, Davidson & Steinberg, 1982; Hoffman, 1983; Bartness & Wade, 1984; Wade & Bartness, 1984); this change was also noted in the IB hamsters exposed to restricted photoperiods. The body weights of the RB females also exhibited a significant increase after melatonin administration. Again, this change is consistent with earlier reports (Reiter, 1980).

Female hamsters with atrophic gonads may have normal circulating LH and FSH levels as do their long photoperiod controls (Reiter, 1980); that was the case in the present study as well. Pituitary and plasma concentrations of prolactin usually exhibit the most marked changes and are typically reduced (Reiter & Johnson, 1974a; Goldman & Brown, 1979) as in the present study. The reduction in prolactin may be of paramount importance in ensuring ovarian atrophy in female Syrian hamsters since, at least in males of the species, it seems to be involved in the maintenance of the LH and FSH receptors at the gonadal level (Bartke, 1980).

The marked rise in pituitary FSH levels observed in the short-day-exposed and melatonin-treated hamsters is a common feature of hypothalamic-photoperiod and gonadal suppression induced by these conditions in female Syrian hamsters (Reiter, 1980). However, in the current studies the rise in the pituitary levels of this gonadotrophin was somewhat greater than previously observed and, in fact, occurred in RB animals that had not yet exhibited vaginal acyclicity. Such a marked rise in pituitary FSH levels has also been previously noticed (Hauser & Benson, 1987c; Benson & Hauser, 1988) and these authors, too, observed that it occurs in advance of interruption of oestrous cyclicity.

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REFERENCES


