EXPERIMENTAL NEUROLOGY 27, 194–205 (1970)

Retinal and Optic Nerve Serotonin and Retinal Degeneration as Influenced by Photoperiod

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Received November 25, 1969; Revision Received January 7, 1970

The environmental light stimulus, which controls neuroendocrine events associated with reproductive cycles, also influences the serotonin cyclicity in the central nervous system. A possible relationship between photoperiod and serotonin in the retina has been determined by liquid scintillation spectrometry after intraocular injection of tritium-labelled amine precursor, 5-hydroxytryptophan (^{3}H•5-HTP), into adult female rats kept for varying time periods in either constant light, constant darkness, or a cyclic photoperiod of 14 hours of light and 10 hours of darkness. Constant illumination suppressed retinal and optic nerve serotonin stores after 16 days, but had no effect after 4 or 30 days exposure. Radioactivity in the retina and optic nerves was reduced after 30 days of constant darkness. Exposure to constant illumination under ordinary laboratory conditions for 4, 16, and 30 days resulted in a reduction in the thickness of the retina, accompanied by degenerative changes in the photoreceptor cells. Terminal segments of these cells fragmented during early exposure periods and were absent, first in the posterior, and later, in the more peripheral areas of the retina after 16 and 30 days of constant light. Neither the cyclic photoperiod nor continuous darkness initiated degeneration in the retina. The results demonstrate that serotonin is influenced by photoperiod fluctuations and suggest that the biogenic amine might be related to light-induced changes in neuroendocrine function.

Introduction

The retina of the rat eye can synthesize serotonin (5-hydroxytryptamine) from its metabolic precursor, 5-hydroxytryptophan. The radioautographic observation of serotonin storage sites in the retina after injection of the labelled precursor assumes that the enzyme necessary for the chemical conversion, 5-hydroxytryptophan decarboxylase, exists in the tissue. Following endogenous serotonin synthesis, the biogenic amine was transported along the optic nerve and contralateral optic tract towards the central ner-

[1] This project was supported by grant No. M68:164 from The Population Council and grant No. HDO4102 from USPHS. Grateful acknowledgement of research assistance from Mrs. Janice Herbert. Publication No. 935, Department of Anatomy, Division of Basic Health Sciences, Emory University.

vous system (25). Serotonin has been demonstrated previously in the retina of adult rats and frogs (11), mice (10), rabbits (30), and man (16).

The role of serotonin in the retina, whether it is associated with optic phenomena or neuroendocrine events coupled to photoperiodicity, is presently unknown. However, intraocular injections of serotonin resulted in decreased weights of male pituitary and adrenal glands (23) and in increased weights of female pituitary glands (24).

The functional modification of the hypothalamic-pituitary-gonadal axis by manipulation of the photoperiod has established that light influences sexual development and synchronization of rhythmic changes in the endocrine physiology of several species, including the rat (13, 35). For example, the pineal gland of the rat exhibits a diurnal rhythm in its content of serotonin, which is eliminated when the animals are exposed to constant illumination, but which persists in animals deprived of visual stimulation (32). The pineal may function in mediating the effects of light on the gonads, since the pineal enzyme, hydroxyindole-*o*-methyl transferase (1), and estrous cycle response (36) to light are abolished by both enucleation and superior cervical ganglionectomy. Similarly, bilateral lesions of the inferior accessory optic tracts of rats, which spared the primary retinal projections, eliminated the pineal and gonadal responses to environmental light in rats (19). Bilateral destruction of the primary optic pathways alone had no effect on the two responses. These observations establish a separate function for the inferior accessory optic tract in the regulation of light-influenced neuroendocrine responses (19).

The results of the present experiments provide evidence that ordinary laboratory lighting or darkness for prolonged periods suppresses the synthesis of serotonin in the retina from its labelled precursor, tritiated 5-hydroxytryptophan, and that the continuous light regimen produces progressive degeneration of retinal receptor cells.

Materials and Methods

Adult female rats were kept in an environment of either constant light (L:L), constant darkness (D:D), or cyclic light (L:D, 14 hours light:10 hours darkness) for varying time periods, ranging from 4 days to 4 weeks. The constant light environment had an illumination, measured as reflected light from the cage floor, of 18 ft-c (=194 lux) from two GE (F15T8. CW) 15-w fluorescent tubes (waves of 3800–7500 Å, peaking at 6000) located 40 cm from the top of the cage. Constant darkness was interrupted twice weekly for cleaning of cages and feeding by a very dim light emitted from a Kodak Safelight, equipped with a 15-w incandescent bulb and a Kodak Wratten series 2 filter (dark red color), suspended 80 cm over the cages. Room temperature was 25 ± 1°C, and food and water were given

ad libitum. At the end of the light:darkness period, the vitreous body of each eye was injected with 4 μc (4 μl) of tritiated 5-hydroxytryptophan (^{3}H · 5-HTP; sp act 13.8 c/mM), using a microsyringe and 30-gauge needle while the animal was lightly anesthetized with ether; rats kept in constant darkness were anesthetized in the dark and brought individually into a lighted room for injection. Following the isotope injection, all animals were returned to their former environments for 4 days prior to autopsy. Animals exposed to constant light or darkness for only 4 days were kept previously in cyclic light (14 hours light:10 hours darkness). At autopsy, the rats were anesthetized with ether, and the eyes and entire brain with optic nerves attached were removed and rinsed with physiolgic saline solution. The cornea of each eye was resected, and the lens and vitreous humor were extruded from the remainder of the eye. The vitreous chamber was rinsed with saline, and the eye was blotted gently with moistened toweling. The optic nerves, which had been trimmed from the posterior part of the eye, were removed from the brain at the rostral margin of the optic chiasm. All samples were rinsed for 1 hour in three changes of cold physiologic saline in a refrigerator (5°C). Rinsing, according to Gershon and Ross (14), removed the unincorporated ^{3}H · 5-HTP and metabolites from the tissues, and labelled serotonin (5-HT) remains at sites of its synthesis.

Following the rinses, the retinas were separated from the sclera and choroid coats with forceps, and they and the optic nerves were blotted on moistened toweling and placed in preweighed glass scintillation counting vials, which then were reweighed to determine the sample weight.

The tissues then were solubilized, a process requiring 12–24 hours in 0.2 ml of 0.1 N NaOH. Fifteen milliliters of liquid scintillation fluid (667 ml toluene, 6 g PPO, 0.1 g bis-MSB, 333 ml Triton X-100; ingredients mixed and allowed to stand for 12 hour prior to use) and 1 ml distilled water were added to each solubilized sample. Radioactivity was counted in a Packard Model 3324 Tri-Carb liquid scintillation spectrometer, and counts per minute (cpm) were calculated from 10-min counts (Table 2). Statistical significance of the results was determined by the Student t test.

Several eyes from each group of rats were fixed in Bouin's solution and processed routinely with a hematoxylin and eosin stain for histologic examination. Average thickness of retinas was calculated from five ocular micrometer measurements, taken 520 μ from the optic nerve, on each of four midsagittal eye sections (Table 1).

Results

Retinal Degeneration. After only 4 days of exposure to constant illumination, terminal segments of retinal receptor cells were fragmenting or absent, and nuclei of these cells were pyknotic. The thickness of the retina was re-

duced as compared with that of the L:D and D:D animals (Table 1). Considerable variability in the occurrence of degenerative signs and thickness, as evident in the standard error of the means (Table 1), was noted among the retinas of this group as compared to others.

Retinas of rats exposed to constant light for 16 days had extensive degenerative changes, especially in the receptor cell layer. Receptor cells had disappeared from the most posterior pole of the retina, but in the more rostral, peripheral areas the nuclei of these cells and some external terminal segments remained; the thickness of the peripheral receptor cell layer was approximately six times that measured in the posterior areas where cells had not completely degenerated (average thicknesses, 30.7 μ peripherally; 5.2 μ posteriorly). The neurons in the bipolar and ganglion cell layers showed no changes, but there was an increase in interfiber spaces (Fig. 1).

After 30 days of exposure to constant light, scattered receptor cell nuclei remained in the most anterior, peripheral retina, but they were pyknotic. More posteriorly, receptor cells were completely absent. An apparent increase in the internuclear spaces in the bipolar cell layer (Fig. 1) possibly indicates either a degeneration of these cells or a nuclear displacement as a result of retinal changes, such as edema. The thickness of the retinas in this group, although significantly less than that in the L:D or D:D groups, was statistically greater than the retinas of the 16 day L:L animals (Table 1). No degenerative or other histologic changes were noted in the retinas of rats exposed to constant darkness or to the 14:10 hour schedule of cyclic light.

The results of all experiments related to the effects of light and darkness on retinal uptake and optic nerve transport of the isotope, 5-hydroxytryptophan, are summarized in Table 2. A control group exposed to 14 hours of

TABLE 1

THICKNESS OF RETINAS FROM CONTROL RATS (CYCLIC LIGHT, L:D) AND RATS KEPT IN EITHER CONSTANT LIGHT OR CONSTANT DARKNESS FOR DIFFERENT TIME PERIODS

Photoperiod	Retinal thickness (Avg. $\mu \pm$ SE)
Cyclic (L:D, 14:10 hours)	227.7 ± 9.6
Constant light (4 days)	142.8 ± 14.3 [a,b]
(16 days)	113.9 ± 8.1 [a]
(30 days)	170.6 ± 7.3 [a,b]
Constant darkness (30 days)	243.4 ± 10.3

[a] $p < 0.001$, compared with cyclic and constant darkness groups.
[b] $p < 0.05$, compared with constant light (16-day) group.

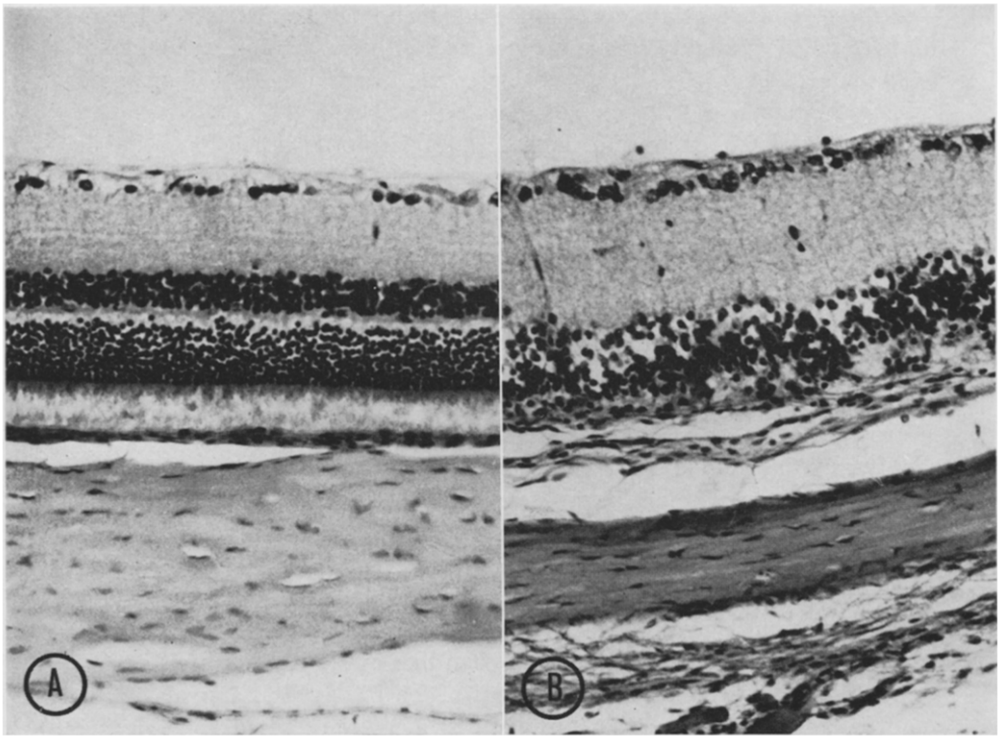

FIG. 1. Sections of the retina; H and E stain; x200. A. Retina of control rat kept in cyclic light; all layers are intact. B. Retina of rat kept in constant light for 30 days; photoreceptor cells absent, nuclei of bipolar cells dispersed, leaving large intercellular spaces, but inner retinal layers appear as in control rats.

light and 10 hours of darkness was included for each time period of exposure to light and darkness; the 4 and 30-day exposures were completed simultaneously with the same control group. Retinal and optic nerve weights and counts per minute per milligram tissue weight (cpm/mg) were comparable in all control groups receiving intraocular injections of tritiated 5-HTP. In animals given the same total dosage of isotope (8 μc) subcutaneously, the radioactivity in the retina averaged less than 1 cpm/mg and was undetectable in one retina, while that in the optic nerve was slightly, but consistently, higher than in the retina when individual tissue counts were compared.

Retinas and optic nerves of animals exposed to constant light for 4 days did not differ from their control groups in weight or isotope uptake. However, after 16 days of constant illumination, a significant reduction in retinal weight was recorded; there was an overall comparable reduction in radioactivity in these tissues, which, when corrected for tissue weight, was signi-

TABLE 2

RADIOACTIVITY IN THE RETINA AND OPTIC NERVES OF RATS KEPT IN CYCLIC LIGHT, CONSTANT LIGHT, OR CONSTANT DARKNESS AFTER INTRAOCULAR INJECTION OF TRITIATED 5-HYDROXYTRYPTOPHAN. TWO GROUPS (16 DAYS) RECEIVED AN IDENTICAL SUBCUTANEOUS DOSAGE (SC)

Light cycle, Days exposed, L:D (14:10 hour)	No. rats	Retina			Optic nerves		
		Wt (mg)	cpm	cpm/mg	Wt (mg)	cpm	cpm/mg
Control group for							
4	8	23.9 ± 1.5	1451.0	60.2 ± 5.4	4.8 ± 0.4	264.7	55.2 ± 2.8
16	10	26.7 ± 2.5	1624.3	59.8 ± 3.2	5.9 ± 0.2	275.3	56.5 ± 5.8
30	8	23.9 ± 1.5	1451.0	60.2 ± 5.4	4.8 ± 0.4	264.7	55.2 ± 2.8
sc 16	6	23.4 ± 3.9	14.8	0.6 ± 0.3	6.6 ± 0.3	24.3	3.7 ± 1.5
L:L (constant light)							
4	8	28.6 ± 2.1	1449.0	55.6 ± 8.0	4.2 ± 0.1	203.9	48.7 ± 2.4
16	10	19.2 ± 1.7[a]	905.6	50.8 ± 7.1[c]	6.5 ± 0.4	162.5	36.4 ± 8.5[c]
30	12	13.5 ± 0.8[b]	919.5	62.3 ± 14.5	5.3 ± 0.3	336.6	62.5 ± 8.1
sc 16	6	16.7 ± 1.8	4.5	0.4 ± 0.1	7.3 ± 0.5	29.9	4.2 ± 1.2
D:D (constant darkness)							
4	8	28.8 ± 1.6	1621.0	58.3 ± 7.1	3.9 ± 0.3	231.1	61.9 ± 3.2
30	12	28.5 ± 1.7	1131.9	34.1 ± 1.7[a]	5.8 ± 0.1	156.5	27.2 ± 4.8[b]

[a] $P < 0.001$.

[b] $P < 0.01$.

[c] $P < 0.05$, as compared with control groups (L:D).

ficantly less than that of the L:D exposed retinas (Table 2). Labelled serotonin had decreased significantly in the optic nerves of L:L rats as compared to that of L:D animals. Retinas of animals on the same lighting schedule (L:L) given identical dosages of the isotope subcutaneously had an average activity of less than 1 cpm/mg tissue, and no radioactivity was detectable in 50% of the retinas. Although the cpm/mg retina in the L:D and L:L rats are so reduced as to be almost uncountable, the lack of detectable labelled material in one-half of the retinas in the latter group might possibly be interpreted as decreased synthesis resulting from constant light exposure, somewhat similar to that found in retinas after intraocular dosage of the isotope.

Exposure to 30 days of constant light caused a reduction in retinal weight as was seen after 16 days. The radioactivity again was reduced as at 16 days, but when the cpm were adjusted for tissue weight, there was no significant difference in labelled serotonin between the L:L and the L:D retinas. The radioactivity in the optic nerves of 30 day L:L rats was unchanged from that in nerves of L:D animals.

The effect of constant darkness for 4 and 30 days on the weight, histology, and incorporation of tritiated 5-HTP in the retina and optic nerve of rats are summarized in Tables 1 and 2. Retinal weight and histology were unaffected by 4- and 30-day periods of exposure to constant darkness. However, there was a significant reduction in retinal radioactivity as compared to that in rats exposed to cyclic or to constant light. The quantity of labelled serotonin passing through the optic nerve had decreased from 55.2 cpm/mg in L:D rats and 62.5 cpm/mg in L:L rats to 27.2 cpm/mg in D:D animals.

Discussion

The effects of light stimulus on neuroendocrine function, as have been determined by studies on the hypothalamus-pituitary-gonadal axis during modifications of the photoperiod, are frequently considered to be direct through photically generated impulses passing to neural centers capable of influencing the hypothalamus. Exactly which anatomic pathways are involved, and whether direct retinohypothalamic connections exist, are unknown, but the optic system somewhere along its course contributes fibers to centers controlling a variety of autonomic and endocrine functions (6, 29). Degeneration studies of the rat brain have failed to demonstrate the presence of retinohypothalamic fibers (15), although there is physiologic evidence for an optic influence on the rostral hypothalamus (7). Whether this influence is strictly electrophysiologic or neurohumoral has yet to be determined.

Serotonin is found normally in the retina of several species, and the enzymes associated with its synthesis from 5-HTP, 5 hydroxytryptophan

decarboxylase, and with its degradation, monoamine oxidase, have been isolated from the retina (2, 21). A possible role for serotonin as a neurotransmitter in the optic pathways was suggested from observations that intracarotid injections of lysergic acid diethylamide (LSD), a serotonin antagonist, depressed evoked responses in the lateral geniculate body of the cat (3, 12). Serotonin, when applied directly to the lateral geniculate nucleus, elicited a similar depression (8).

A preliminary radioautographic study demonstrated serotonin localization in the rat retina and optic pathways (25), and the present results demonstrate that light and dark photoperiods influence the synthesis of serotonin in the optic system. Exposure of rats to constant illumination for 16 days significantly depressed the radioactivity in the retina. The activity, expressed as counts per minute (cpm), was greatly reduced after 30 days exposure to constant light, but when calculated on a cpm/mg tissue basis, the difference was statistically insignificant (Table 2). Apparently, the absolute value (cpm) for serotonin synthesis was not reduced during the period of 16–30 days exposure, although the retinal receptor cells continued to degenerate further. However, there was a resulting decrease in serotonin passing into the optic nerves from the retina at the 16-day period and a recovery to control level in the optic nerve at the 30-day period (Table 2). Since the retinal degeneration seemed to be limited to the photoreceptor cells, serotonin synthesis in the retina must have been dependent on intact bipolar cells and the ganglion cells, the axons of which form the optic nerve. In each instance, where a reduction in radioactivity occurred in the retina, a comparable decrease was found in the optic nerves. This observation was made among individual animals, as well as for entire groups.

The reduction in retina and optic nerve serotonin content after both constant illumination for 16 days and constant darkness for 30 days suggests that the abnormal photoperiod, whether it is increased light or darkness, depresses the uptake of the isotope, tritiated 5-hydroxytryptophan, or the synthesis and storage of serotonin in the retina. Rüther, Halaris, and Matussek (28) found that both serotonin and norepinephrine content in the rat brain fluctuated in the same direction in response to either constant light or darkness, but instead of a decrease, as reported here for the retina, they found increased concentrations in whole brain homogenates.

Retinal weights were markedly reduced after the 16- and 30-day light exposure periods; examination of histological preparations indicated a gross, progressive degeneration of the visual receptor cell layer of the retina, which most likely explains the reduction in retinal weight at these time periods. The visual receptor cell degeneration was extensive enough to suggest that these animals had lost their central receptor cell (visual) function

by 16 days and all vision by 30 days. The retinal degeneration described here as resulting from exposure to ordinary laboratory illumination in some ways resembles that described by Noell and his co-workers (22) after exposure of rats to filtered green and monochromatic light of much greater intensity and at a higher, abnormal body temperature, for shorter periods of time than described here. These investigators concluded that the damaging effect of light on the retina was extremely dependent on body temperature, and, at normal body temperature, ordinary laboratory illumination apparently was either not strong enough or was not maintained continuously for a sufficient length of time to induce an irreversible effect.

The possibility that the tritiated 5-hydroxytryptophan injected or the serotonin synthesized in the eye might be injurious to the retina was suggested by the results of Tammisto (33), which showed that low microgram doses of serotonin, when injected into the common carotid artery, caused a profound contraction of the retinal vessels for 2 min. The close similarity of radioactivity levels, both in cpm and cpm/mg tissue, of cyclic light control retinas and the absence of degenerative changes in cyclic light control, 4-day constant light, and both constant dark groups repudiate this possibility; in addition, the pattern of degeneration discounts this possibility of drug effect on retinal vessels, since the photoreceptor cells are nourished by a choriocapillary blood supply, and the ganglion cells, which are contiguous to the vitreous and did not degenerate, are nourished by the retinal vessels. Since the radioactivity in 30-day light exposed retinas had returned to control level, it is unlikely that the decreased activity in the 16-day light-exposed retinas, under identical conditions, resulted from a vasoconstrictive action of serotonin on retinal vessels.

Exposure of rats to constant darkness for 4 days did not affect serotonin synthesis in the retina or optic nerve. However, the radioactivity was reduced significantly in the retinas and optic nerves of rats kept in constant darkness for 30 days, as compared to both the control groups and the 16- and 30-day groups exposed to constant illumination. There was no reduction in retinal weight or any degeneration of retinal components in rats exposed to darkness under the prescribed conditions; therefore, the decreased uptake of isotope, or serotonin synthesis and storage, apparently resulted from a functional modification of the retina by darkness. Rasch *et al.* (27) reported that the retinas of rats kept in total darkness from birth to 65 days of age were identical to those of control rats on cyclic light, but that the retinal RNA level in the control rats was seven to ten times greater than that in the groups exposed to total darkness. Chow, Riesen, and Newell (5) had previously shown atrophy and loss of ganglion cells and optic nerve fibers in retinas of dark-reared chimpanzees. Similarly, Maraini *et al.* (18) found no histologic changes in the retina, lateral genic-

ulate nucleus, or visual cortex and no differences in leucine incorporation in the rat retina or visual cortex with monocularly light-deprived eyes; however, a consistent decrease in leucine uptake was detected in the ganglion cells of the lateral geniculate nucleus receiving input from the closed eye. DeRobertis and Franchi (9) reported that exposure of rabbits to darkness for 24 hours causes the synaptic vesicles in photoreceptor cells to accumulate near the synaptic membrane, and that after 9 days of darkness, a sharp decrease in the size of the synaptic vesicles occurred at both rod and cone synapses. In guinea pigs, there appears to be no effect of light or darkness adaptation on the average synaptic vesicular diameter (20). These electron microscopically observed changes in rods and cones cannot be resolved with the light microscope. The function of the retina in constant light or darkness most probably varies among different species and depends on whether the animal is inherently nocturnal or diurnal in habit.

Photic stimuli influence the physiology and behavior of animals in many ways, a subject recently reviewed by Wurtman (35). Constant illumination stimulates early pituitary-ovarian function in rodents (13, 17), and darkness or blinding (4, 34) retards sexual development. Quay and Halevy (26) found a 50% reduction in the serotonin and related amine content of the pineal gland of rats constantly illuminated for over 4 weeks, but the metabolic by-product of its metabolism, 5-hydroxyindoleacetic acid, was unaffected. Continuous light treatment for 8 weeks significantly increases the thyroid hormone secretion rate (TSR) of rats. The TSR in cyclic light or total darkness was unaffected (31). Whether these instances of photically modified endocrine functions are related to serotonin synthesis in the retina and its subsequent passage through optic pathways to central nervous system centers, cannot be ascertained from the present results. However, experiments in which rats are exposed to ordinary, continuous laboratory illumination for long periods of time probably should be interpreted in relation to the occurrence of extensive degeneration of the photoreceptor cells.

References

1. AXELROD, J., R. J. WURTMAN, and S. H. SNYDER. 1965. Control of hydroxyindole -0- methyl transferase activity in the rat pineal gland by environmental lighting. *J. Biol. Chem.* **240**: 949–954.
2. BAKER, P. C., and W. B. QUAY. 1969 5-hydroxytryptamine metabolism in early embryogenesis and development of the brain and retinal tissue. A review. *Brain Res.* **12**: 273–295.
3. BISHOP, P. O., G. FIELD, B. L. HENNESSY, and J. R. SMITH. 1958. Action of D-lysergic acid diethylamide on lateral geniculate synapses. *J. Neurophysiol.* **21**: 529–549.
4. BROWMAN, L. G. 1940. The effects of optic enucleation on the male albino rat. *Anat. Rec.* **78**: 59–73.

5. CHOW, K. L., A. H. RIESEN, and F. W. NEWELL. 1957. Degeneration of retinal ganglion cells in infant chimpanzees reared in darkness. *J. Comp. Neurol.* **107**: 27–42.
6. CRITCHLOW, V. 1963. The role of light in the neurosecretory system, pp. 377–402. *In* "Recent Advances in Neuroendocrinology." A. V. Nalbandov [ed.]. University of Illinois Press, Urbana.
7. CRITCHLOW, V., and DE GROOT, J. 1960. Experimental investigations of pathways involved in light-induced constant estrus in the rat. *Anat. Rec.* **136**: 179.
8. CURTIS, D. R., and R. DAVIS. 1962. Pharmacological studies upon neurons of the lateral geniculate nucleus of the cat. *Brit. J. Pharmacol.* **18**: 217–246.
9. DE ROBERTS, E., and C. M. FRANCHI. 1956. Electron microscope observations on synaptic vesicles of the retinal rods and cones. *J. Biophys. Biochem. Cytol.* **2**: 307–318.
10. ERÄNKÖ, O., and E. MERENMIES. 1961. *In vivo* inhibition of histochemically demonstrable amino oxidase in the retina of the mouse. *Acta Ophthalmol. (Kbn)* **39**: 335–352.
11. ERÄNKÖ, O., M. NIEMI, and E. MERENMIES. 1961. Histochemical observations on esterases and oxidative enzymes of the retina, pp. 159–171. *In* "The Structure of the Eye." G. K. Smelser [ed.]. Academic Press, New York.
12. EVARTS, E. V., W. LANDAU, W. FREYGANG, and W. MARSHALL. 1955. Some effects of LSD-25 and bufotenine on electrical activity in the cats visual system. *Amer. J. Physiol.* **182**: 594–598.
13. FISKE, V. M. 1941. Effect of light on sexual maturity, estrous cycles and anterior pituitary of the rat. *Endocrinology* **29**: 187–196.
14. GERSHON, M. D., and L. L. ROSS. 1966. Location of sites of 5-hydroxytryptamine storage and metabolism by radioautography. *J. Physiol. London.* **186**: 477–492.
15. HAYHOW, W. R., C. WEBB, and A. JERVIE. 1960. The accessory optic system in the rat. *J. Comp. Neurol.* **115**: 187–216.
16. KOJIMA, K., M. IIDA, Y. MAJIMI, and S. OKADA. 1961. Histochemical studies of the human retina. *Jap. J. Ophthalmol.* **5**: 205–210.
17. LUCE-CLAUSEN, E. M., and E. F. BROWN. 1939. The use of isolated radiation in experiments with the rat. III. Effects of darkness, visible and infra-red radiation on three succeeding generations of rats. *J. Nutrition* **18**: 551–562.
18. MARAINI, G., F. CARTA, R. FRANGUELLI, and M. SANTORI. 1967. Effect of monocular light deprivation on leucine uptake in the retina and the optic centers of the newborn rat. *Exp. Eye Res.* **6**:299–302.
19. MOORE, R. Y., A. HELLER, R. K. BHATNAGER, R. J. WURTMAN, and J. AXELROD. 1968. Central control of the pineal gland: visual pathways. *Arch. Neurol.* **18**: 208–218.
20. MOUNTFORD, S. 1963. Effects of light and dark adaptation on the vesicle population of receptor-bipolar synapses. *J. Ultrastruct, Res.* **9**: 403–418.
21. MUSTAKALLIO, A. 1967. Monoamine oxidase activity in the various structures of the mammalian eye. *Acta Ophthalmol. Suppl.* **93**: 1–62.
22. NOELL, W. K., V. S. WALKER, B. S. KANG, and S. BERMAN. 1966. Retinal damage by light in rats. *Invest. Ophthalmol.* **5**: 450–473
23. O'STEEN, W. K., and J. L. BARNARD. 1967. A possible neural effect of serotonin on the rat hypophysis. *Anat. Rec.* **157**: 295.
24. O'STEEN, W. K., and J. L. BARNARD. 1969. Intraocular serotonin: its influence on endocrine organs of the adult female rat. *Anat. Rec.* **163**: 239.

25. O'STEEN, W. K., and G. M. VAUGHAN. 1968. Radioactivity in the optic pathway and hypothalamus of the rat after intraocular injection of tritiated 5-hydroxytryptophan. *Brain Res.* **8**: 209–212.
26. QUAY, W. B., and A. HALEVY. 1963. Experimental modification of the rat pineal's content of serotonin and related amines. *Physiol. Zool.* **35**: 1–7.
27. RASCH, E., H. SWIFT, A. H. RIESEN, and K. L. CHOW. 1961. Altered structure and composition of retinal cells in dark-reared mammals. *Exp. Cell Res.* **25**: 348–363.
28. RÜTHER, E., A. HALARIS, and N. MATUSSEK. 1967. Norepinephrine and 5-hydroxytryptamine in the CNS of rats under constant illumination and total darkness. *Med. Pharmacol. Exp.* **17**: 139–143.
29. SCHARRER, E. 1964. Photo-neuro-endocrine systems: General concepts. *Ann. N.Y. Acad. Sci.* **117**: 13–22.
30. SHANTHAVEERAPPA, T. R., and G. H. BOURNE. 1964. Monoamine oxidase distribution in the rabbit eye. *J. Histochem. Cytochem.* **12**: 281–287.
31. SINGH, D. V., and C. W. TURNER. 1969. Effect of light and darkness upon thyroid secretion rate and on endocrine glands of female rats. *Proc. Soc. Exp. Biol. Med.* **131**: 1296–1299.
32. SNYDER, S. H., M. ZWEIG, J. AXELROD, and J. E. FISCHER. 1965. Control of circadian rhythm in serotonin content of the rat pineal gland. *Proc. Nat. Acad. Sci. U.S.* **53**: 301–305.
33. TAMMISTO, T. 1965 The effect of 5-hydroxytryptamine (serotonin) on retinal vessels of the rat. *Acta Ophthalmol.* **43**: 430–433.
34. TRUSCOTT, B. L. 1944. Physiological factors in hypophysial-gonadal interaction. I. Light and follicular mechanism of the rat. *J. Exp. Zool.* **95**: 291–305.
35. WURTMAN, R. J. 1967. Effects of light and visual stimuli on endocrine function, pp. 19–59. *In* "Neuroendocrinology," vol. **2**. L. Martini and W. F. Ganong [eds.]. Academic Press, New York.
36. WURTMAN, R. J., J. AXELROD, E. W. CHU, and J. E. FISCHER. 1964. Mediation of some effects of illumination on the rat estrous cycle by the sympathetic nervous system. *Endocrinology* **75**: 266–272.