ALTERATION IN PINEAL - TESTICULAR AXIS OF THE INSECTIVOROUS BAT - RHINOPOMA KINNEARI (MICROCHIROPTERA : MAMMALIA) EXPOSED TO VARIOUS WAVELENGTH OF LIGHT.

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Abstract

The pineal gland of insectivorous microchiropteron - Rhinopoma kinneari subserves dual function photo sensory and endocrine. Its secretions play a pivotal role in complex reproductive processes and functions. Using normal light as control for the basis for comparison, groups of bats were exposed to continuous blue, green, yellow red light and total darkness for eighteen day to assess their impact on pineal gland testicular morphology and histology of sexually mature male bats. Blue and green light induced significant increase in the length and cross section area of pineal gland. This inverse trend was not discerned in bats exposed to yellow and red color light; or total darkness. The results indicate that the observed changes in pineal gland are probably mediated via the optic pathway, while the alterations in the testes and seminiferous tubules are due to modulation in the secretary functions of the pineal gland.

Key Words : Rhinopoma kinneari, Bats, Pineal gland, Pineal-testicular axis, Light induced changes.

Introduction

The mammalian pineal gland is said to be an endocrine organ in most species, but it seems to have photo sensory role as well in many placentals. It has anatomical connections via axon and hemal vessels with the brain. The pineal gland of over 110 species of bats has been examined. The pineal gland is comprised of light and dark pinealocytes, neuroglial cells, unmyelinated nerve fibers, blood vessels, occasional intrapineal skeletal muscle, secretary vesicles, synaptic ribbons and melanin pigment (1-10). Cyclic secretary activity to occurs in the pinealocytes. Coordination of circadian and diurnal rhythms are believed to be mediated via the hypothalamus and hypophyseal gonadal axis. (11-14). Seasonal factors and photoperiod markedly effect breeding cycles physiological milieu and behaviour (15). Photic stimulations in nocturnal species cause inductive changes in pineal gland - testicular axis of many placentals (16, 17)). Very little is known about the effect of different wave length of light in the pineal gland - testicular structural relationships (3, 8, 18-23). Hence the present report deals with the comparison of alterations induced by various forms and regimen of light on mophometrics of pineal - testicular axis in the insectivorous nocturnal bat rhinopoma kinneari.

Materials and Methods

Sexually mature adult males of Rhinopoma kinneari weighing approximately 20+2 gm. were collected from Baghor ki Haveli, Udaipur India (latitude 24/34 N, longitude 73/42 E)on 23rd Oct. at 3 pm. Six groups of five bats each were subjected to following light regimen. Group I bats were exposed to (13 hr. L. : 11 hr. D) as normal control, Group II comprised of bat maintained in total darkness, bats of Group III, IV, V and VI were exposed to continuous blue, green, yellow or red light for eighteen days. Bats were maintained in wire cages, kept in wooden enclosure to provide complete light insulation. However, they had ad libitum access to food and water. After eighteen days, bats were sacrificed by cervical dislocation between 11 am. to 12 noon. The total weight of body and brain was recorded. The pineal gland was carefully excised and fixed in aceto-acetic-formalin (4h.) for reduced silver staining by Bodian protargal technique (24-26). The testes were carefully excised and fixed in aqueous Bourin's fixative (18hr.) The pineal gland were processed for routine light and electron microscopy. The size of the pineal gland and testes were determined by Zeiss oculometer. The data were analyzed using a one way ANOVA (27). Every alternate section (7 μm) of pineal gland testes were visually appraised and micro photographed to record the various changes in them.

Result

The pineal gland of Rhinopoma kinneari located on the dorsal aspect of the brain just posterior to the third ventricle. It is supported by the habenular commisure. The stalkers pineal gland of Rhinopoma kinneari conforms to type A (7, 17).

The effect of various forms of light on pineal - testicular axis of rhinopoma kinneari shows significant variations. Using normal (13 hr. L. : 11 hr. D) as control for comparison.

A. Morphology

1. **Effect of normal light (13h. L : 11h. D) :** A significant increase in brain weight, pineal width, and cross section area of the pineal gland occurred ($P<0.0.5$). A reverse trend was seen in length of pineal, diameter of testes and seminiferous tubules.
2. **Effect of total darkness :** A significant increase in body, brain weight and width of pineal glad was seen ($P<0.05$). However, length of pineal and diameter of seminiferous tubules decreased considerably.
3. **Effect of red light :** A significant increase in body, brain weight and width of pineal was seen ($P<0.05$). However, length of pineal and diameter of seminiferous tubules decreased considerably.
4. **Effect of yellow light :** A significant increase in body weight, brain weight, pineal width and testicular

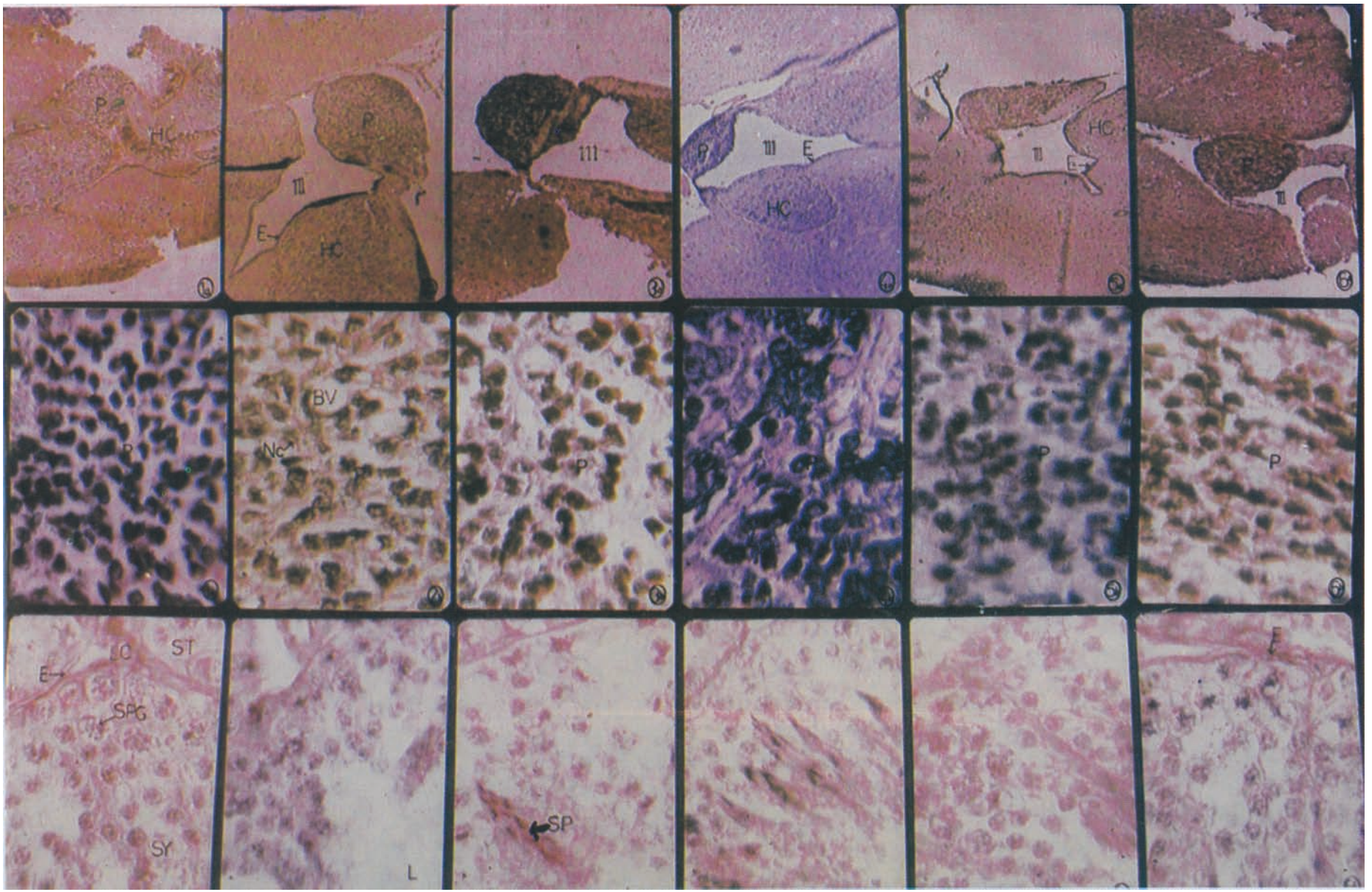

Plate-A Showing effect of Normal (13hr.L : 11hr. D) (fig.1), Blue (fig.2), Green (fig3) Yellow (fig.4) Red (fig.5) Light and total darkness (fig.6) regimen on the morphometrics and histology of pineal gland and testes of Rhinopoma Kinneari (la,b to 6 a,b-x100 and x 1000 (pineal gland) lc tp VI c-100 x (Testes) BV Vessel, Ct-connective Tissue, E-Epithelium, HC-Habenular complex, HN-Habenular nuclei, it-interstitail cell, L-Lumen, Nc-Nerve cell, P-Pineal gland, PC- Pinealocytes, Sp-sperm, SPG-Spermatogonia, St-Seminiferous tubules III-Thrd ventricle.

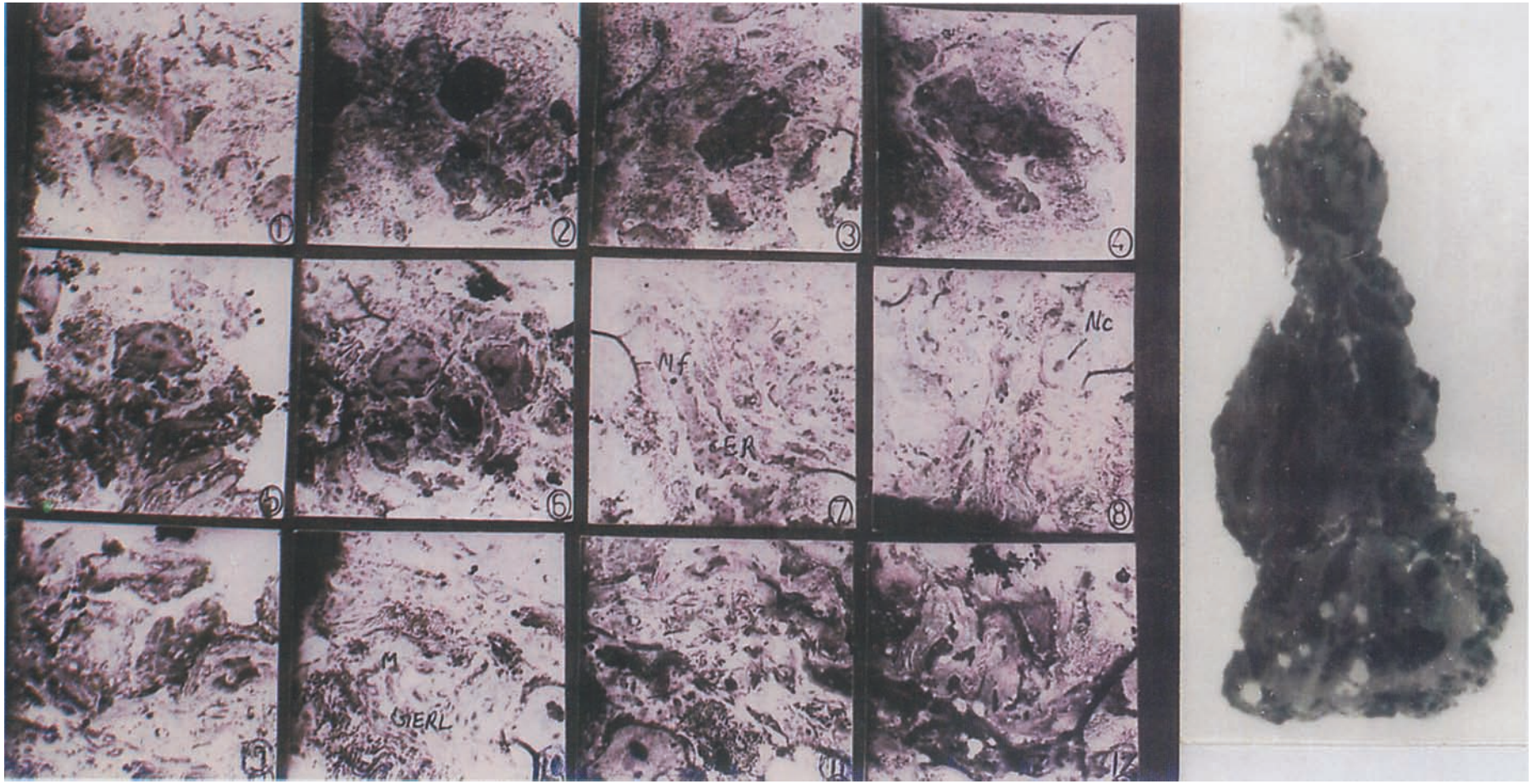

Plate 2 - Showing Electron Micrograph of Breeding Adult Female Pineal with habenular nuclei of Rat Tailed bat "Rhinopoma Kinneari" in Joel 100 S electron microscope. Fig. 1 to 8 at x 7000 and fig. 9 to 12 at x 12000 in large format. Unmyelinated Nerve cell- having axon and dendrite, Dense Core vesicle, Mitochondria, Golgi Body, rough endoplamic reticulum, Smooth endoplasmic reticulum glycogen granules, Lipid droplets, dividing cell, vacuoles glial cell, melanin pigment, astrocytes, mast cell, corpora arenacia and skeletal fibers are seen.

morphometrics was seen (P>0.05). However, length of pineal gland and diameter of testes were greatly reduced.

5. **Effect of green light:** The width, length and cross section area of pineal showed significant increase (P<0.05) although no discernible alterations occurred in the testes and seminiferous tubules.
6. **Effect of blue light :** A significant increase (P<0.05) in brain weight, width, length and in cross section area of pineal was observed but the testicular diameter was reduced.

B. Histology :

The pineal gland of Rhinopoma kinneari consists of neuroglial cells and pinealocytes of two types light and dark. Secretary granules were observed in the gland and in supra pineal recesses. Effect of total darkness and various forms of light on histology of pineal gland showed variations in size. Thus, the population of dark pinealocytes, and melanin pigmentation was much more lighter than the pineal gland of bats maintained in total darkness. However, in normal yellow dark type of pinealocytes were observed. In case of bats exposed to normal light the pinealocytes were uniformly scattered and no clustering was observed.

The pineal gland of bats exposed to blue, green yellow and normal light manifested maximal number of blood vessels and vacuoles. Concomitant evaluation of testicular structure of bats subjected to aforesaid treatments showed patterns of alterations. In bats exposed to yellow light, active spermatogenesis was seen. All germs cell types spermatogonia, premeiotic spermatocytes, differentiating spermatids and fully formed spermatozoa could be observed. This was true for bats kept under chronic, scoptic conditions. Bats exposed to green and red light showed active spermatogenesis. Contrarily, blue light, normal light (13h. L.: 11h. D.) had an attenuating effect on spermatogenic activity in the testes.

Discussion

The present studies on Rhinopoma kinneari highlight on a comparative basis, the effect of total darkness, normal and various forms of light on the morphometrics and histology of pineal gland and testes correlatively. The morphology and histology of pineal gland and testes was greatly influenced by various photic stimulation.

In the present studies blue light caused significant increase in the brain weight, length, width and in cross section area of pineal gland. However, inverse relationship occurred with reference to diameter of testes, green light increased the width, length and cross section area of pineal gland but had no significant effect on diameter of testes and seminiferous tubules and active spermatogenesis. On the other hand yellow light showed a significant decrease in the length of pineal and cross sectional areas of pineal. The diameter of testes and seminiferous tubules also decreased. Yellow light seems to have an injurious effect on brain weight, and pineal - testicular morphology. The aggressive behavior of these bat observed during experiment was probably due to hyperxcitability caused by yellow light. A comparison with vampire bats shows that these bats forage in moonless nights and leave their roosts when darkness is complete. Brief light flashes of 0.0.63 - 3.33 m.sec. phase shift the circadian flight activity of the bat Hipposideros speoris (28); and readily suppress pineal melatonin production in hamster pineal (29). The inhibition of hamster pineal melatonin after 1- or - 5- second light pulse at night has been reported by Reiter et al., (30). The brightness and wave length of the light was suggested to play an important role in regulating melatonin biosynthesis and elaboration (31-34). In the present study, red light induced maximum changes in the brain weight, and width of pineal gland. However, no significant reductions were discrned in the diameter of testes and seminiferous tubules. Significant increase in brain weight, pineal width and cross section of pineal gland were observed in bats exposed to normal light (13hr. L: 11hr. D.) with concomitant significant reduction in the diameter of testes and seminiferous tubule. The results of the present study demonstrate that continuous exposure of bats to yellow light stimulated active spermatogenesis. Bats kept under chronic scoptic condition or exposed to green and red light also showed active spermatogenesis, blue colored light, and 13h. L: 11h. D. of normal light had attenuating effect on spermatogenic activity in the testes.

A comparison of these findings with other placentals and vertebrates reveals many interesting similarities and differences. Thus, Reiter stated that the pineal gland of nocturnally active mammals (with rod dominated retina) is more sensitive to inhibition by light vis-a-vis diurnal species which have a cone-dominate retina (33). Further, melatonin secreted by pinealocytes causes oscillations in the function of other endocrine.

In the present studies on Rhinopoma kinneari certain wave lengths of light had no effect on testicular functions which are regulated by FSHRSH-FSH and LHRSH-LH secretions from hypothalamus and hypophysis. This may mean that melatonin secretion was augmened by blue light, which had an antigonadotrophin effect, thus reducing spermatogenic activity in the testes. It may mean that melatonin secretion did no acquire the threshold levels to cause inductive aberrations or yellow light had an inhibitory effect on melatonin biosynthesis. This is corroborated by reduction in the size of pineal gland of these bats.

Lewy et al., observed that acute light onset during darkness leads to a decline in pineal melatonin secretion (35). Further, circulating melatonin is related to brightness of the light to which the animals (including humans) are exposed. Animals differ in their sensitivity to light.

Wave length of light to produced different morphological and histological effects on the pineal gland and testes of Rhinopoma kinneari. The most significant incremental effect was observed with reference to blue light and decremental in relation to yellow light. Brainard et al., observed that rod-dominated mammals are more sensitive to blue light than the ones devoid of rods (36, 37). The visual pigment in rods (rhodopsin) is sensitive to blue light. It was proposed without adequate proof that visible light has inhibitory effect on pineal. Reiter showed that exposure of rats to red light effected melatonin rhythm which was equivalent to duration of darkness (36). However, sun et al. differed with this and emphasized that high intensity of red light cannot be

Table 1 : Effect of total darkness and different colours of light on the pineal gland and testicular orphometrics of Rhinopoa kinneari

S.No.	Treatment	Mean Bat wt. (gm)* N=5	Mean Brain wt. (gm)** N=5	Mean width of pineal gland between 2 HC (μm)** N=5	Mean length of pineal gland anterio post. (μm)** N=5	Mean area of transvers section of pineal gland (sq.μm)** N=5	Mean dia. of testis (mm)* N=5	Mean dia. of seminiferous tubules (μm)** N=5
1.	Normal (Control) (12h.L : 12h.D)	19.76a	0.277a	524.4a	272.6a	112198.7a	3.5	210.8
2.	Blue light 4358A^0	19.68a	0.294a	460.4a	392.0b	141484.48b	4.5	254.2
3.	Green light 5461A^0	17.65a	0.217a	411.8ab	389.0bc	126096.82ab	5.5	336.0
4.	Yellow light 5770A^0	16.97ab	0.255a	293.6c	196.8b	45305.8c	4.0	238.8
5.	Red light 6234A^0	22.07a	0.321ab	547.0a	178.0da	76439.4d	5.0	312.6
6.	Total darkness	18.94ab	0.254a	327.0bd	346.6f	88931.2ad	6.0	355.0
standard error		0.9495	0.0076	24.93	4.7486	7162.7611	0.2958	11.7152
Critical diff. 5%		2.771	0.0222	72.792	13.860	20907.4465	0.8634	34.1956
Critical diff. 1%		3.759	0.030	98.71	18.80	28362.8151	1.1716	46.3895
Coefficient of variance (%)		11.068	6.105	13.048	3.5893	16.2752	13.9250	9.2055

* - Significant at 5% ; ** - Significant at 1%
Within a coloumn followed by the same letters are not significantly different.

Legend HC = Habenular commissure
dia = diameter
wt = weight

regarded as 'safe' light, as it affects the profile of N-acetyl transferase (38). In the present studies, red light caused a distinct increase in the morphometrics of pineal gland, which may have correlation with elevated output of melatonin.

Brainard et al. found that green, blue or ultraviolet light did not cause significant alteration in the function of the reproductive tract of hamster (29, 36). However, when they were exposed to red or yellow light for 11h. for 12 weeks partial reproductive collapse was noticed. Axelrod, suggested that pineal gland responds directly to long wave length but not to light of short wave length (39). Bright light (2500 lux) can suppress the night time secretion of melatonin while dim light (500 lux) has little or no effect (40,41).

Wurtman, observed that pineal activity of rats was suppressed by continuous exposure to light (14). Further, green light was found to be more effective than light of other spectral colors in this process. However, no valid comparison of their result can be made with chiropterans due to lack of data. Photochromatic stimulation of pineal gland induces gonadal development and changes, thus acting as mediator of light.

Thus the size of the pineal appeared to be governed by the principle that darkness enhances the pineal activity and, therefore, increases the size of the pineal gland (42). This principle appears to be applicable to the nocturnal R. kinneari. The enlarged pineal gland of Rhinopoma kinneari may mean greater biosynthesis and elaboration of hormone (melatonin), which would evidently suppress spermatogenesis (43).

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Appendix 2.1
Anova of effect of total darkness and different colours of light on the pineal gland and testicular aorphometrics of Rhinopoa kinneari

Source	dt	Bat weight (gm)			Brain weight (gm)			Width of pineal between 2 HC (um)			Length of pineal antero posterior (um)			Area of transverse section of pineal gland (um)			Diameter of testis (um)			Diameter of sesiniferous tubules (um)		
		SS	MS	f cal	SS	MS	f cal	SS	MS	f cal	SS	MS	f cal	SS	MS	f cal	SS	MS	f cal	SS	MS	f cal
Stage	5	81.5	16.5	3.6155*	0.0149	0.00298	10.2758**	5689616.6	1137983.3	365.93**	223687.8	44737.56	396.785**	31023532139.407	6204706427.8814	24.1874**	21.88	4.37	10.00*	84251.815	16850.30	24.5548**
Error	24	108.2	4.5083		0.007	0.00029		74630.4	3109.6		2706.2	112.75		6156617697.796	256525737.40817		10.50	0.4375		16469.6	686.23	
Total	29	189.7			0.0219			5764247.0			226394.0			37180149837.203				32.38		100721:4		

** Significant difference at 1%

*Significant difference at 5%

HC Habenlar commissure