

Melatonin Synthesis in the Pineal Gland: Effect of Light Mediated by the Sympathetic Nervous System

Abstract. Exposure to light reduces the ability of the rat pineal gland to synthesize melatonin and decreases the weight of the gland. When the sympathetic nerves to the pineal gland are cut, light no longer has an effect on melatonin synthesis or pineal weight. The response of the gland does not require that the gonads or the pituitary gland be present.

Melatonin (5-methoxy *N*-acetyl tryptamine) (1) is synthesized in the mammalian pineal gland by the *N*-acetylation and subsequent *O*-methylation of serotonin (2). It has recently been shown that the activity of hydroxyindole-*O*-methyl transferase (HIOMT), the enzyme responsible for the *O*-methylation step, is influenced by illumination. Rats kept in constant darkness have 3 to 10 times as much melatonin-synthesizing-ability in their pineal glands as littermates kept in continuous light (3).

The effect of illumination on the pineal transferase, which is accompanied by smaller but consistent changes in pineal weight (4), is demonstrable within 24 hours (5) and appears to be specific, in that other enzymes in the pineal gland such as monoamine oxidase are unaltered (3). Since evidence has been presented that melatonin is secreted by the mammalian pineal gland (6) and inhibits ovary growth and the subsequent incidence of estrus in rats (7), it has been suggested that the light-induced inhibition of HIOMT activity may constitute a mechanism of neuroendocrine regulation of gonad function (3).

There are several ways in which information about lighting could reach the pineal gland of the rat. (i) Light could penetrate the skull and impinge directly upon the pineal. It has recent-

ly been shown that sufficient light penetrates the mammalian skull to activate photoelectric cells implanted within the brain (8). (ii) Light could act, through the hypothalamus and pituitary body or other neuroendocrine organs, to alter the level of a circulating hormone, which might in turn influence pineal HIOMT. Although the effect of light upon pineal weight does not require pituitary, gonad, adrenal, or thyroid function (9), ovarian hormones and the estrous cycle do influence the phospholipid content of the pineal gland (10). (iii) Information about lighting could be transmitted to the pineal via a neural route.

It has recently been demonstrated that the major, if not the only, innervation of the rat pineal gland is sympathetic, and consists of fibers whose cell bodies are in the superior cervical ganglia (11). It will be shown that the pathway by which information about lighting reaches the pineal gland involves the eyes and the sympathetic nervous system, and is independent of the pituitary body or gonads. The experiments described here will also demonstrate that it is possible to trace

Table 1. The effect of bilateral orbital enucleation or superior cervical ganglionectomy on the response of the rat pineal gland to constant light or darkness. Groups of 36 mature female animals were placed under experimental lighting conditions for 11 days, beginning 1 day after surgery.

Condition	Pineal weight (mg)	HIOMT activity ($\mu\mu\text{mole/gland}$)
<i>Sham-operated</i>		
Dark	1.20 \pm 0.02	13.8 \pm 1.3
Light	0.95 \pm 0.03*	4.8 \pm 2.1*
<i>Enucleated</i>		
Dark	1.17 \pm 0.07	9.7 \pm 1.0
Light	1.15 \pm 0.08	11.6 \pm 2.3
<i>Ganglionectomy</i>		
Dark	1.02 \pm 0.06	6.1 \pm 2.0
Light	1.03 \pm 0.05	7.1 \pm 1.0

* $p < .001$

a neuroanatomic tract by using an appropriate enzymatic indicator.

To determine whether a given organ was participating in mediating the effect of light upon pineal weight and HIOMT activity, the organ was removed and the subsequent capacity of the pineal gland to respond to light was examined. Sprague-Dawley rats weighing 160 to 180 g were subjected to various surgical procedures under ether anesthesia. One or two days later, groups of 8 to 36 animals were placed in constant-light or constant-dark rooms equipped with double-door light baffles and air conditioning. After 6 to 11 days, animals were killed by neck fracture while still in light or darkness. Pineal glands were quickly removed, weighed, and assayed for HIOMT activity (12). All animals were killed between 9:00 and 10:00 A.M.

Removal of both eyes resulted in a complete loss of the capacity of the pineal gland to respond to altered illumination with the accompanying changes in weight or HIOMT activity (Table 1). This indicates that the action of light upon the rat pineal gland is not direct, but is mediated by retinal receptors.

To determine whether pituitary or ovarian hormones are required for light to influence the HIOMT activity of the pineal gland, the effects of continuous illumination or darkness were examined in adult males, immature females (21 days old), adult females in which both ovaries had been removed, and hypophysectomized adult female rats (13). In all cases, light produced its characteristic inhibitory effect on pineal

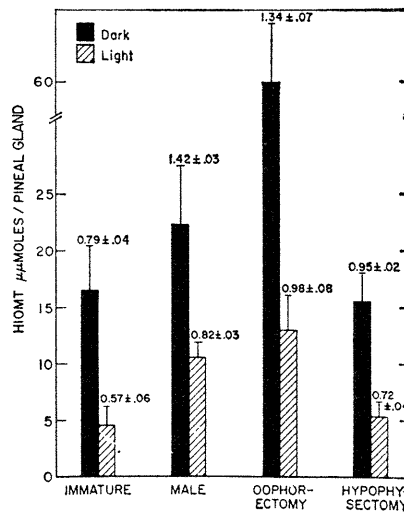


Fig. 1. The effect of constant light or darkness on the weight of the pineal gland and HIOMT activity in the rat. Immature 21-day-old female rats, and bilaterally-oophorectomized or hypophysectomized adult females were maintained under experimental lighting conditions for 6 days. Adult males were kept in light or darkness for 42 days. The HIOMT activity is expressed as micromicromoles of C^{14} -melatonin formed per whole pineal gland per hour. Average pineal weights \pm standard error are given over the appropriate bars.

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weight and melatonin-synthesizing ability (Fig. 1). In rats exposed to constant light, the pineal glands weighed significantly less ($p < .001$), and there was significantly less HIOMT activity per gland ($p < .001$ for all groups except males: $p < .01$) than those of littermates exposed to constant darkness. These observations show that although ovarian and pituitary hormones may influence the pineal gland, their presence is not required to mediate the effect of light upon HIOMT activity in the pineal gland.

The sympathetic innervation of the pineal gland was interrupted by removal of both superior cervical ganglia. After this procedure, rats placed in continuous light no longer responded with decreased pineal weight or HIOMT activity (Table 1). Thus the transmission of information about illumination to the pineal gland, or the capacity to respond to such information, requires the presence of an intact sympathetic innervation.

After superior cervical ganglionectomy, rats develop some degree of ptosis; this sign may be used to assay the success of the operative procedure. To rule out the possibility that ptosis blocked the passage of light into the eyes, and thereby rendered ganglionectomized animals unresponsive to illumination, two experiments were performed. In the first, it was found that removal of one eye, which reduced the orbital surface by an amount approximately equal to that covered in ptosis, did not interfere with the effects of light on pineal weight and HIOMT activity (Table 2). Next, it was demonstrated that prevention of ptosis in ganglionectomized rats by removal of the eyelids did not restore the capacity of the pineal gland to respond to light (Table 2).

These observations indicate that the sympathetic nervous system is directly involved in the regulation of melatonin synthesis in the rat pineal gland. Furthermore, the actions of the sympathetic nervous system on the pineal gland are influenced by environmental lighting. Changes in illumination could alter the rate of release of a transmitter substance from the sympathetic nerve endings in the pineal gland, and this neurotransmitter could then influence the activity of the melatonin-synthesizing enzyme. It is well established that sympathetic nerves release norepinephrine (14). Recently it has been

Table 2. The effect of unilateral orbital enucleation or bilateral superior cervical ganglionectomy with the removal of the eyelids on the response of the rat pineal gland to constant light or darkness. Groups of seven littermate mature female animals were kept under experimental lighting conditions for 11 days, beginning 1 day after surgery.

Condition	Pineal weight (mg)	HIOMT activity ($\mu\text{mole/gland}$)
<i>Unilateral enucleation</i>		
Dark	1.64 \pm 0.13	25.5 \pm 0.7
Light	1.02 \pm 0.05*	9.3 \pm 0.8*
<i>Ganglionectomy and eyelid removal</i>		
Dark	1.03 \pm 0.05	17.3 \pm 2.2
Light	0.99 \pm 0.06	14.6 \pm 1.1

* $p < .001$

shown that sympathetic nerves in the pineal gland also contain serotonin (15). It is possible that the liberation of these amines could affect HIOMT activity. If one way by which light influences the rat estrous cycle (16) is by controlling the synthesis and secretion of pineal melatonin (3), it would be expected that removal of the sympathetic innervation of the pineal gland might alter the effect of light upon the gonads of the rat. Further experiments are in progress to test both of these hypotheses.

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12. The HIOMT activity was assayed by a modification of the method of J. Axelrod, P. D. MacLean, R. W. Albers, H. Weissbach, in *Regional Neurochemistry*, S. S. Kety and J. Elkes, Eds. (Pergamon, New York, 1961), pp. 307-311. Two or three glands were homogenized in 1.0 ml of 0.05M (pH 7.9) phosphate buffer. For the enzyme assay, 0.6 ml of the pineal homogenate, 10 μg of

N-acetylserotonin, and 0.1 μc of C^{14} -methyl-5-adenosylmethionine (3.7 μmole , 10,000 count min) were incubated at 37 C for 1 hour. The C^{14} -melatonin formed was extracted with 8 ml of chloroform, which was washed twice with borate buffer, pH 10.0. A 6-ml portion of the chloroform extract was evaporated to dryness, and counted in a liquid scintillation spectrophotometer after the addition of ethanol and phosphor.

13. Hypophysectomized animals were obtained from Hormone Assay Laboratories, Inc., Chicago, Illinois.
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