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Dark exposure inhibits the mitotic activity of thyroid follicular cells in male mice with intact pineal

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Key words. Mice, male; dark exposure; activation, light-restriction-induced; pineal; thyroid follicular cells, mitotic activity.

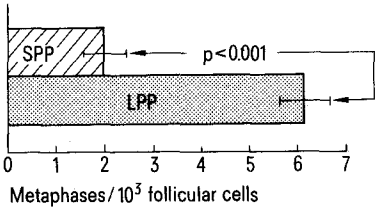
Pineal control of thyroid secretion and growth has been recently a subject of intensive research. An inhibitory effect of the pineal gland on thyroid function has been suggested[2]. However, the site of inhibition of hypothalamo-pituitary-thyroid axis has not been determined precisely, although data supporting both hypothalamic and peripheral direct effects are available[3,4].

Studies dealing with the influence of the pineal on the histological appearance and growth of the thyroid gland, primarily relating to the indices of hypertrophy, have produced contradictory results. Following pinealectomy (PX), enlargement of the thyroid, resulting in increase of its weight, has been reported by many authors[4–9]; however, negative reports also exist[10–12]. Histologial changes in the thyroid after PX, suggesting increased activity of the gland and similar to those observed after thyroid stimulating hormone (TSH) administration, have been reported[8,9]. Other investigators have not found the marked histological alterations in the thyroid following PX[4]; others even have shown changes which would be unlikely to accompany the increased activity of the thyroid[3].

Until now, the influence of the pineal gland on thyroid hyperplasia has been examined in only one report; it has been shown that PX increases the mitotic activity of thyroid follicular cells[14]. The role of lighting conditions in the control of thyroid growth remains, so far, unclear[11,12,15]. The aim of the present study was to examine the influence of different lighting conditions on the mitotic activity of thyroid follicular cells in mice with an intact pineal.

Materials and methods. Eleven adult male mice of C57 bl strain (mean weight = 38 ± 5 g) were used in the study. Six mice were subjected to long photoperiod (LPP) (16 h light: 8 h darkness) and the remaining 5 mice were exposed to short photoperiod (SPP) (10 h light: 14 h darkness). The animals were maintained in lighting conditions specified above for 10 weeks. In order to evaluate the thyroid mitotic activity, the metaphase-arrest technique was employed. On the day of termination of the experiment the mice were injected with Colchicine (Sigma) at a dose of 1 µg/l g BW, i.p. Two hours later the mice were anesthetized with methoxyflurane (Metofane, Pittman-Moore) and bled by cardiac puncture. Thyroid glands were collected from all the animals and fixed in 10% formalin. After histological processing, paraffin sections (6 µm thick) were stained with hematoxylin and eosin. In the microscopic preparations the mitotic activity rates (number of metaphases per 1,000 scored follicular cells from the randomly selected sections of each thyroid lobe) were evaluated. Groups to be compared were tested for equality of variances using the F-test. A difference in mean mitotic activity rates (MMARs) between groups was estimated by means of the Student's t-test.

Results and discussion. The results are presented in figure 1. As shown, the MMAR of the thyroid follicular cells is significantly lower in mice which were maintained in SPP



Mean mitotic activity rates in the mice subjected to short photoperiod (SPP) and long photoperiod (LPP). Bars represent means ± SEM; p, level of significance.

(2.00 ± 0.44, $X \pm SEM$) than is that in mice kept in LPP (6.16 ± 0.47, $p < 0.001$). It has been previously well established that the activity of the pineal gland is dependent on lighting conditions and increases in animals subjected to longer dark-exposure[16]. On the basis of our present data the question of whether lighting conditions themselves affect the proliferation of thyroid fillicular cells or if the observed phenomenon reflects only the dark-induced pineal activation or light-induced pineal blockade, cannot be adequately answered. However, an assumption may be offered that thyroid mitotic incidence is under the inhibitory control exerted by the pineal gland, and this pineal effect can be blocked by the maintenance of animals in LPP. Such an interpretation of our present data is compatible with the previous report[14] demonstrating the increase of mitotic activity of thyroid follicular cells following PX in rats and with the series of reports showing thyroid hypertrophy after PX in rats[4,8,9]. mice[7], and cats[5]. Furthermore, the present results are in compliance with the earlier study[15] demonstrating attenuation of thiourea-induced thyroid enlargement in hamsters by maintaining the animals in SPP. Additionally, in the same report[15] the authors demonstrated that the inhibitory effect of longer dark-exposure on thyroid hypertrophy is independent on the presence of the pineal gland.

Inconsistently, in other studies constant darkness has been shown not to affect the thyroid weight[10] or even to cause thyroid enlargement[11,12].

Our hypothesis that the pineal gland decreases the proliferation of thyroid follicular cells, an effect which can be revealed by subjecting the mice to SPP, is supported by the experiments utilizing the pineal hormone, melatonin. Administration of melatonin has been reported to inhibit thyroid hypertrophy after methylthiouracil (MTU)-treatment[17] or after PX[6,7], but not to affect the thyroid weight in untreated controls with intact pineal[6,17,18]. Melatonin has been shown to decrease the height of the thyroid follicular cells not only in MTU-treated rats but also in untreated controls[17], which is in compliance with the PX-induced increase of the height in question[14]. Administration of pineal extracts induced signs of thyroid involution in rats[8,19]. In contrast, a goitrogenic effect of melatonin on the thyroid has been postulated[6,20]. Surprisingly, histological signs of thyroid hyperactivity after melatonin treatment have been observed in one laboratory[18]. It is noteworthy that melatonin exhibits antimitotic activity in some in vitro tests for antimitotic drugs[21,22], although it presumably does not compete with colchicine for tubulin binding sites[23].

In summary, the present results prove that, in mice with intact pineals, subjecting animals to SPP results in inhibition of mitotic activity of thyroid follicular cells when compared to animals reared in exposure to LPP. This effect is most probably related to the light restriction-induced activation of the pineal gland; however, direct effect of lighting conditions, independent on the presence of the pineal, cannot be excluded.

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Effect of photoperiod and all-female grouping on the estrous cycle of the bandicoot rat, *Bandicota bengalensis*[1]

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Summary. Female bandicoot rats showing irregular cycles in 12L:12D were exposed to light-darkness cycles of 1L:23D, 4L:20D and 8L:16D. Significant regularization of the estrous cycle was observed in 8L:16D with most of the rats exhibiting a regular 3-day cycle and the regularity was further enhanced by all-female grouping (4/cage).
Key words. Bandicoat rat; *Bandicota bengalensis;* photoperiod; estrous cycle; all-female grouping.

Photoperiod is the primary environmental influence on reproduction of various vertebrate species[2-4] including rats[5-8] but not house mice[9]. While short photoperiod (8L:16D) inhibits reproductive tract growth in young female white-footed mice[10], 6L:18D fails to reduce reproductive success in adult female deer mice[11]. In golden hamsters, short photoperiod causes gonadal regression in adult[12], but does not affect the gonadal maturation of young ones[13].

All-female grouping causes pseudopregnancy (the Lee-Boot effect) or continuous diestrus in mice[14-16], but it has no effect on the estrous cycle in the Norway rat[17,18]. On the contrary, all-female grouping induces cyclicity in the non-cyclic female bandicoot rat[19], while individually housed bandicoot rats show extremely irregular estrous cycles with 12L:12D light-darkness cycle[20,21]. Thus, in the present study the effect of photoperiod and all-female grouping on the estrous cycle of the bandicoot rat was evaluated.

Materials and methods. Adult female bandicoot rats, *Bandicota bengalensis* (GRAY), weighing about 150–220 g each, were trapped in grain storage warehouses in Calcutta. They were