EVIDENCE FOR A NONRETINAL PATHWAY OF LIGHT TO THE PINEAL GLAND OF NEWBORN RATS

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The pineal gland of rats is markedly affected by exposure of the animals to varying periods of light and darkness. After maintenance of adult rats in continuous lighting, there is a significant decrease in pineal gland weight and in the enzymatic capacity of the pineal gland to synthesize melatonin, and an increase in the activity of the serotonin-synthesizing enzyme, 5-hydroxytryptophan decarboxylase, in the pineal gland. These effects of light exposure are abolished by bilateral orbital enucleation.

Circadian, or 24-hr rhythms, have been demonstrated in the content of serotonin in the rat pineal gland and in the activity of the melatonin-forming enzyme. The pineal content of serotonin is maximal at about 1 P.M. (lights on from 5 A.M. to 7 P.M.), and declines rapidly after the lights are turned off to reach a trough at about 11 P.M. We found that this rhythm appears to be endogenous, since it persists in blinded animals and in rats kept in continuous darkness for up to 2 weeks. However, the nocturnal decline in pineal serotonin content can be prevented if illumination is extended an additional 4 hr to 11 P.M. Additional light exposure does not prevent the nocturnal decline in pineal serotonin in adult rats subjected to bilateral orbital enucleation, indicating that this effect required intact retinae.

Intact retinae appear to be required for lighting information to influence most endocrine and circadian systems in mammals. However, Ganong et al. have found that measurable amounts of light can penetrate the skull to the brains of mammals, without the intervention of the eyes. Other workers have obtained evidence suggesting that light can directly affect hypothalamic neurons in the duck and rat.

The present work was undertaken to study the development of the circadian rhythm in pineal gland serotonin content in newborn rats and its control by environmental lighting. Experiments were designed to examine the possibility that lighting information might influence the pineal gland by an extraretinal route in newborn rats.

Methods.—Sprague-Dawley rats, both male and female, of varying ages were maintained with their mothers under diurnal lighting conditions in clear plastic cages at a constant temperature of 25°C for at least 3 days prior to experimental treatment. An overhead fluorescent lamp provided about 110-150 ft-c of illumination at the level of the cages. Unless otherwise noted, lights were kept on from 5 A.M. to 7 P.M. daily. Rats were killed by anesthesia with chloroform at 1 P.M. and 11 P.M. Pineal glands were removed immediately, placed on paper towels impregnated with cold, isotonic saline, and weighed on a 25-mg Roller Smith balance.

Two pineal glands were used for serotonin assay. After weighing, the pineal glands were homogenized in 0.5 ml of ice-cold 0.01 N hydrochloric acid with a conical ground-glass homogenizer and frozen. Serotonin assays were performed on the following day by the method of Snyder et al.
FIG. 1.—Twenty-four-hr serotonin rhythm in pineal glands of 6-day-old and 12-day-old rats and the effect of additional lighting. Each group contained 16 rats. Vertical bars show the magnitude of the standard error of the mean.

FIG. 2.—Persistence of a 24-hr rhythm in pineal serotonin in blinded rats after additional lighting. Each group contained 12–16 rats. Vertical bars show the magnitude of the standard error of the mean.

Complete bilateral orbital enucleation was carried out under light ether anesthesia.

Results.—Presence of the serotonin rhythm in pineal of newborn rats: Groups of 6-day-old and 12-day-old rats kept under diurnal lighting conditions were killed at 1 P.M. (8 hr of light) or 11 P.M. (4 hr of darkness). Some groups were transferred at 7 P.M. on the day of killing to a room in which illumination was extended an additional 4 hr to 11 P.M., at which time they were killed. Pineal glands of rats in all groups were examined for serotonin content (Fig. 1). The serotonin concentration in pineal glands of 6-day-old rats at 1 P.M. was about half that of 12-day-old rats, confirming earlier results. Negligible amounts of serotonin were detected in ten pooled pineal glands from 1- and 3-day-old rats. In both 6- and 12-day-old rats, pineal serotonin levels were 2–3 times higher at 1 P.M. than at 11 P.M. in darkness. Exposure to 4 additional hr of lighting prevented the nocturnal decline of pineal serotonin content in both of these groups. The extent of the day-night changes in pineal serotonin and the effect of additional lighting were essentially the same as observed earlier in adult rats. These effects of lighting were found despite the fact that the eyelids of all 6- and 12-day-old rats in this study were firmly shut.

Effect of blinding on the serotonin rhythm and on the response to additional lighting in pineals of 12-day-old rats: The presence of the pineal serotonin rhythm and the response to additional lighting in newborn rats whose eyelids have not yet opened can be explained in several ways: light may penetrate the closed eyelids; lighting information might be communicated to the newborn rats by the mother. Alternatively, lighting information could reach the newborn rat directly, affecting the
pineal gland by a pathway that does not require the retinas. To investigate these possibilities, groups of rats were subjected to bilateral orbital enucleation when 11 days old and were killed at 12 days of age at 1 P.M. and 11 P.M. along with normal controls. Some normal and blinded rats were exposed to 4 additional hr of light (7-11 P.M.) on the day they were killed. It was found earlier\textsuperscript{11} that additional lighting was unable to prevent the nocturnal decline in pineal serotonin content in blinded adult rats. To examine the possibility that the influence of additional lighting in 12-day-old rats might be mediated by the mother, the mother of one group of blinded rats exposed to additional lighting was herself blinded by bilateral orbital enucleation on the day before killing. The circadian rhythm in pineal serotonin of blinded rats did not differ from that of the normal controls (Fig. 2). When the blinded animals were exposed to 4 additional hr of lighting, their pineal serotonin content at 11 P.M. was significantly (\(p < 0.001\)) higher than that of blinded rats in darkness at 11 P.M. Their pineal serotonin concentration was lower (\(p < 0.001\)) than that of normal controls exposed to 4 additional hr of lighting. Serotonin levels for the blinded group with a blinded mother were the same as for the corresponding group with a normal mother.

Thus, unlike earlier findings in adult blinded rats,\textsuperscript{11} additional lighting clearly had an effect in partially preventing the nocturnal decline in pineal serotonin in 12-day-old blinded rats. In addition, these results indicate that this effect is not mediated by the mother. Since additional lighting completely abolished the nocturnal decline in pineal serotonin in newborn rats with intact eyes but closed eyelids and blinding partially eliminated this effect, some lighting information must enter the eyes through the closed eyelids to affect the pineal gland. However, the partial abolition of the nocturnal decline in pineal serotonin in blinded 12-day-old rats by additional lighting would indicate that some lighting information reaches the pineal gland by an extraretinal pathway.

Effect of blinding and hooding on the pineal serotonin rhythm and the response to additional lighting in 12-day-old rats: In order to ascertain whether the presumed extraretinal pathway of light to the pineal gland in 12-day-old rats involved the head, the following experiment was designed. Groups of 11-day-old rats were blinded and removed from their mothers on the 12th day of age. Rats were killed at 1 P.M. or 11 P.M. or transferred on the day of killing to a room in which the lights were on until 11 P.M., providing 4 additional hr of lighting. Rats exposed to additional lighting were subdivided into hooded and nonhooded groups. Hooded rats were clothed in a black corduroy hood which covered the head and extended down to the inferior border of the rib cage with holes for the forelimbs. Animals were able to breathe and move about freely while wearing the hoods. The hoods did not permit the entry of any detectable light under the experimental conditions. Animals were hooded at 5 P.M. on the day they were killed.

As previously observed, 4 additional hr of lighting partially eliminated the nocturnal decline in pineal serotonin of the 12-day-old blinded rats (Fig. 3). Pineal serotonin values at 11 P.M. of hooded rats exposed to additional lighting did not differ from those of blinded rats at 11 P.M. in darkness, indicating that hooding completely abolished the effect of additional lighting. These results show that lighting information can reach the pineal gland by a pathway that involves the head but not the eyes.
Fig. 3.—Effect of hooding on the pineal gland response to additional lighting in 12-day-old blinded rats. Each group contained 16 rats. Vertical bars show the standard error of the mean.

Fig. 4.—Effect of blinding on the pineal gland response to additional lighting in 27-day-old rats. Groups contained 16 rats. Vertical bars indicate the standard error of the mean.

**Pineal serotonin rhythm in 27-day-old blinded rats:** Groups of 24-day-old rats whose eyelids were completely open were blinded by bilateral orbital enucleation. When they were 27 days old, animals were killed at 1 P.M. and 11 P.M. along with normal controls and their pineal glands examined for serotonin (Fig. 4). On the day they were killed, some groups were transferred to a room in which the lights were kept on until 11 P.M. when they were killed. In the control animals, 4 additional hr of light completely prevented the nocturnal decline in pineal serotonin. Unlike findings in 12-day-old rats, additional lighting had no effect in blinded 27-day-old rats. These results indicate that in 27-day-old rats, the nonretinal pathway of light to the pineal gland is no longer operative.

**Discussion.**—Results of the present study indicate that the 24-hr rhythm in pineal serotonin content is present at 6 days of age, the earliest time at which serotonin is detectable in the rat pineal gland. Since blinding did not completely abolish the influence of lighting, it is not clear whether or not the pineal serotonin rhythm in newborn rats is "endogenous," i.e., persists in the absence of environmental cues. In the 27-day-old rats as well as the adult rat the serotonin rhythm has been found to be endogenous, since a nocturnal fall in this amine occurs in blinded animals at 11 P.M. It would appear that as early as the 6th day of life, lighting has a synchronizing influence on the pineal serotonin rhythm.

The complete abolition and the partial loss, after blinding, of the response of pineal serotonin to additional lighting suggests that some light penetrates the closed eyelids of these animals and is transmitted to the pineal gland.

The most striking finding in this study is the demonstration that lighting information can be conveyed to the pineal gland of the 12-day-old rat by a nonretinal route involving the head. By 27 days of age, this nonretinal pathway does not appear to be functional. The possibility of nonretinal photoreception in mammals has been observed by other workers. Benoit, in numerous experiments, has shown that the photosexual reflex in immature male ducks can take place after bilateral
orbital enucleation. The response in blinded ducks can be accentuated by placing a quartz rod deep into the orbit at an area overlying the hypothalamus. As a result of these experiments, Benoit has concluded that light can activate the photossexual reflex in ducks by a direct action on hypothalamic photoreceptors.

Lisk and Kannwischer have obtained evidence for a direct effect of light on hypothalamic neurons of the adult rat. In their experiments, continuous light, impinging by means of a stereotaxically implanted glass rod, on the suprachiasmatic region of enucleated rats resulted in a constant estrouslike vaginal cycle. If the glass rods were covered by black masking tape, the effect of continuous light on the estrous cycle was obliterated.

The location of the presumed extraretinal photoreceptor described here is not clear. In amphibians, the pineal complex itself has been shown to contain photoreceptive elements, and to transduce lighting information into nerve impulses. In the adult rat, however, histological studies have failed to reveal the presence of photoreceptive cells in the pineal gland. It is possible, however, that in the very young rat there is a photoreceptive function for the pineal gland. It should be noted that the pineal gland of the rat has a superficial location, just beneath the skull at the junction of the sagittal and occipital sutures.

Another possibility is that photoreception might take place via hypothalamic receptors as suggested by the work of Benoit and of Lisk and Kannwischer. We have obtained evidence that the pathway by which lighting changes influence the pineal gland in the adult rat involves the medial forebrain bundle as well as the eyes. The reduction of melatonin-synthesizing capacity in the pineal gland by constant light exposure can be obliterated by lesions in the medial forebrain bundle. The circadian changes in pineal serotonin concentration are also abolished by these lesions, suggesting that the central mechanisms controlling the pineal serotonin rhythm and central components of the pathway of light to the pineal gland may have the same or similar anatomic representation in the brain.

The present demonstration of extraretinal photoreception in very young rats would suggest that lighting information may be utilized in controlling physiological processes in mammals some time before visual function can be demonstrated.

The pineal gland is an end-organ of the sympathetic nervous system and has been implicated in gonadal regulation. The ability of lighting information to influence the pineal gland prior to the development of visual function would suggest that photic control of autonomic and endocrine events in mammals may be separate from and developmentally precede retinal photoreception.

Summary.—A 24-hr rhythm has been found in serotonin content of the pineal gland of newborn rats. Four additional hr of lighting can prevent the nocturnal decline in pineal serotonin in 12-day-old rats whose eyelids are closed. There is a partial response to additional lighting in 12-day-old blinded rats. Hooding of 12-day-old rats completely abolishes the response to additional lighting. Additional lighting does not prevent the nocturnal decline in pineal serotonin in 27-day-old blinded rats. These data indicate that in the very young rat, light can influence the pineal gland by an extraretinal pathway which involves the head.

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