ACTA ENDOCRINOLOGICA
36, 617–624, 1961

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INTERACTIONS OF THE PINEAL AND EXPOSURE TO CONTINUOUS LIGHT ON ORGAN WEIGHTS OF FEMALE RATS

By

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ABSTRACT

Either exposure to constant light for 80 days or pinealectomy produced similar changes in the weights of the ovaries and adrenals of female rats. These were not additive when both procedures were employed. Pinealectomy did not share with light-exposure the capacity to induce uterine hypertrophy.

Rats exposed to constant light for 56 days had lighter pineals than animals kept in darkness; this decrease was not affected by administration of bovine pineal extracts.

The increase in ovarian weight produced in rats by exposure to light for 56 days was prevented by bovine pineal extracts, but these extracts were without effect on the uterine hypertrophy produced under the same conditions.

These data suggest that the effect of light upon the weight of the ovary is mediated via the pineal.

Many observers have shown that the onset of gonadal maturation and the degree of gonadal activity can be affected in normal animals in at least two ways: a) Environmental factors may be varied: Food, temperature, humidity, light, the presence of eggs or young, and the presence of a companion or mate have all been found to influence gonadal function in different species (*Harris*

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Supported in part by grants from The Josiah Macy Jr. Foundation, the Commonwealth Fund, the Wilson Laboratories and Grant A-1943 (C1) of the Unted States Public Health Service.

1955). b) Lesions may be caused in the central nervous system: Gellert and Ganong produced true precocious puberty in rats by making electrolytic lesions in the arcuate nucleus of the hypothalamus. Animals so treated showed premature opening of the vagina, followed by normal oestrous cycles (*Gellert & Ganong* 1960). Kitay observed that surgical removal of the pineal gland in the rat produced ovaries larger than those of sham-operated animals (*Kitay* 1954). *Wurtman et al.* (1959) showed that bovine pineal extracts inhibited this ovarian enlargement, and further demonstrated that daily administration of these extracts to intact animals produced a decrease in ovarian weight which varied with dose.

There is also considerable evidence that both environmental factors and neurosurgical procedures act by affecting the neuroendocrine apparatus which controls the secretion of gonadotrophins by the anterior lobe of the pituitary gland. For example, the gonads of hypophysectomized ferrets do not respond to extra illumination (*Hill & Parkes* 1933); similarly the dose of bovine pineal extract which produces a decrease in the ovarian weight of control rats has no effect on hypophysectomized animals (*Wurtman, Altschule & Grave,* unpubl.). *Harris & Jacobsohn* (1952) have shown that both the maturation and the subsequent activity of the anterior lobe of the pituitary gland are controlled by some extrinsic stimulus, which acts upon the pituitary gland when it is placed adjacent to the pituitary stalk or the medium eminence of the hypothalamus. This stimulus is generally regarded as derived from within the central nervous system.

Few attempts have been made to synthesize anatomically the data obtained from different experimental studies of factors that regulate gonadotrophic function. The single environmental variable affecting the state of the gonads whose anatomic basis has been studied in greatest detail is length of exposure to visible light. Continuous exposure to light hastens the onset of the breeding season in ferrets, causes premature opening of the vagina and onset of oestrous, and produces increased ovarian and uterine weight in rats (*Fiske* 1941).

It has been suggested that the effect of light on gonadal activity depends on intact optic nerves, since the oestrous cycles of ferrets in which the optic nerves have been severed are either absent entirely, or not subject to seasonal or photic influence (*Bissonette* 1938; *Clark et al.* 1939). However, *Benoit & Assenmacher* (1959) have claimed the existence of a deep light receptor within the hypothalamus, declaring that a »photosexual reflex« may be elicated by illuminating the hypothalamus directly through an eviscerated eye socket. *Clark et al.* (1939) studied the more central pathways of the photosexual reflex in the ferret. They observed that destruction of the superior colliculi or interruption of retinal impulses to the midbrain, the dorsal nucleus of the lateral geniculate body, and the visual cortex did not interfere with the gonadal

response to added light. They concluded that this response depended upon impulses which passed either to the ventral nucleus of the lateral geniculate body, or to the subthalamus by way of the accessory optic tracts. However, when the brains of the ferrets were later studied by *Jefferson* (1940), he could find no evidence of accessory optic tracts or of optic connections with the ventral nucleus of the lateral geniculate body in this species. There are no reports of optic fibers terminating directly in the hypothalamus of the ferret, though *Frey* (1951) has described a hypothalamic optic root in the dog. *Harris* (1955) summarized this subject and concluded »... at the moment, it seems likely that light acts via optic nerve fibers which transmit a stimulus in some way to the hypothalamus and the pituitary gland, but the details of this path are still doubtful«.

In 1959, *Fiske & Greep* reported that exposure of rats to continuous light produced characteristic changes in the hypothalamus. Although the paraventricular nuclei seemed to be unaffected by this treatment, the cells of the supraoptic nuclei were larger and appeared to be more active than those of animals kept in darkness. More Gomori-positive substance was found along the hypothalamo-hypophyseal tract in the continuously-lighted animals. In light-treated rats large amounts of neurosecretory material appeared to be moving along the axones, whereas the pars nervosa of such animals was partially depleted of Gomori-positive substance. This suggested that when under the influence of added light, the rat hypothalamus secretes and releases increased amount of neurosecretory material. The increased activity appeared to reside solely in the supraoptic nuclei. One effect of continuous lighting upon the central nervous system is evidently upon the hypothalamus. It has not been determined whether the effect of light upon the hypothalamus is direct, or, in addition, whether the effect of illumination upon the hypothalamus involves the pathway which relates light and the gonads.

Two phenomena already described suggest a link between light and the pineal gland; they are: a) the similar effects of constant exposure to artificial light and of removal of the pineal gland on the weight of the rat ovary (*Wurtman et al.* 1960), and b) the role of a pineal hormone in the control of melanophores in vertebrates (*Lerner & Case* 1959). *Fiske et al.* (1960), and *Wurtman et al.* (1960), recently reported that animals exposed to light for long periods had abnormally small pineal glands. Such small pineal glands were found whether animals remained under the experimental conditions for a few weeks or for a year. This finding differs from the time-course of light-induced ovarian and uterine enlargement: The latter changes are evident in maturing rats after seven weeks of continuous light, but disappear soon afterwards even though the experimental conditions are maintained (*Fiske* 1941).

In addition, *Roth, Wurtman & Altschule* (unpubl.) have observed that pinealectomy produces changes in the hypothalamus which are similar to those

caused by light exposure, and which are consistent with increased secretory activity within the supraoptic nucleus.

These findings suggested that perhaps some of the effects of variations in light exposure or alterations in the availability of pineal hormone might operate via the same neuroendocrine pathway. Experiments were therefore undertaken to test this hypothesis. Since pinealectomy causes ovarian hypertrophy, and exposure to light leads to both ovarian hypertrophy and a decrease in pineal weight, it seemed possible that light and also pinealectomy prevent the elaboration of that pineal hormone which causes a decrease in ovarian weight. The effects of light exposure and pinealectomy on the ovary should, therefore, be similar, but not additive. Similarly, the dose of bovine pineal extract which produces small ovaries in control rats and which prevents the ovarian hypertrophy which follows pinealectomy (*Wurtman et al.* 1959) should also prevent the ovarian hypertrophy that is produced by light.

Although pineal extracts produce a decrease in pineal weight, and evidence of decreased pineal secretory activity in normal animals (*Kitay* 1954 *b*, and *Holmgren et al.* 1960), administration of pineal extracts to animals maintained in constant light would not be expected to cause a further decrease in pineal weight if exposure to constant light had already produced maximal pineal atrophy. However, if the atrophy produced by light were not maximal, injection of extracts would produce a further decrease in pineal weight.

This paper reports the results of experiments designed to study these possibility.

MATERIAL AND METHODS

Part I. Thirty-four female rats of the Charles River CD strain were divided into four groups of eight or nine each, and either sham-operated or pinealectomized on their 26th day; pinealectomy and sham-operation were performed as described by *Kitay & Altschule* (1954). Thereafter they were maintained either in constant light or in almost-constant darkness for 80 days. At the end of this period, the rats were weighed and autopsied. The ovaries, adrenals, and uteri were weighed separately and organ weights were expressed as mg per 100 g of body weight, so that they would be comparable to the data reported previously in papers from this laboratory. Since previous experience indicated that none of the experimental techniques used here would have an effect on the body weight of any group of animals, comparisons drawn between these ratios would be expected to have the same validity as those drawn between absolute organ weights.

Light was provided by two 100-watt fluorescent bulbs, each three feet long, suspended one foot above the floor of the cages. The total darkness was interrupted for about five minutes every second day while the cages were cleaned.

Part II. Forty-seven female rats of the Charles River CD strain were treated as follows from their 26th to their 82nd day (after which they were weighed and autopsied): 17 were exposed to constant light and given 1.0 ml per day of 0.9 % saline; 15 were

exposed to constant light and given 1.0 ml per day of bovine pineal extract, made essentially as described before (*Altschule* 1957), all injections were administered intraperitoneally. In addition, 15 rats were maintained in almost constant darkness. Constant light and darkness were provided as described above. The ovaries, adrenals and uteri were weighed separately on a Roller-Smith balance, and the pineals were weighed individually on a VDF Micro-torque balance (this apparatus has a sensitivity of 0.005 mg).

RESULTS

There was no statistically significant difference in body weight between any of the experimental groups.

Part I. Rats which were either pinealectomized or exposed to constant light showed ovarian hypertrophy ($P < 0.01$) (Table 1). The changes produced were not additive when both procedures were applied to a given animal. The experimental procedure generated two distinct populations: sham-operated rats kept in continual darkness, and all other rats ($P < 0.01$). This indicated that pinealectomy added nothing to the effects of light on ovarian weight, and vice versa.

Rats which were either pinealectomized or exposed to light showed adrenal hypertrophy ($P < 0.001$) (Table 1). Here also, the changes produced by the two experimental procedures were not additive. Constant light produced uterine hypertrophy, but pinealectomy had no effect on the weight of this organ (Table 1).

Part II. Rats exposed exposed to 56 days of continuous light had ovaries larger than those of animals maintained in the dark ($P < 0.001$); this ovarian

Table 1.
Effect of illumination and pinealectomy in rats.
Organ weight expressed in mg/100 g body weight ± standard error.

		Sham-operated	Pinealectomized
Ovary:	Dark	18.4 ± 1.93*	24.9 ± 2.12
	Light	25.3 ± 1.78	23.8 ± 1.98
Adrenal:	Dark	38.9 ± 3.24**	47.3 ± 3.69
	Light	53.1 ± 3.08	53.4 ± 3.45
Uterus:	Dark	20.1 ± 3.41***	19.6 ± 3.86
	Light	36.9 ± 3.22	41.2 ± 3.61

* Sham-operated and dark differ from all others: $P < 0.01$.
** Sham-operated and dark differ from all others: $P < 0.001$.
*** Dark differs from light: $P < 0.001$.

Table 2.
Effect of illumination and pineal extracts in rats.
Organ weight expressed in mg/100 g body weight ± standard error.

	Pineal	Ovary	Adrenal	Uterus
Dark	0.68 ± .03	28.4 ± 1.5	27.3 ± 1.0	39.5 ± 2.5
Light and saline	0.55 ± .03	36.0 ± 1.5	29.2 ± 1.0	49.7 ± 2.3
Difference	0.13**	7.6***	1.9	10.2***
Light and saline	0.55 ± .03	36.0 ± 1.5	29.2 ± 1.0	49.7 ± 2.3
Light and pineal extract	0.58 ± .03	29.4 ± 1.5	25.8 ± 1.0	54.2 ± 2.5
Difference	0.03	6.6***	3.4*	4.5

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

hypertrophy was completely inhibited by the dose of bovine pineal extract used ($P < 0.001$) (Table 2).

Animals maintained in constant light and given pineal extracts had adrenals which were significantly smaller than those of animals kept in light and given saline ($P < 0.05$) (Table 2). The adrenals of animals kept in darkness were not significantly smaller than those of animals maintained in constant light.

Exposure to constant light produced uterine hypertrophy ($P < 0.001$) pineal extracts were ineffective in preventing this hypertrophy (Table 2).

Exposure to constant light produced a decrease in the weight of the pineal gland ($P < 0.01$); administration of pineal extracts to animals kept in constant light did not enhance this decrease (Table 2).

DISCUSSION

The data are consistent with the concept that the effects of light upon the ovary and adrenal, but not the uterus, are mediated via the pineal.

Exposure of rats to constant light for the experimental period appears to have produced maximal inhibition of pineal growth, and of the pineal's inhibitory influence upon ovarian weight: this concept explains the finding that animals kept in constant light and given a dose of bovine pineal extract sufficient to cause in control rats a decrease in pineal weight (*Kitay* 1954 *b*) and

secretory activity (*Holmgren et al.* 1960) exhibit no further decrease in the mass of their pineals.

Two types of evidence suggest that the ovary-weight-inhibiting-factor of the pineal operates via the anterior lobe of the pituitary gland: a) *Wurtman et al.* (1959) have shown that pinealectomy produces pituitary enlargement, and pineal extracts produce small pituitary glands; b) pineal extracts have no effect upon ovarian weight in hypophysectomized animals given a dose of a standard FSH preparation (Armour) large enough to maintain normal ovarian size (*Wurtman, Altschule & Grave,* unpubl.).

The ovarian enlargements produced by pinealectomy or by exposure to constant light are not additive, and have in common the fact that either or both together are similarly inhibited by administration of the same dose of a bovine pineal extract (*Wurtman et al.* 1959). However, pinealectomy or pineal extracts do not reproduce the uterine hypertrophy or atrophy which is associated with exposure to constant light or darkness (*Fiske* 1941). This discrepancy cannot yet be explained. On the other hand the fact that light alters both ovarian and uterine weight, whereas pinealectomy affects only that of the ovary, suggests that the influence of the pineal upon gonadotrophins is primarily upon FSH secretion, whereas light seems to influence the secretion of both FSH and LH. On the other hand, the pineal has been reported to influence other secondary sex characters which are dependent upon circulating oestrogen, such as the time of opening of the vaginal introitus (*Kitay & Altschule* 1954), and the vaginal oestrous cycle of senile rate (*Meyer et al.*, in press). These findings suggest effects on both FSH and LH activity. Moreover, several groups have recently suggested that FSH alone may have a positive influence on uterine weight, without added LH (*Squire & Li* 1958; *Steelman et al.* 1959). In the absence of general agreement on the actions of the pituitary gonadotrophins, it is impossible at present to define the mechanisms of the differing influences of the pineal gland and light on uterine weight.

The nature of the relation between light, the pineal, and the weight of the adrenal is also not clear. *Farrell* (1959) has suggested that the canine pineal secretes a hormone, *glomerulotrophin,* which stimulates the zona glomerulosa of the adrenal cortex to release aldosterone. However, *Wurtman et al.* (in press) have shown that removal of the pineal or administration of bovine pineal extracts to the rat produces no significant specific alteration in the size of this region. It appears that the pineal influences the weight of the adrenal cortex uniformly, much as does corticotrophin. The data presented here and elsewhere (*Wurtman et al.* 1959) indicate that this influence is inhibitory and suggest but do not prove, that the adrenal-weight-stimulating effect of light is somehow related to the pineal. Oestrogens stimulate adrenal hypertrophy in the rat (*Korenchevsky & Dennison* 1936; *Bourne & Zuckerman* 1941; *Gompertz* 1958). The effects of the pineal on the weight of the rat adrenal may

therefore be secondary to the effects of the pineal upon the gonads, but as described above, the nature of this relation awaits definition. Thus any such suggestions must be recognized as preliminary and uncertain.

ACKNOWLEDGEMENTS

We wish to acknowledge with thanks the assistance of Dr. Mindel C. Sheps and Miss Rita Nickerson, of the Department of Preventive Medicine, Harvard Medical School, in statistically analyzing and interpreting these data.

REFERENCES

Altschule M. D.: New Engl. J. Med. *257* (1957) 919.
Benoit J. & Assenmacher I.: Recent Progr. Hormone Res. *15* (1959) 143.
Bissonnette T. H.: Res. Publ. Ass. nerv. ment. Dis. *17* (1938) 361.
Bourne G. & Zuckerman S.: J. Endocr. *2* (1941) 283.
Clark W. E. L., McKeown T. & Zuckerman S.: Proc. roy. Soc. B. *126* (1939) 449.
Farrell G. L.: Recent Progr. Hormone Res. *15* (1959) 275.
Fiske V. M.: Endocrinology *29* (1941) 187.
Fiske V. M. & Greep R. O.: Endocrinology *64* (1959) 175.
Fiske V. M., Bryant G. K. & Putnam J.: Endocrinology *66* (1960) 489.
Frey E.: Bull. Schweiz. Akad. med. Wiss. *7* (1951) 115.
Gellert R. J. & Ganong W. F.: Acta endocr. (Kbh.) *33* (1960) 569.
Gompertz D.: J. Endocr. *17* (1958) 107.
Harris G. W.: Neural Control of the Pituitary Gland. London (1955).
Harris G. W. & Jacobsohn D.: Proc. roy. Soc. B. *139* (1952) 263.
Hill M. & Parkes A. S.: Proc. roy. Soc. B. *113* (1933) 537.
Holmgren U., Altschule M. D. & Wurtman R. J.: Nature (Lond.) *186* (1960) 393.
Jefferson J. M.: J. Anat. (Lond.) *75* (1940) 106.
Kitay J. I.: Endocrinology *54* (1954 *a*) 114.
Kitay J. I.: Thesis, Harvard Medical School, Boston, Mass. (1954 *b*).
Kitay J. I. & Altschule M. D.: The Pineal Gland, Cambridge, Mass. (1954).
Korenchevsky V. & Dennison M.: J. Path. Bact. *41* (1936) 323.
Lerner A. B. & Case J. O.: Invest. Derm. *32* (1959) 211.
Squire P. G. & Li C. H.: Science *127* (1958) 32.
Steelman S. L., Segaloff A. & Anderson R. N.: Proc. Soc. exp. Biol. (N. Y.) *101* (1959) 452.
Wurtman R. J., Altschule M. D. & Holmgren U.: Amer. J. Physiol. *197* (1959) 108.
Wurtman R. J., Roth W., Altschule M. D. & Wurtman J. J.: Fed. Proc. *19* (1960) 53.