

PINEAL N-ACETYLTRANSFERASE AND HYDROXYINDOLE-O-METHYLTRANSFERASE: CONTROL BY THE RETINOHYPOTHALAMIC TRACT AND THE SUPRACHIASMATIC NUCLEUS

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SUMMARY

The visual pathway and central neural structures involved in the photic and endogenous regulation of the activity of pineal N-acetyltransferase and hydroxyindole-O-methyltransferase were investigated. The results indicate that the visual pathway regulating both enzymes is the retinohypothalamic tract, and that the inferior accessory optic tract is clearly not involved in the regulation of hydroxyindole-O-methyltransferase activity, as has been previously thought. In addition, the supra-chiasmatic nucleus was found to be necessary for the generation of a rhythm in N-acetyltransferase activity in blinded animals, and to be responsible for the tonic elevation of hydroxyindole-O-methyltransferase activity in blinded animals. Finally, it was concluded that the rapid and large daily changes in N-acetyltransferase activity seen in a normal lighting cycle and the much slower and smaller changes in hydroxyindole-O-methyltransferase activity seen only after weeks in constant lighting conditions are mediated by the same neural tract; the different time courses of the effects of environmental lighting may be explained on the basis of different intracellular regulatory mechanisms.

INTRODUCTION

Neural signals mediate the photic control of the two pineal enzymes which convert serotonin to melatonin^{12,21}, N-acetyltransferase³⁶ (EC 2.3.1.5) and hydroxyindole-O-methyltransferase¹ (EC 2.1.1.4). The final portion of the neural pathway regulating these enzymes in the rat, and probably in mammals in general^{2,12,18,27,29}, is composed of axons from postganglionic cells in the superior cervical ganglia. These ganglia are innervated by fibers from cell bodies in the upper thoracic intermedio-

lateral cell column; these in turn are innervated by central structures^{12,21}. In contrast to the clear picture which has developed regarding the peripheral neural regulation of these enzymes, a puzzling situation exists regarding the identity of the visual pathways, nuclei, and other central structures which regulate the activity of these pineal enzymes.

On one hand, the retinohypothalamic tract has been thought to transmit photic signals which control pineal N-acetyltransferase activity^{12,21,25}. This visual pathway terminates in the suprachiasmatic nucleus^{18,26}, which appears to be essential to maintain the 20- to 70-fold circadian rhythm in N-acetyltransferase activity²⁵, and might in fact be the central oscillator driving this rhythm, and influencing others^{22,33}.

The effects of light on the activity of N-acetyltransferase are complex. First, light entrains the circadian clock mechanism to the environmental lighting cycle^{15,25}. Second, light acts by an unidentified pathway to rapidly block transmission of neural signals to the pineal gland¹⁶. As a result, when N-acetyltransferase activity is elevated during the night, an exposure to light results in an extremely rapid 'turn-off' of enzyme activity.

On the other hand it has been generally assumed^{23,24}, until recently²¹, that the inferior accessory optic tract, which terminates in the medial terminal nucleus of the accessory optic system, transmits photic signals which control hydroxyindole-O-methyltransferase activity. Whereas a daily rhythm in this enzyme is not readily apparent^{20,30,31}, light has a distinct tonic effect on this enzyme. After animals have been in a normal lighting cycle, exposure to constant light causes a gradual decrease in activity occurring over a period of weeks^{2,12,39}.

The puzzling aspect of these observations is obvious when they are considered together. It is difficult to conceive of a simple scheme through which two sets of visual pathways and central structures could act *via* a single peripheral innervation to control remarkably different dynamic changes in two enzymes probably located in the same cell. In addition, this issue has recently become more complex. First, attempts to replicate the original effect of inferior accessory optic tract transection on the photic control of hydroxyindole-O-methyltransferase were unsuccessful²¹. Second, a re-examination of the anatomy of the central retinal projections using the autoradiographic tracing method demonstrated that the inferior accessory optic tracts, rather than being completely crossed as previously described²³, also contained an uncrossed component²¹. This finding invalidated a basic assumption used in our conclusion that the retinohypothalamic tract, not the inferior accessory optic tract, regulated N-acetyltransferase activity²⁵ because the visual pathway lesions we used to study the neural control of pineal N-acetyltransferase activity left this crossed component of the inferior accessory optic tract intact. Thus, our assumption that the only visual projection surviving following the lesion was the retinohypothalamic tract is incorrect.

In the present report the question of the central neural structures and visual pathway regulating pineal N-acetyltransferase and hydroxyindole-O-methyltransferase activities is re-examined using blinded and sighted rats kept in constant light. Our results indicate that one series of central and peripheral neural structures and a single visual pathway, the retinohypothalamic projection, are responsible for the neural regulation of both enzymes.

MATERIALS AND METHODS

Protocols

Experiment I. The effects of blinding, suprachiasmatic nucleus ablation, retrochiasmatic hypothalamic deafferentation on pineal enzyme responses to constant light (Figs. 2-6; 10, 11)

Female albino rats (Sprague-Dawley, Zivic Miller Co.) were obtained at 180-200 g body weight during the early spring. They then were subjected to a sham operation, bilateral suprachiasmatic nucleus ablation or retrochiasmatic hypothalamic deafferentation as described below. Following surgical treatment the animals were housed either in a room providing constant environmental lighting or one with a light/dark 14/10 h lighting cycle; lights were turned on at 06.00 h. Animals were decapitated 4 weeks later at 05.00, 11.00, 17.00 and 23.00 h. When animals were killed during the dark period all procedures were performed under dim red light (15 W ruby lamp, Sylvania). Pineal glands were removed, placed in tubes on solid CO₂, and stored (-72 °C). Brains were removed and fixed in 10% formalin for histological preparation and examination.

Experiment II. The effects of postchiasmatic optic tract transection on pineal enzyme responses to constant light (Figs. 7-9)

Female albino rats (Hilltop Farms, Scottsdale, Pa.) were obtained at 180-200 g body weight during the late spring. After surgical treatment (sham operation or optic tract transection) the animals were housed in a room providing constant lighting. Animals were decapitated 4 weeks after operation at 08.00, 13.00, 19.00 and 01.00 h. Pineal glands and brains were obtained as described above.

Experiment III. The effects of a postchiasmatic optic tract transection on pineal N-acetyltransferase responses to diurnal light and the acute response to light at night (Table I)

Female albino rats similar to those used in Experiment II were used. After surgical treatment (sham operation or postchiasmatic optic tract transection) the animals were housed in a room with a light/dark 12/12 h lighting cycle; lights were on from 06.00 to 18.00 h. After 4 weeks in this lighting cycle each operated groups was divided into three subgroups. One was sacrificed at 09.00 h with the lights on, one at 24.00 h in darkness, and one at 24.00 h after a 30 min exposure to light. Pineal glands and brains were prepared as described above.

Surgical and histological procedures

The details of surgical preparation, of the procedures for sham operation and blinding and the methods used for lesion localization and histological examinations have been given²⁵.

Postchiasmatic optic tract transection (Fig. 1C)

Large bilateral radiofrequency lesions were placed in the optic tract caudal to the optic chiasm using a Kopf radiofrequency lesion maker and insect pins insulated except at the tip. The coordinates for these lesions were incisor bar, 0; lateral, 1.2 mm from bregma and ventral 9.5 mm below the skull. A radiofrequency current of 10 V was passed for 45 sec. This lesion totally transects both the primary and accessory optic tracts as they leave the optic chiasm and in this regard significantly differs from the operative procedure used in earlier studies^{23,25}. In each case a lesion was judged successful one week later if the rat had fixed, dilated pupils in bright light at that time.

Retrochiasmatic hypothalamic deafferentation (Fig. 1D)

This was made by placing animals in the stereotaxic apparatus with tooth bar at horizontal zero, the sagittal sinus was exposed and incised and a modified Halasz knife (vertical and horizontal components 2.4 mm) was stereotaxically lowered through the midline at a level 1 mm caudal to the bregma until it touched the skull base. It was then rotated approximately 90° in each direction and removed vertically through the midline. These lesions are similar to the postchiasmatic hypothalamic knife cuts described in a previous study²⁵

Enzyme analysis

Pineal glands were prepared for enzyme analysis by sonicating each gland in 75 µl of 0.1 M sodium phosphate buffer, pH 6.8; N-acetyltransferase activity and hydroxyindole-O-methyltransferase activity were measured in 25 µl samples of this homogenate as described^{3,28}

Data presentation

All data are based on 5–6 determinations, except where indicated. The vertical bars represent the standard error in groups of 3 or more animals or the range of values in groups of 2 animals.

Statistical analysis was performed using a two-tailed 't'-test for differences.

The results of enzyme assays from Experiment I are presented in Figs. 2, 6 and 11. The data for sighted or blinded animals with no central neural lesion and maintained in constant light are presented first in Fig. 2 and again in Figs. 6 and 11 to facilitate comparison of lesion data with the control groups.

As indicated above, only a single group of animals for any one treatment was obtained at each of the 4 chosen times. However, in all figures the data for the 11.00 or 12.00 h groups are presented at both the beginning and end of the 24-h sampling period.

RESULTS*Blinding*

The normal rhythm in pineal N-acetyltransferase and the activity of hydroxyindole-O-methyltransferase during a 24-h period in a light/dark 14/10 h lighting cycle

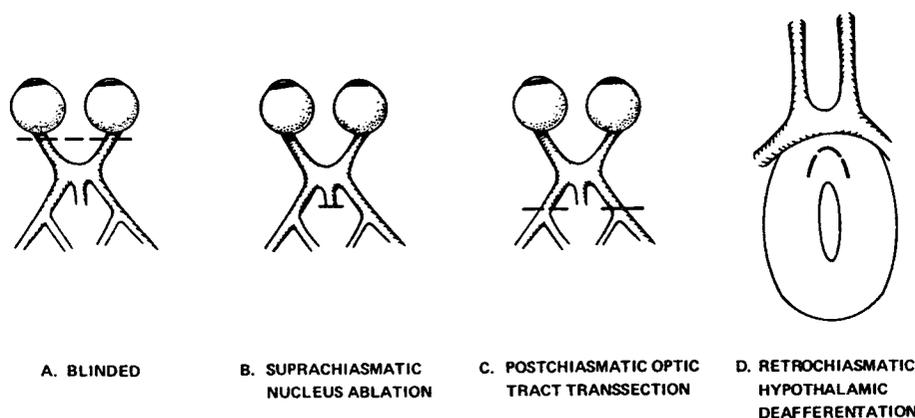


Fig. 1. Diagrammatic representation of the lesions used in this study. A: blinding by bilateral orbital enucleation. B: suprachiasmatic nucleus ablation. C: postchiasmatic optic tract transection. D: retrochiasmatic hypothalamic deafferentation.

is presented in Fig. 2B. Exposure to constant light for 30 days resulted in a reduction of hydroxyindole-O-methyltransferase and the disappearance of the rhythm in N-acetyltransferase activity (Fig. 2A). Destruction of all three known visual pathways, by blinding (Fig. 1A), prevented the suppressive effects of light on hydroxyindole-O-methyltransferase activity and allowed the rhythm in N-acetyltransferase activity to persist, in agreement with earlier reports^{2,12,14,15,21,24,25,39}

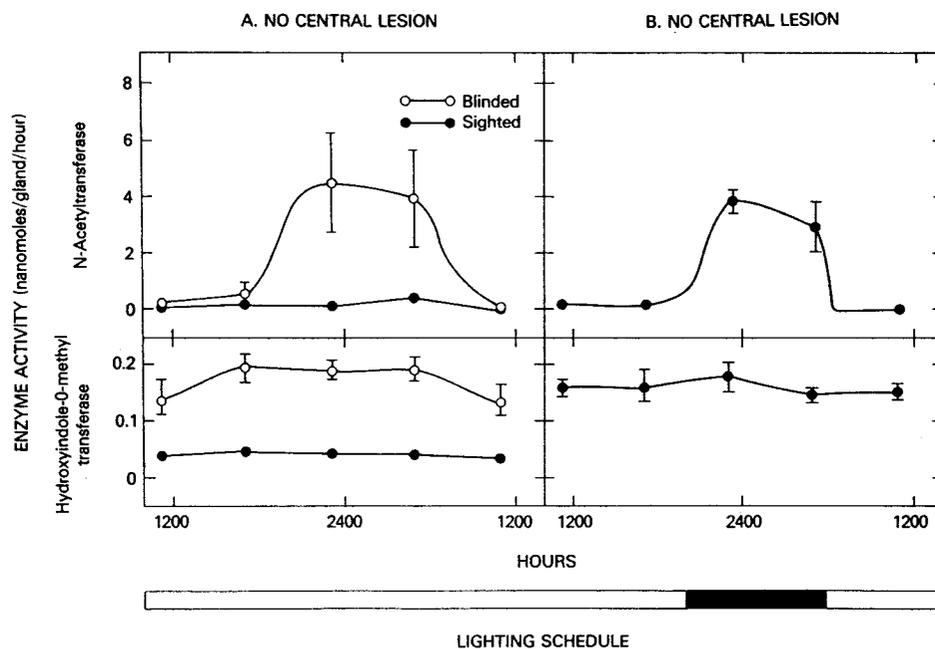


Fig. 2. Pineal N-acetyltransferase and hydroxyindole-O-methyltransferase activities in sighted and blinded animals bearing no central lesions. Data is presented as the mean (\pm S.E.) of 4-6 determinations. The lighting cycles are indicated below the figure. A: sighted and blinded animals with no central lesion. B: sighted animals in a normal lighting cycle.

Suprachiasmatic nucleus ablation

We determined whether lesions of the suprachiasmatic nucleus alter the activity of hydroxyindole-O-methyltransferase (Fig. 1B). The 43 animals prepared fell into two distinct groups according to histological analysis of the lesions. One was composed of 13 animals bearing incomplete lesions of the suprachiasmatic nuclei. Even though some of these lesions also destroyed descending projections from the suprachiasmatic nuclei³⁵, apparently sufficient numbers of neurons survived to allow for the elevation of N-acetyltransferase activity to occur. All data from these animals with incomplete lesions as judged histologically was eliminated. The remaining animals had complete bilateral lesions of the suprachiasmatic nuclei. In some the lesions were small and almost exclusively destroyed the suprachiasmatic nuclei, with only minimal and varying extension into the anterior hypothalamus area, the periventricular nucleus, the optic chiasm and the retrochiasmatic area. In other animals the lesions were significantly larger (Fig. 3) and, in addition to ablating the suprachiasmatic nuclei, also transected the optic chiasm, extended into the anterior hypothalamic area and nucleus and into the retrochiasmatic area. No significant difference between the enzyme values from animals with large and small suprachiasmatic nuclei lesions could be detected; the data from all animals with complete suprachiasmatic nucleus ablation were pooled. A diagrammatic representation of a coronal section through the anterior

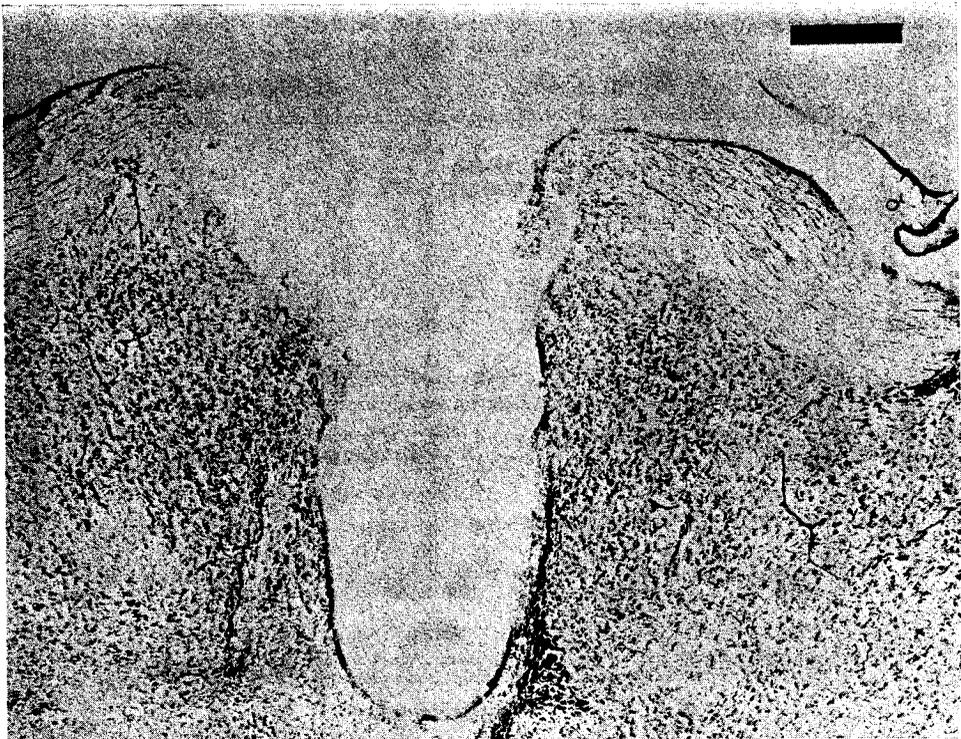


Fig. 3. Photomicrograph of a suprachiasmatic nucleus lesion. The lesion is large, extending into the anterior hypothalamic area and nucleus. The optic chiasm is transected. Marker bar = 0.5 mm.

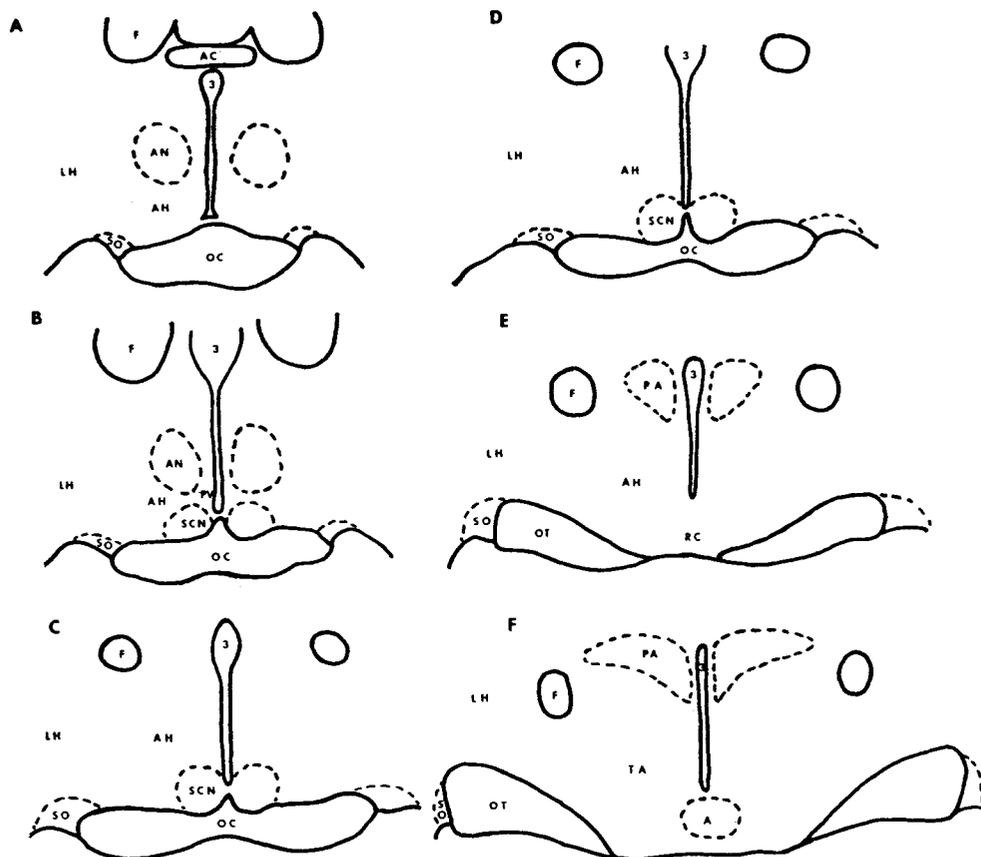


Fig. 4. Diagram of the suprachiasmatic region of the hypothalamus showing structures involved in lesion of the suprachiasmatic nucleus and optic tract (Figs. 3, 6, 7). Abbreviations: A, arcuate nucleus; AC, anterior commissure; AH, anterior hypothalamic area; AN, anterior hypothalamic nucleus; F, fornix; LH, lateral hypothalamic area; OC, optic chiasm; OT, optic tract; PA, paraventricular nucleus; PV, periventricular nucleus; SCN, suprachiasmatic nucleus; SO, supraoptic nucleus; TA, tuberal area; 3, third ventricle.

hypothalamus is shown in Fig. 4. Representative large and small suprachiasmatic lesions are shown diagrammatically in Fig. 5.

Destruction of the suprachiasmatic nuclei abolished the rhythm in pineal N-acetyltransferase activity in blinded animals²⁵ and resulted in low levels of hydroxyindole-O-methyltransferase activity (Fig. 6B), significantly lower than that in blinded animals without the lesion. A significant difference was not detectable between the group of sighted and the group of blinded animals, both with bilateral suprachiasmatic nucleus ablation.

Postchiasmatic optic tract transection

As observed above, when all visual pathways are destroyed by blinding, the rhythm in pineal N-acetyltransferase persists and the suppressive effects of light on hydroxyindole-O-methyltransferase activity are blocked. To determine if the effect of

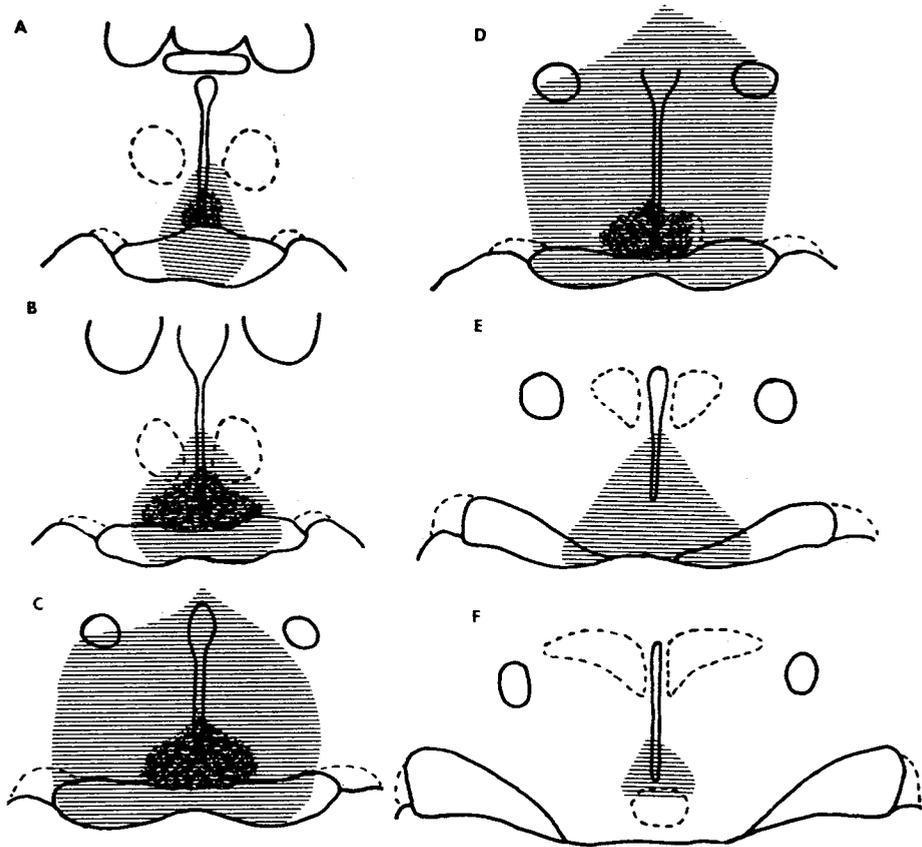


Fig. 5. Diagram showing the locus and extent of typical small (stipple) and large (horizontal lines) suprachiasmatic nucleus lesions. Structures as in Fig. 4.

light is mediated only by the retinohypothalamic tract, lesions were made which spare this tract but destroy all components of the accessory and primary optic tracts (Fig. 1C). Histological inspection indicated that the optic tracts leaving the chiasm were destroyed (Fig. 7) by the large bilateral lesions used, which were placed lateral to the suprachiasmatic nuclei. The lesions extend into adjacent anterior hypothalamic area and, to a varying extent, into the lateral hypothalamic area. Diagrams indicating the extent of the lesions in this group are shown in Fig. 8.

N-Acetyltransferase and hydroxyindole-O-methyltransferase activities in these animals were identical to sighted animals in constant light indicating that the retinohypothalamic pathway could mediate the chronic suppressive effects of light on both pineal enzymes (Fig. 9), and that neither the primary nor accessory optic tracts are required for these photic effects.

To determine if the acute and rapid effects of light on pineal N-acetyltransferase activity were also mediated by the retinohypothalamic tract, and if the rhythm in N-acetyltransferase activity persisted in animals with this lesion, a second group of animals was prepared in which the only visual pathway left intact was the retino-

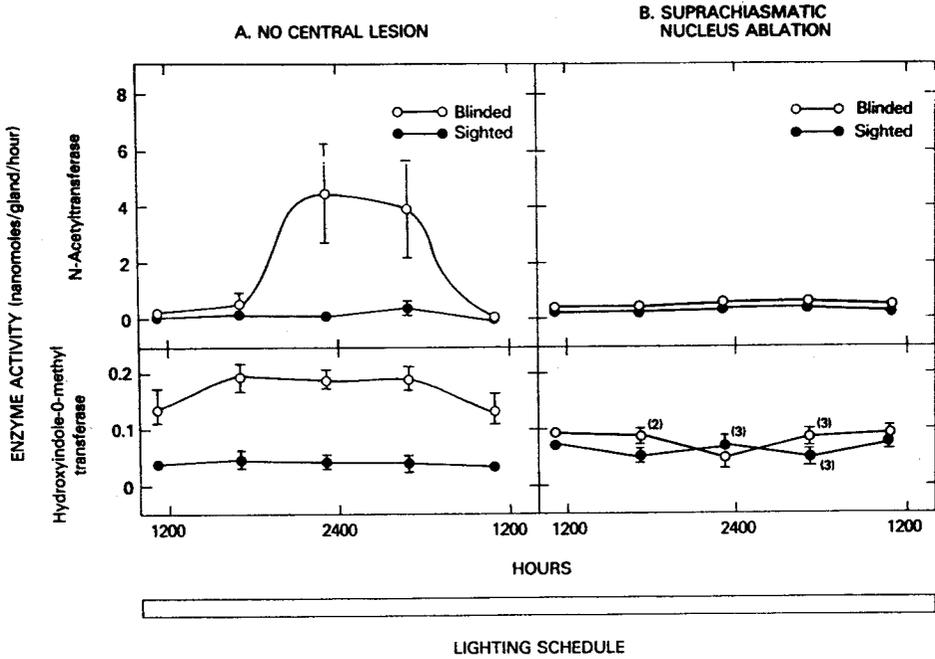


Fig. 6. Pineal N-acetyltransferase and hydroxyindole-O-methyltransferase activities in sighted and blinded animals with a lesion of the suprachiasmatic nucleus and retinohypothalamic projection. Data is presented as the mean (\pm S.E.) of 4-6 determinations except where indicated in brackets. The vertical bars indicate the S.E. in all cases except where there were only two animals in a group; in that case the vertical bar represents the range of values. The lighting cycle is indicated below the figure. A: sighted and blinded animals with no central lesion (from Fig. 2A). B: sighted and blinded animals with suprachiasmatic nuclei ablation.

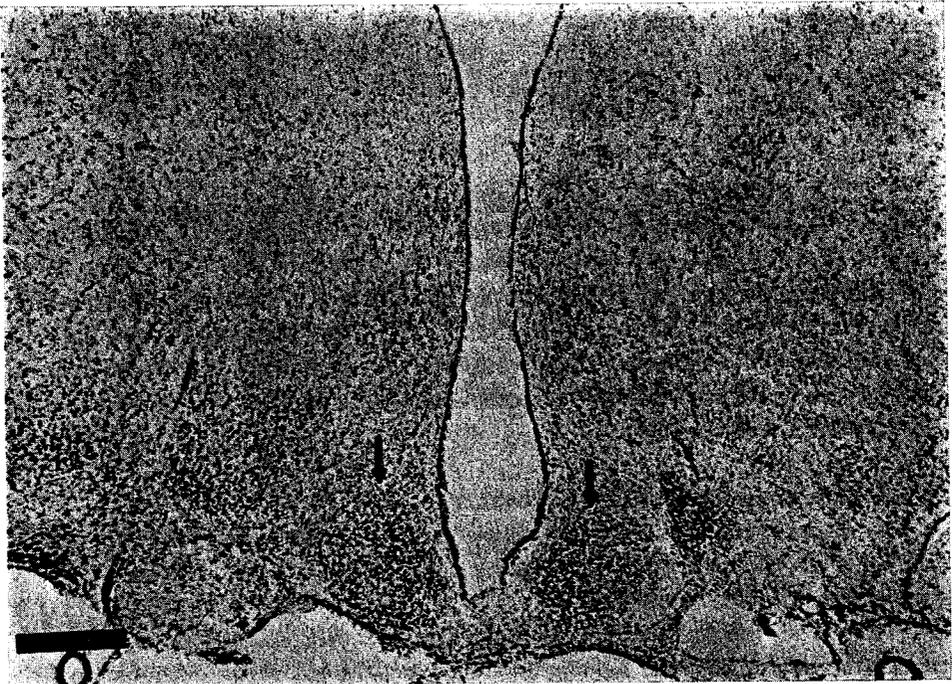


Fig. 7. Photomicrograph of a representative postchiasmatic optic tract transection. The lesion completely transects the optic tracts immediately lateral to the suprachiasmatic nuclei (arrows). Marker bar = 0.5 mm.

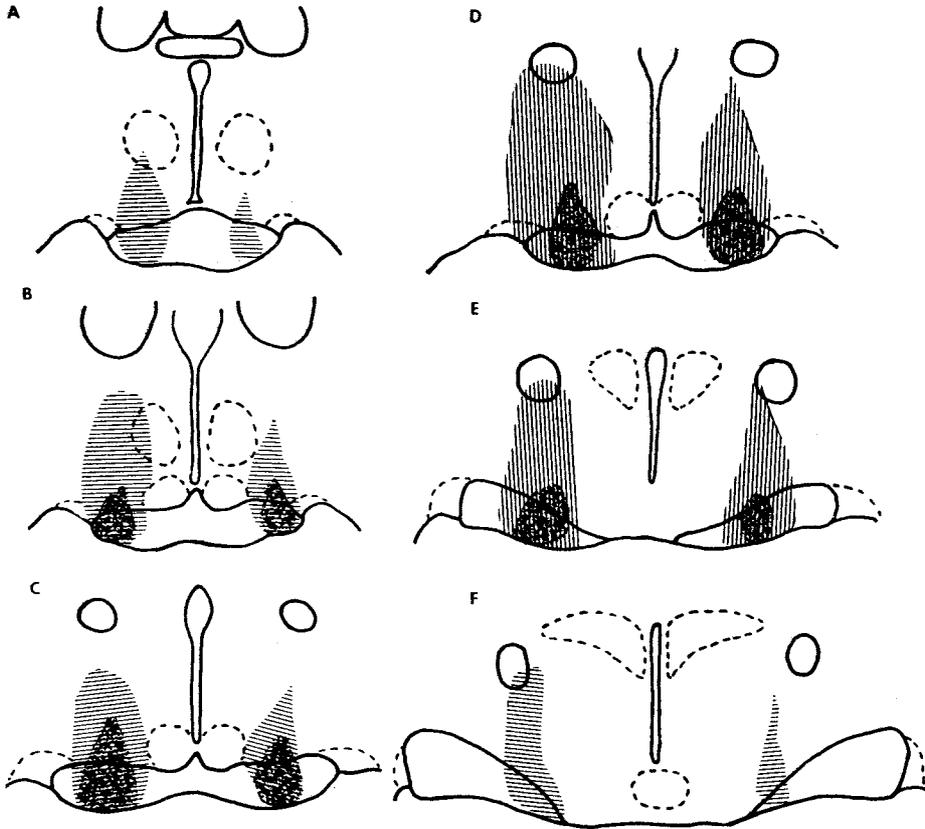


Fig. 8. Diagrams showing the extent of a typical small (stipple) and large (horizontal lines) postchiasmatic optic tract transection. Structures as in Fig. 4.

hypothalamic tract (Table I). These animals were maintained in a light/dark 12/12 h lighting cycle. N-Acetyltransferase activity at 24.00 h was 14.29 ± 1.82 nmol/gland/h; a 30-min light exposure decreased enzyme activity 95% to a value equivalent to that observed during the light period (09.00 h) in both sham-operated and optic tract transection animals (Table I). This indicates that the retinohypothalamic tract mediates the rapid effects of light on N-acetyltransferase activity, and that the lesion used does not destroy the suprachiasmatic nucleus or other structures required to generate a rhythm in N-acetyltransferase activity.

Retrochiasmatic hypothalamic deafferentation

To determine if retrochiasmatic projections from the suprachiasmatic nucleus are involved in the regulation of pineal hydroxyindole-O-methyltransferase activity, animals were prepared bearing a knife cut through this area of the hypothalamus (Fig. 1D). The tip of the knife passed through the retrochiasmatic hypothalamic area between the chiasm and the median eminence, cutting known projections from the suprachiasmatic nucleus³⁵. It appeared as a thin necrotic zone about 2 mm long (Fig. 10). This was a very reproducible lesion and was successful in all but one animal.

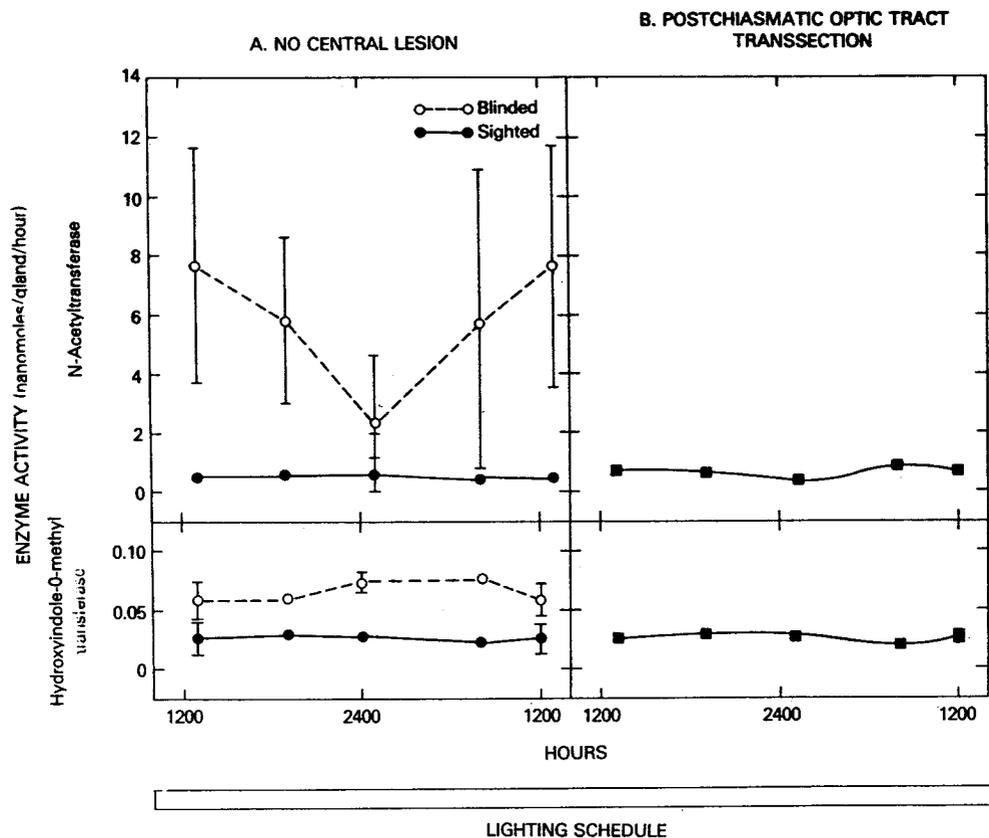


Fig. 9. Pineal N-acetyltransferase and hydroxyindole-O-methyltransferase activities in sighted and blinded animals with a postchiasmatic optic tract transection. Data is presented as the mean (\pm S.E.) of 4–6 determinations. The lighting cycle is indicated below the figure. A: sighted and blinded animals with no central lesion. B: animals with a postchiasmatic optic tract transection.

TABLE I

Postchiasmatic optic tract transection: effects of the acute response of N-acetyltransferase activity to light and on the N-acetyltransferase system

Adult female albino rats, 180 g, were subjected to either sham operation or bilateral postchiasmatic optic tract transection. They were maintained in light/dark (LD) 12/12 h (lights on 06.00 to 18.00 h) for 45 days and then sacrificed under one of these conditions; at 09.00 with lights-on (L), at 24.00 with lights-off (D) and at 24.00 following a 30-min exposure to light (D \rightarrow L30). Pineals were removed and analyzed for N-acetyltransferase activity. Each value is the mean (\pm S.E.) of 7 or 8 determinations.

Operated group	N-Acetyltransferase activity (nmol product formed/gland/h)		
	Time of day and lighting condition		
	09.00 (L)	24.00 (D)	24.00 (D \rightarrow L30)
Sham-operated	0.80 \pm 0.09	13.68 \pm 1.87	0.87 \pm 0.06
Postchiasmatic optic tract transection	0.80 \pm 0.08	14.29 \pm 1.82	0.87 \pm 0.11

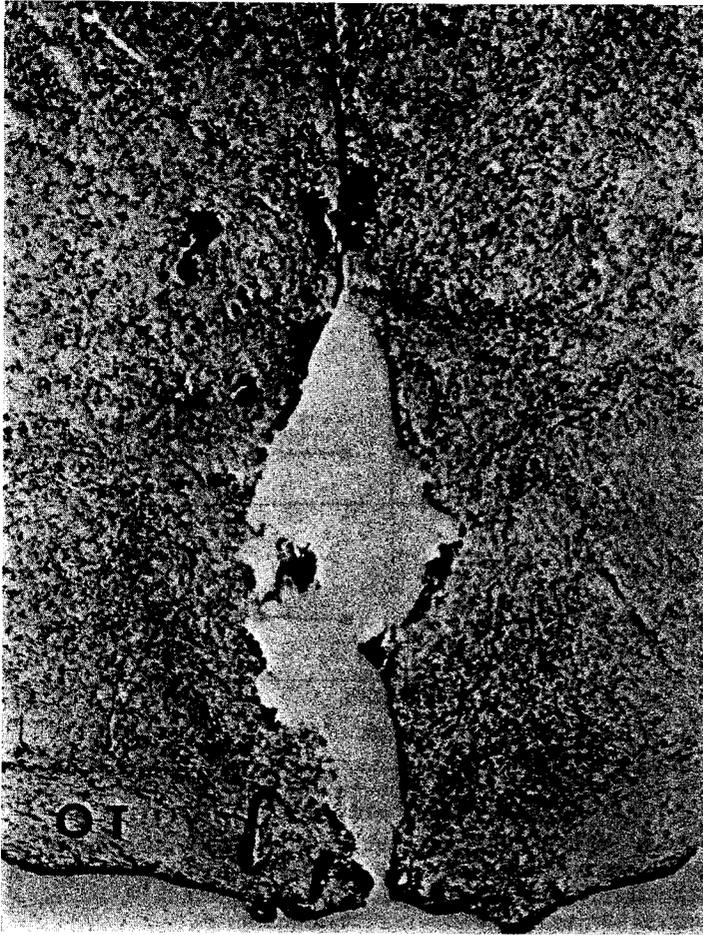


Fig. 10. Photomicrograph of the knife cut lesion produced by the retrochiasmatic hypothalamic deafferentation. The cut crosses the medial hypothalamus, extending from the fornix on one side to that on the other in the retrochiasmatic area just caudal to the point where the optic tracts (OT) separate. Marker bar = 0.5 mm.

The knife cut had an effect on pineal enzymes similar to that of the supra-chiasmatic nucleus lesion (Fig. 11). It blocked the maintenance of the elevated levels of hydroxyindole-O-methyltransferase in blinded animals and the rhythm in N-acetyltransferase in both the intact and blinded animals with this lesion; the activity of hydroxyindole-O-methyltransferase was equivalent to that of the sighted animals in constant light.

DISCUSSION

On the basis of observations presented in this report and in previous ones it appears reasonable to reach the following three conclusions.

- (1) *Photic regulation of pineal N-acetyltransferase and hydroxyindole-O-methyl*

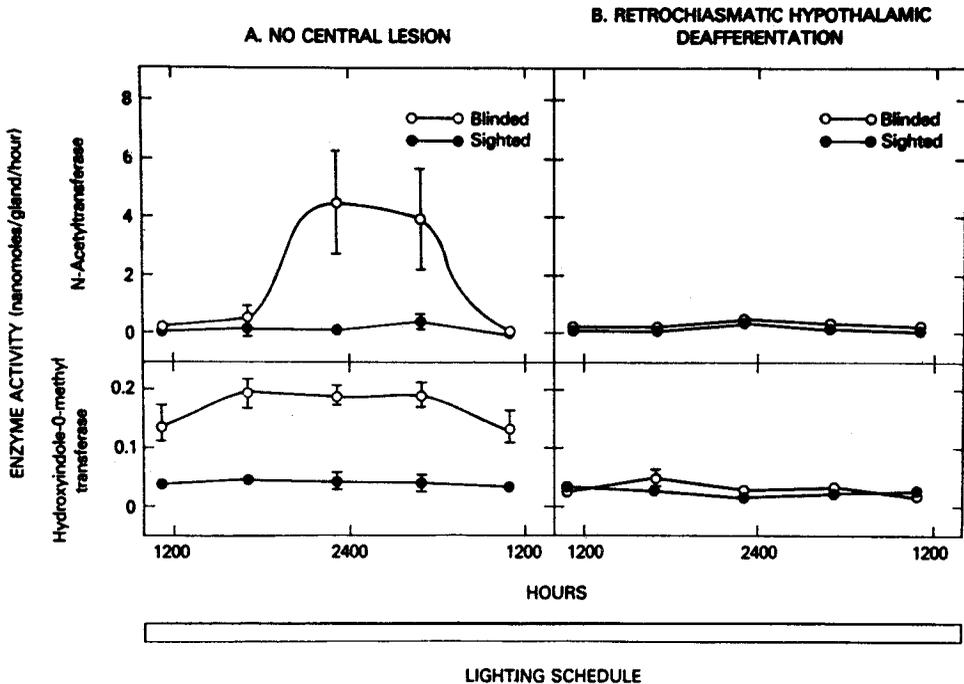


Fig. 11. Pineal N-acetyltransferase and hydroxyindole-O-methyltransferase activities in sighted and blinded animals with a retrochiasmatic hypothalamic cut. Data is presented as the mean (\pm S.E.) of 4–6 determinations. The lighting cycle is indicated below the figure. A: sighted and blinded animals with no central lesion (from Fig. 2A). B: sighted and blinded animals with a retrochiasmatic hypothalamic cut.

transferase is via the retinohypothalamic tract, not the inferior accessory optic tract. It is quite clear that the activities of the two pineal enzymes studied are regulated by photic input. In the present study pineal glands were analyzed from animals in which all components of the primary and accessory optic tracts were transected at the level of the optic chiasm and the only known surviving central retinal projection is the retinohypothalamic tract. Light clearly acts in these animals, as it does in intact animals, to suppress the activity of hydroxyindole-O-methyltransferase, to entrain the rhythm in N-acetyltransferase in animals maintained in diurnal light, and block the rhythm in constant light. In addition, exposure to light during the dark period of a diurnal cycle causes a rapid decrease in the activity of N-acetyltransferase. None of these effects of light are detectable in animals in which the retinohypothalamic tract is also destroyed, as in the case of blinded animals¹⁶. Based on these observations it seems appropriate to conclude that the visual pathway involved in the photic regulation of both pineal N-acetyltransferase and hydroxyindole-O-methyltransferase activities is the retinohypothalamic projection.

(2) *The suprachiasmatic nucleus is the endogenous source of signals which generate the circadian rhythm in pineal N-acetyltransferase activity and tonically elevate hydroxyindole-O-methyltransferase activity.* Evidence to support the idea that these signals are endogenously generated derives from the observation that the activities of N-acetyl-

transferase and hydroxyindole-O-methyltransferase in blinded animals as compared to sighted animals maintained in a light/dark 14/10 h lighting cycle are the same^{14,15,25}, except that the circadian rhythm in N-acetyltransferase activity in the blinded animals is free-running and is not entrained to the light-dark cycle as it is in the sighted animals. There are no major quantitative differences in the magnitude of the peak levels of N-acetyltransferase or the high levels of hydroxyindole-O-methyltransferase^{2,14,39}. These findings clearly indicate that the mechanisms required for generating the N-acetyltransferase rhythm and for maintaining high levels of hydroxyindole-O-methyltransferase activity are endogenous and fully functional in the absence of environmental lighting.

Evidence to support the idea that the location of this endogenous signal generator appears to be the suprachiasmatic nucleus is that lesions which destroy a number of projections to this area do not block the generation of the rhythm in N-acetyltransferase activity²⁵, whereas lesions that ablate the suprachiasmatic nucleus do abolish the rhythm²⁵. In the present study this latter finding was confirmed and it was also determined that lesions of the suprachiasmatic nucleus prevent the endogenous maintenance of hydroxyindole-O-methyltransferase activity at an elevated level. Accordingly, it appears appropriate to conclude that both enzymes are regulated by signals originating in the suprachiasmatic nuclei.

Two aspects of the function of the suprachiasmatic nucleus and its relationship with the retinohypothalamic pathway require additional comment. First, in view of both the functional evidence reviewed above and the anatomical evidence^{9,18,26}, it is highly likely that the retinohypothalamic projection alters pineal function by directly interacting with structures in the suprachiasmatic nucleus. In this regard, light can be viewed as having an entraining function and a transmission function. The effect of light in entraining the endogenous oscillator to the environmental lighting cycle is apparently slow; it takes approximately a week to entrain the N-acetyltransferase rhythm to a 12-h phase shift^{4,6}. The effect of light on signal transmission appears to be extremely rapid, and probably accounts for the "turn-off" of N-acetyltransferase by light, the blocking of a circadian rhythm in N-acetyltransferase activity by constant light, and eventually in the gradual decrease in the activity of hydroxyindole-O-methyltransferase activity in constant light^{2,9,16}. The nature of these interactions is not known. One of us (R.Y.M.) believes that they represent direct effects on the endogenous oscillating mechanism. In contrast, the other (D.C.K.) believes that acute alterations of the oscillating mechanism are not required for these effects, but that signal transmission is blocked independently.

The second aspect of this proposal that should be discussed is the question of how a single hypothalamic nucleus can regulate two pineal enzymes that exhibit diverse dynamic responses. In the case of N-acetyltransferase, central neural stimulation results in rapid 20- to 100-fold increases in enzyme activity over a period of hours and cessation of neural stimulation results in an immediate turn-off of enzyme activity. In contrast, hydroxyindole-O-methyltransferase decreases gradually over a series of weeks^{2,39} following tonic cessation of neural stimulation, and varies little on a daily basis. The best explanation we can offer is that the nature of the intracellular

mechanisms regulating N-acetyltransferase, perhaps the stability of the required mRNA and the stability of the enzyme protein, are significantly different than those regulating the activity of hydroxyindole-O-methyltransferase. As a result, in response to daily neural stimulation there is a large rapid daily increase in the number of active molecules of N-acetyltransferase, but only a slight change in the number of active molecules of hydroxyindole-O-methyltransferase, one that is sufficient to maintain a steady state^{20,30}.

(3) *A single neural pathway from the suprachiasmatic nucleus to the pineal gland regulates pineal hydroxyindole-O-methyltransferase and N-acetyltransferase activities.* It is apparent from previous studies and the present report that removal of the superior cervical ganglia, lesions of the medial forebrain bundle, and lesions of the retrochiasmatic area of hypothalamus, through which axons from the suprachiasmatic nucleus project³⁵, block the stimulatory effects of the suprachiasmatic nucleus on pineal enzymes^{18,23,24,25,27}. On this basis we propose that there is a common neural pathway which transmits all signals that regulate the enzymes involved in the control of the formation of melatonin from serotonin in the pineal. Details concerning the precise localization of hypothalamic connections regulating the autonomic innervation of the pineal gland are not well known; interestingly, recent observations³² have indicated that the pathway from the eye to the pineal may require no more than 5 synapses²¹. Thus, in view of all of the data available, it appears that pineal N-acetyltransferase and hydroxyindole-O-methyltransferase activities are regulated by a single neural system. A schematic representation of this regulatory pathway is presented in Fig. 12.

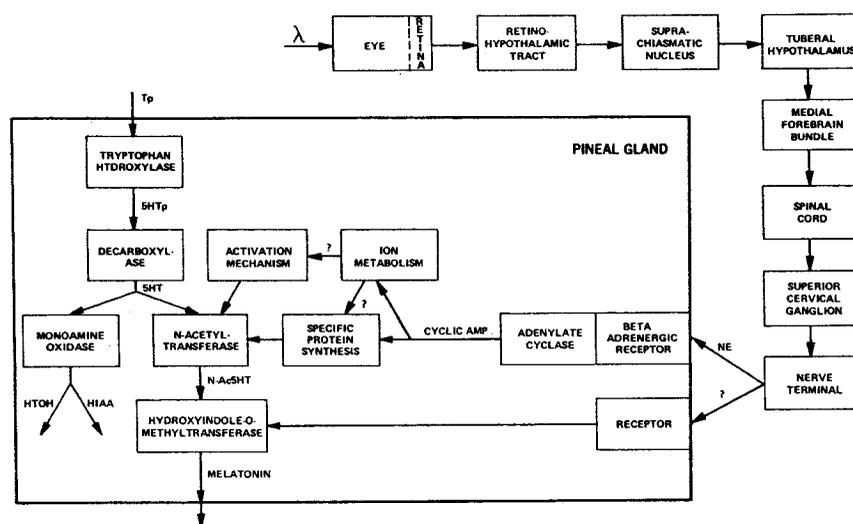


Fig. 12. A schematic representation of the neural control of the pineal gland. NE, norepinephrine; cyclic AMP, adenosine 3',5'-cyclic monophosphate; Tp, tryptophan; OHTp, 5-hydroxytryptophan; 5-HTp, 5-hydroxytryptamine; serotonin; HIAA, 5-hydroxyindole acetic acid; HTOH, 5-hydroxytryptophol; N-Ac5HT, N-acetylserotonin. The question marks indicate unproven hypotheses. This scheme is a modification of one that was previously published¹⁹.

The case has been made that N-acetyltransferase is a key neurally controlled 'regulating enzyme' that specifically turns one biochemical pathway on and off¹²; as such it may be a general model for neural control of cell-specific unique metabolism. This idea evolved when it became apparent that the entire neural pathway from the eye to the pineal gland and the mechanisms involved in converting neural signals into biochemical signals appeared to exist and function for the single purpose of controlling this one key regulating enzyme. In view of our findings here, it would appear necessary to expand this to include hydroxyindole-O-methyltransferase as well. Interestingly, these enzymes together provide distinct yet complementary types of responses to neural stimulation. One is a dynamic minute-to-minute response which is relatively rapid reflection of neural stimulation. The other is a tonic, dampened response that is an integrated measure of the amount of neural stimulation the tissue has received during the previous week or so. Simultaneously, therefore, these enzymes provide both an index of ongoing neural stimulation and an integrated measure of previous neural stimulation of the pineal gland. As such this system offers an intriguing demonstration of how dynamically different biochemical responses can be generated in a cell by the same central neural stimulation.

As a final point it would appear appropriate to comment on the nature of the transsynaptic and intracellular regulation of these two enzymes (Fig. 12). Pineal N-acetyltransferase activity appears to be regulated by the release of norepinephrine from presynaptic stores. This transmitter stimulates N-acetyltransferase activity through a mechanism which depends upon interaction with β_1 -adrenergic receptors, the generation of cyclic AMP, a change in membrane physiology resulting in hyperpolarization, the synthesis of new protein and stabilization of N-acetyltransferase molecules^{3,5,7,8,12,13,14,17,28,34}. In contrast there is little evidence available to explain how neural signals stimulate hydroxyindole-O-methyltransferase activity. Organ culture studies have produced only negative results in this area^{3,13} perhaps because of the slow response time of hydroxyindole-O-methyltransferase activity. In addition no report of in vivo effects of adrenergic agonists on the activity of this enzyme are known to us. It is possible that this enzyme is regulated by a β_1 -adrenergic cyclic AMP mechanism similar to that regulating N-acetyltransferase activity. Alternatively, it also is possible that another transmitter is released from pineal sympathetic nerves with norepinephrine, perhaps octopamine, dopamine, or serotonin, and that it is involved in the regulation of hydroxyindole-O-methyltransferase enzyme^{10,11,29}. Future studies using long-term drug treatment may clarify this issue.

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REFERENCES

- 1 Axelrod, J. and Weissbach, H., Enzymatic O-methylation of N-acetylserotonin to melatonin, *Science*, 131 (1960) 1312-1313.
- 2 Axelrod, J., Wurtman, R. J. and Snyder, S. H., Control of hydroxyindole-O-methyltransferase activity in the rat pineal gland by environmental lighting, *J. biol. Chem.*, 240 (1965) 949-954.
- 3 Berg, G. R. and Klein, D. C., Pineal gland in organ culture. II. Role of adenosine 3',5'-monophosphate in the regulation of radiolabelled melatonin production, *Endocrinology*, 89 (1971) 453-461.
- 4 Binkley, S., Klein, D. C. and Weller, J., Dark induced increase in pineal serotonin N-acetyltransferase activity: A refractory period, *Experientia (Basel)*, 29 (1974) 1339-1340.
- 5 Deguchi, T., Role of the beta adrenergic receptor in the elevation of adenosine cyclic 3',5'-monophosphate and induction of serotonin N-acetyltransferase in rat pineal glands, *Molec. Pharmacol.*, 9 (1973) 184-190.
- 6 Deguchi, T., Circadian rhythms of enzyme and running activity under ultradian lighting schedule, *Amer. J. Physiol.*, 232 (1977) 375-381.
- 7 Deguchi, T. and Axelrod, J., Induction and superinduction of serotonin N-acetyltransferase by adrenergic drugs and denervation in the rat pineal, *Proc. nat. Acad. Sci. (Wash.)*, 69 (1972) 2298-2211.
- 8 Deguchi, T. and Axelrod, J., Control of circadian change in serotonin N-acetyltransferase activity in the pineal organ by the β -adrenergic receptor, *Proc. nat. Acad. Sci. (Wash.)*, 69 (1972) 2547-2550.
- 9 Hendrickson, A. E., Wagoner, N. and Cowan, W. M., An autoradiographic and electron microscopic study of retino-hypothalamic connections, *Z. Zellforsch.*, 135 (1972) 1-26.
- 10 Jaim-Etcheverry, G. and Zieher, L. M., Localizing serotonin in central and peripheral nerves. In G. Schmidt (Ed.), *Neurosciences*, 3rd ed., MIT Press, Cambridge, Mass., 1974, pp. 917-923.
- 11 Jaim-Etcheverry, G. and Zieher, L. M., Octopamine probably coexists with noradrenaline and serotonin in vesicles pineal adrenergic nerves, *J. Neurochem.*, 25 (1975) 915-918.
- 12 Klein, D. C., The pineal as a model of neuroendocrine regulation. In S. Richlein, J. Martin and R. Baldasserini (Eds.), *The Hypothalamus*, Raven Press, New York, 1978, pp. 303-327.
- 13 Klein, D. C. and Berg, G. R., Pineal gland: Stimulation of melatonin production by norepinephrine involves cyclic AMP-mediated stimulation of N-acetyltransferase, *Advanc. Biochem. Psychopharm.*, 3 (1970) 241-263.
- 14 Klein, D. C., Reiter, R. J. and Weller, J. L., Pineal N-acetyltransferase activity in blinded and anosmic rats, *Endocrinology*, 89 (1971) 1020-1023.
- 15 Klein, D. C. and Weller, J. L., Indole metabolism in the pineal gland: A circadian rhythm in N-acetyltransferase, *Science*, 169 (1970) 1093-1095.
- 16 Klein, D. C. and Weller, J. L., A rapid light-induced decrease in pineal serotonin N-acetyltransferase activity, *Science*, 177 (1972) 532-533.
- 17 Klein, D. C. and Weller, J. L., Adrenergic-adenosine 3',5'-monophosphate regulation of serotonin N-acetyltransferase activity and the temporal relationship of serotonin N-acetyltransferase activity to synthesis of ^3H -N-acetylserotonin and ^3H -melatonin in the cultured rat pineal gland, *J. Pharmacol. exp. Ther.*, 186 (1973) 516-527.
- 18 Klein, D. C., Weller, J. L. and Moore, R. Y., Melatonin metabolism: Neural regulation of pineal serotonin N-acetyltransferase, *Proc. nat. Acad. Sci. (Wash.)*, 68 (1971) 3107-3110.
- 19 Klein, D. C. and Yuwiler, A., Beta-adrenergic regulation of indole metabolism in the pineal gland. In E. Usdin and S. Snyder (Eds.), *Frontiers in Catecholamine Research*, Pergamon Press, London, 1973, pp. 321-326.
- 20 Lynch, H. J. and Ralph, C. L., Diurnal variation in pineal melatonin and its nonrelationship to HIOMT activity, *Amer. Zool.*, 10 (1970) 300.
- 21 Moore, R. Y., The innervation of the mammalian pineal gland. In R. J. Reiter (Ed.), *The Pineal and Reproduction*, Karger Press, Basel, 1978, pp. 1-29.
- 22 Moore, R. Y. and Eichler, V. B., Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat, *Brain Research*, 42 (1972) 201-206.
- 23 Moore, R. Y., Heller, A., Bhatnagar, R. K., Wurtman, R. J. and Axelrod, J., Central control of the pineal gland: Visual pathways, *Arch. Neurol. (Chic.)*, 18 (1968) 208-218.
- 24 Moore, R. Y., Heller, A., Wurtman, R. J. and Axelrod, J., Visual pathways mediating pineal response to environmental light, *Science*, 155 (1967) 220-223.
- 25 Moore, R. Y. and Klein, D. C., Visual pathways and the central neural control of a circadian rhythm in pineal serotonin N-acetyltransferase activity, *Brain Research*, 71 (1974) 17-33.

- 26 Moore, R. Y. and Lenn, N. J., A retinohypothalamic projection in the rat, *J. comp. Neurol.*, 146 (1972) 1-14.
- 27 Moore, R. Y. and Rapport, R. L., Pineal and gonadal function in the rat following cervical sympathectomy, *Neuroendocrinology*, 7 (1970) 361-374.
- 28 Parfitt, A., Weller, J. L., Sakai, K. K., Marks, B. H. and Klein, D. C., Blockade by ouabain or elevated potassium ion concentration of the adrenergic and adenosine cyclic 3',5'-monophosphate-induced stimulation of pineal serotonin N-acetyltransferase activity, *Molec. Pharmacol.*, 11 (1975) 241-255.
- 29 Pelligrino de Iraldi, A. and Zieher, L. M., Noradrenaline and dopamine content of normal decentralized, and denervated pineal glands of the rat, *Life Sci.*, 5 (1966) 149-154.
- 30 Quay, W. B., Lack of rhythm and effect of darkness in rat pineal content of N-acetylserotonin-O-methyltransferase, *Physiologist*, 10 (1967) 286.
- 31 Reiter, R. J. and Klein, D. C., Observations on the pineal glands, the Harderian glands, the retinas, and the reproductive organs of adult female rats exposed to continuous light, *J. Endocrinol.*, 51 (1971) 117-125.
- 32 Saper, C. B., Loewy, A. D., Swanson, L. W. and Cowan, W. M., Direct hypothalamoautonomic connections, *Brain Research*, 117 (1976) 305-312.
- 33 Stephan, F. K. and Zucker, I., Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions, *Proc. nat. Acad. Sci. (Wash.)*, 69 (1972) 1583-1586.
- 34 Strada, S., Klein, D. C., Weller, J. and Weiss, B., Norepinephrine stimulation of cyclic adenosine monophosphate in cultured pineal glands, *Endocrinology*, 90 (1972) 1470-1476.
- 35 Swanson, L. W. and Cowan, W. M., The efferent connections of the suprachiasmatic nucleus of the hypothalamus, *J. comp. Neurol.*, 160 (1975) 1-12.
- 36 Weissbach, H., Redfield, B. G. and Axelrod, J., The enzymatic acetylation of serotonin and other naturally occurring amines, *Biochim. Biophys. Acta (Amst.)*, 54 (1961) 190-192.
- 37 Wolfe, D., Potter, D., Richardson, K. and Axelrod, J., Localizing norepinephrine in sympathetic axon by electron microscopic autoradiography, *Science*, 138 (1962) 440-442.
- 38 Wolfe, D. E., The epiphyseal cell: An electron microscopic study of its intercellular relationships and intracellular morphology in the pineal body of the albino rat. In J. Ariens Kappers and J. P. Schadé (Eds.), *Structure and Function of the Epiphysis Cerebri*, Progr. Brain Res., Vol. 10, Elsevier, Amsterdam, 1965, pp. 332-386.
- 39 Wurtman, R. J., Axelrod, J. and Phillips, L., Melatonin synthesis in the pineal gland: Control by light, *Science*, 142 (1963) 1071-1072.