# MORPHOMETRIC AND HISTOCHEMICAL ALTERATIONS IN THE PINEAL GLAND OF RHINOPOMA KINNEARI DURING VARIOUS DEVELOPMENTAL AND REPRODUCTIVE STATES

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## Abstract

In placental mammals, the pineal gland is known to play a significant role. Rhinopoma Kinneari, insectivorous micro chiropterans, was used in the present study to delineate the histochemical profile of macromolecule in the pineal gland using developmental and reproductive state as the variable. Size of pineal gland, site and pattern of distribution of protein, DNA and RNA was used as the parameter of evaluation. Pineal size was greater in pregnant and lactating females i.e. up to July. However, it was smallest in the foetal pineal gland. Juvenile pineal size was significantly larger in August. In summer as the temperature increases hypertrophy of pinealocyte nuclei and nucleoli occurs. Mercurobromophenol blue DNA and RNA accumulation was highest in the pineal gland of foetal and torpor stage while lowest in neonate and pregnant state.

**Key Words** : Bats-Rhinopoma kinneari, Pineal gland, Pinealocytes.

## Introduction

The mammalian pineal gland displays considerable degree of morphological, histochemical diversity. The pineal gland is greatly influenced by photoperiod and other variants of the seasonal factors (15) Such changes are cyclic in numerous bat species and are linked with there physiological states (5,7,89,26). Seasonal rhythms are associated with changes in the length of the day (33,34). In addition, developmental and reproductive states are also linked with pineal gland activity.

Studies on the nexus between cyclic activities of the chiropteran pineal gland and gonadal cycles are scant and fragmentary (1,2,3,6). Hence the present report deals with a comparison of the pineal gland morphology and histology during foetal, neonate, juvenile and adult states of male Rinopoma kinneari. Similar studies were carried out on female with pregnancy and lactation as the other variables.

## Materials and Methods

Rhinopoma kinneari were collected from Bagor-ki-Haveli, Udaipur (Raj.), India (latitude 24/34 N, longitude 73/42 E). Bats were sexed and categorized on the basis of weight and reproductive state. They were sacrificed by cervical dislocation between 11 a.m. to 12 noon. The brain was removed. The pineal gland with habenular complex was carefully excised and fixed for routine light and electron microscopy. Histochemical detection of mercurobromophenol positive protein, DNA and RNA as per the method described in the Pearse (1968). The size of the pineal gland was determined by utilizing a Zeiss oculometer. The data were analyzed using a one-way ANOVA (12). Every alternate section (7$\mu$m) of pineal gland was visually appraised and microphotographed.

## Results

Histochemical loci of mercuro-bromophenol positive protein, DNA and RNA in the cellular elements of pineal gland during various developmental and reproductive state of Rhinopoma kinneari manifested characteristic staining profile.

The pineal gland was situated on the dorsal side of the brain between the two cerebral hemispheres, just posterior to the third ventricle and anterior to the optic lobe. It rested upon the dinecephalons and was highly vascularised by numerous superficial blood capilliaries.

Using the classification of pineal gland of Rhinopoma kinneari conformed to type A ( 4 ). It was characterized by an oval to pinecone shape and was located in the deeper aspect of the cerebral hermisphere. The gland was flattened in a dorsoventral direction. A compact and massive habenular complex grossly oversized was observed which coursed through the anterior aspect of the gland. The pineal parenchyma consists of two types of pinealocytes-light and dark make up bulk the gland ( 3 ).

Significant difference ($p<0.01$) in the width of pineal gland between two habenular nuclei was observed from foetal to juvenile stage in male bats. However, no significant difference was observed during various developmental and reproductive stage of female bats.

A significant difference ($p<0.01$) in the length of pineal gland between anterioposterior axes was observed in foetal and neonate, neonate and juvenile, non-breeding and breeding adult male bats. However, in female bats significant difference was observed between foetal and neonate, non-breeding and breeding, breeding and pregnant, pregnant and lactation stage.

Mercurobromo phenol positive protein in the male bats nucleoli of pinealocytes of foetus and torpor bats displayed intense staining. This was also true for nuclear membrane as compared to chromatin network and nucleoplasm. The staining intensity of neonate pinealocytes was observed milder as compared to the pinealocytes of foetal and juvenile state. The pinealocytes of non-breeding and breeding bats stained moderately as compared to ones of the juvenile phase. The nucleus of pinealocytes of non-breeding males stained slightly strongly than the pinealocytes

**TABLE 1 : Relationship between various development and reproductive state of Bat weight, Brain weight and pineal gland morphology in male Rhinopoma Kinneari**

| S.No. | Stages | Mean Bat wt. (gm)**N = 5 | Mean Brain wt. (gm)*N = 5 | Mean width of Pineal gland between two HC ( m)**N = 5 | Mean length of Pineal gland anterio post ( m)** N=5 | Mean area of transverse section of Pineal gland (sq. m)**N=5 |
|---|---|---|---|---|---|---|
| 1. | Foetal (at term) | 1.09 a | 0.104 a | 405.0 a | 197.4a | 62960.9a |
| 2. | Neonate | 4.91 4b | 0.245b | 254.6b | 360.2b | 71909.9a |
| 3. | Juvenile | 8.474c | 0.276bc | 532.4c | 262.4a | 112377.4b |
| 4. | Nonbredding | 12.554 d | 0.273bcd | 421.0ac | 330.0bc | 108392.8c |
| 5. | Breeding Adult | 23.781e | 0.259bcde | 431.2ac | 212.4a | 69818.8a |
| 6. | Hibernated | 13.912df | 0.284bcdef | 444.2ac | 262.8a | 91474.38abc |
| Standard error | | 0.6628 | 0.0348 | 28.81101 | 16.552 | 8653.433 |
| Critical diff. 5% | | 1.9348 | 0.1016 | 84.27 | 48.315 | 25258..582 |
| Critical diff. 1% variance (%) | | 2.66 | 0.1377 | 113.28 | 65.54 | 34265.519 |

* Significant at 5% ** Significant at 1% Legend HC = Habenular commissure
Within a coloum followed by the same letters are not significantly different.

nucleus of breeding stage. In all the stages, the endothelium of the blood vessels mainfested mild protein staining. The pinealocytes of feamles exibited intesne protein staining during both non-breeding and nulliparous stage. The nuclear membrane and nucleoli stained intensely. Mild staining was noticed in the pinealocytes of pregnant female. The nuclear membrance and nucleoli of pinealocytes in juvenile and lactating females exhibited moderate protein staning.

DNA and RNA staining patterns in the pineal gland of male torpor bats nuclear membrane and nucleoli of pinealocytes stained intensely for DNA and RNA. Positive pyronin Y staining was exhibited by cytoplasm. The nucleoli of pinealocytes of foetal bats stained strongly with methyl green and pyronin Y. In juvenile males, methyl green staining was observed in the nucleus of pinealocytes. In non-breeding male, the nucleus showed weak methyl green staining. In breeding males, the meythyl green staining was subdued due to predominance of pyronin Y stain. In case of neonate female the nucleolus of pinealocytes did not manifest methyl green staining. In juvenile female, the nucleoli, nuclear membrane of pinealocytes did not manifest methyl green staining. In non-breeding moderate methyl green staining was observed. However, in breeding female it was intense. The cytoplasm of the pinealocytes showed intense pymonin Y staining. In pregnant stage the nucleoli of pinealocytes no stained by methyl green and intensely with pyronin Y. In lactating stage the pineal gland showed moderate to intense methyl green staining and light pyronin Y staining.

Pinealocytes are not only the principal cellular components of the pineal gland, but they are also the principal synthetic machinary of this enyzmatical gland with highly diverse and often questionable empirical roles assigned to it. Pineal gland is an important integral component of neuro endocrine axis. Pinealocytes were of juvenial stages very large round to oval encetric nucleolus, several large clumps of heterochromatin disperce in the nucleoplasm. Nerve fibers tightly contacting pinelocytes. Infolding of ependy make larger the size ofthe pineal gland at juveniale stage. Fotel pinalocytes & nuclei are large and scatter then the other stage. The pineal gland Rhinopoma kinneari consists neurogilian cells and pinealocytes of two types e.g., (i) light and (ii) dark. Secretory granules are observe in the supra pineal recesses. The population of dark pinealocytes, and melatonin pigmentation are observe it may mean greater biosynthesis and elavoration of hormone. Which would evendtly supress folliculogenesis. The blood supply to the gland is observed from the posterior choroidal branches of the posterior cerebral arteries. The gland has copious blood supply. In advanced age the pineal gland of pregnant and torpor stage shows mulbary shape lobules of calcium deposits (Corpors aranacea). The pineal is innervated by sympathetic axons - post gangolionic sympathetic axons arrive in the pineal from perikariya located in the SCG. The striatd muscle fibers lie primarily near the surface of the gland and their association with blood vessels has been noted.

Two population of pinealocytes have been described which deffered in the contents of organelles. Granular vasicles, cytoplasmic, vacuoles, glycogen granules rarely observed connectric lamellae, gaint mitochonidria with parallel bundles of microfillaments are observed.

### Pineal gland of foetus

The tiny pineal gland of fully devloped foetus was characterised by rosette-like arrangement of cells. Two structurally different types of pinealocytes were observed. These were the light and dark pinealocyte which differed in the degree of their cytoplasmic density and staining affinity for eosin.

The pinealocyte nuclei were oval in shape. They were prominent with very large round to oval nucleus which was eccentrically

placed. Secretory granules of various shapes and size were observed on the pripheral aspects of these cells. The cytoplasm of these cells was weakly eosinophilic. The mean diameter of pinealocyte nucleus was 28 $\mu$m. Intra pineal neurons and blood vessels were also observed.

### Pineal gland of Neonate

The two types of pinealocytes were found and had nuclei with granular chromatin material. Pinealocytes were tightly packed together. The cytoplasm of these cells was strongly eosinophilic. The mean diameter of pinealocytes nucleus was 21 $\mu$m. Intra pineal neurons, cavities, and blood vessels were also observed.

### Pineal gland of juvenile male

The pinealocytes were arranged in rosette pattern and were tightly packed. The mean diameter of pinealocytes nucleus was 31 $\mu$m. Two types of pinealocytes could be recognized i.e. the light and dark types. They differed in the degree of their cytoplasmic density and staining affinity for eosin.

### Pineal gland of juvenile female

The pinealocytes were round to oval in shape and contained granular cytoplasm, which was weakly eosinophilic. Pinealocytes were observed to be highly eosinophilic. Pinealocytes were observed to be tightly packed. The nucleus of pinealocytes was large and contained prominent nucleoli. The mean diameter of nucleus was 27 $\mu$m. Intra pineal neurons, intra-pineal cavities, and blood vessels were also observed.

### Pineal gland of adult male

The pinealocytes were round to oval in shape and were of two types viz. light and dark. In the light pinealocytes cytoplasm was agranular and stained weakly. However, in dark pinealocytes, the cytoplasm was strongly eosinophilic. These pinealocytes were randomly arranged and did not manifest any specific pattern. The mean diameter of nucleus was 30 $\mu$m. The nucei of pinealocytes also exhibited differential basophilia. Intrapineal neurons; intra pineal cavities and blood vessels were also observed.

### Pineal gland of adult female

Pinealocytes were closely packed and were not surrounded by connective tissue element. Non uniform arrangement of pineal parenchymal cells was observed. Two structurally different types of pinealocytes were observed. These were the light - and dark-pinealocytes. The relative population of light pinealocytes was observed to be much higher than the dark pinealocytes. These cells differed in their degree of cytoplasmic density. No inter mediate stages between the light and dark pinealocytes were discernible. Pinealocytes exihbited signs of synthetic activities. Their cytoplasm was weakly eosinophilic.

The pinealocytes were characterized by the presence of a large and oval nucleus. The nucleus was characterised by large quantity of stainable euchromatin and prominent basophilic nucleolus. The mean diameter of pinealocytes nuclei in female during the non breeding stage was 22 $\mu$m, and during breeding stage it was 26 $\mu$m. In pregnant females it ws 27 $\mu$m and in lactating females it was 25 $\mu$m.

**TABLE 2:Relationship between various development and reproductive state of Bat weight, Brain weight and pineal gland morphology in female Rhinopoma Kinneari**

| S.No. | Stages | Mean Bat wt. (gm)**N = 5 | Mean Brain wt. (gm)*N = 5 | Mean width of Pineal gland between two HC ( m)**N = 5 | Mean length of Pineal gland anterio post ( m)** N=5 | Mean area of transverse section of Pineal gland (sq. m)**N=5 |
|---|---|---|---|---|---|---|
| 1. | Foetal (at term) | 1.09 a | 0.104 a | 405.0 a | 197.4a | 62960.9a |
| 2. | Neonate | 5.353b | 0.234b | 509.4a | 276.6b | 110675.4b |
| 3. | Juvenile | 8.602c | 0.252ab | 384.6a | 286.6bc | 86346.8a |
| 4. | Nonbredding | 12.608 d | 0.267ab | 258.8a | 264.0abc | 57570.3a |
| 5. | Breeding Adult | 16.458c | 0.260ab | 376.4a | 375.0d | 110078.8bc |
| 6. | Pregnant | 14.796def | 0.232ab | 497.4a | 227.0ac | 91065.4ab |
| 7. | Lactation | 14.979defg | 0.240ab | 524.2a | 350.6cde | 114925.7d |
| Standard error | | 0.7864 | 0.043 | 90.84 | 18.1199 | 8728.288296 |
| Critical diff. 5% | | 2.277 | 0.1262 | 265.1 | 52.480 | 25279.58079 |
| Critical diff. 1% | | 3.002 | 0.1641 | 346.8 | 69.187 | 34068.185053 |
| Coefficient of variance (%) | | 16.658 | 42.805 | 48.10 | 14.3417 | 20.586858 |

* Significant at 5% ** Significant at 1% Legend HC = Habenular commissure

Within a coloum followed by the same letters are not significantly different.

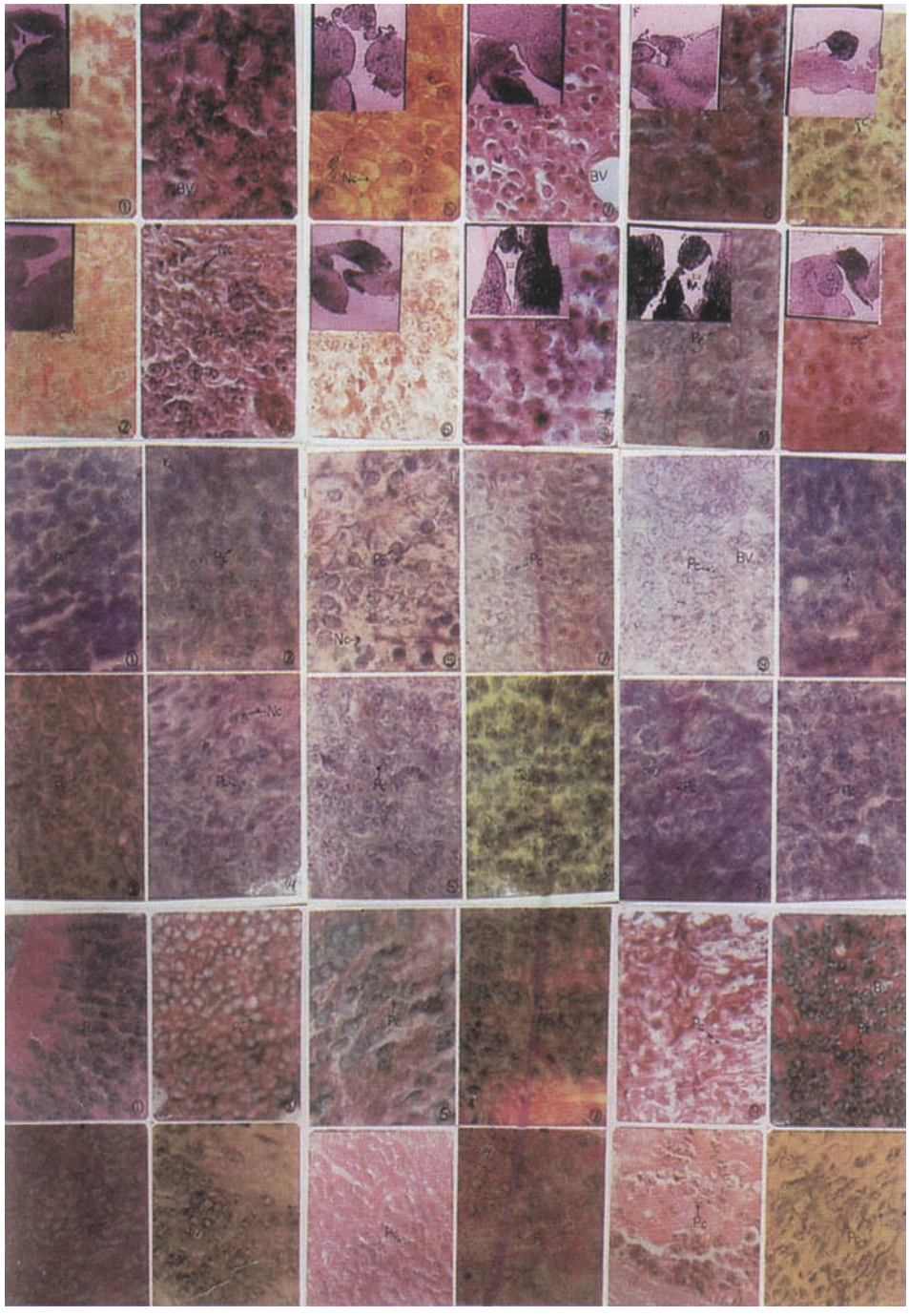

Plate 1- Showing pineal gland histochemical localization of hematoxyline-eosin staining (Plate-A) Protein staining (Plate-B), DNA-RNA staining (Plate-C) in various developmental and reproductive stage of rat tailed but Rhinopoma kinneari atx1000 (insert at x 100) fig. 1 Pinealocytes of fetus at term- Rosette like arrangement of cells. Dark pinealocytes and occasionally light pinealocytesare seen. Oval nuclei are eccentrically placed. Secretary vesicle are observed in supra pineal recess and on peripheral aspect of these cells. Fig. 2 Pinealocytes of pregnant state-Cell are tightly packed. Strongly eosinophilic cytoplasm. Brain sand are lobular and mulberry shapes. fig.3 Pinealocytes of neonate- Two types of pinealocytes are seen. Intra pineal neurons, cavities and blood vessels are seen. fig. 4 Pinealocytes of lactaing state- Relative population of light pinealocytes aare lossely arranges due to folding of cell. Light and Dark pinealocytes are seen. They differed in the degree of their cytoplasmic density and staining affinity fig.6 Pinealocytes of juvenile female-Round to val pinealocyte nucleolus is very charascteristic. Note the nucleolus hidden underneath the large clump of heterochromatin. Nerve fibers of various sizes run in between the pinealocytes Lipid droplet and glycogen granule are seen. fig.7 Pinealocytes of Non-Breeding male-Pinealocytes cytoplasm are a granular and stained weakly. Intra pineal neurons, intra pineal cavities and blood vessels are seen fog. 8 Pinealocytes of Non-Breeding female-Round prineal gland showing less innervations of neuron fig.9 Pinealocytes of Breeding male-Left side of invervations note more thicker than right side. fig. 10 Pinealocytes of breeding female fig. 11 Pinealocytes of torpor stage note corpora arenacia fig. 12 Pinealocytes of tropor female note large round to oval eccentric nuceolus, several large clumps of heterochromatin disperse in the nucleolus.

B.V. - Blood Vessel, E- Ependyma, HC- Habenular commissure, HN- Habenular nuclei, NC- Nerve cell, P-Pineal gland, Pc- Pinealocyte, III- Third ventricle.

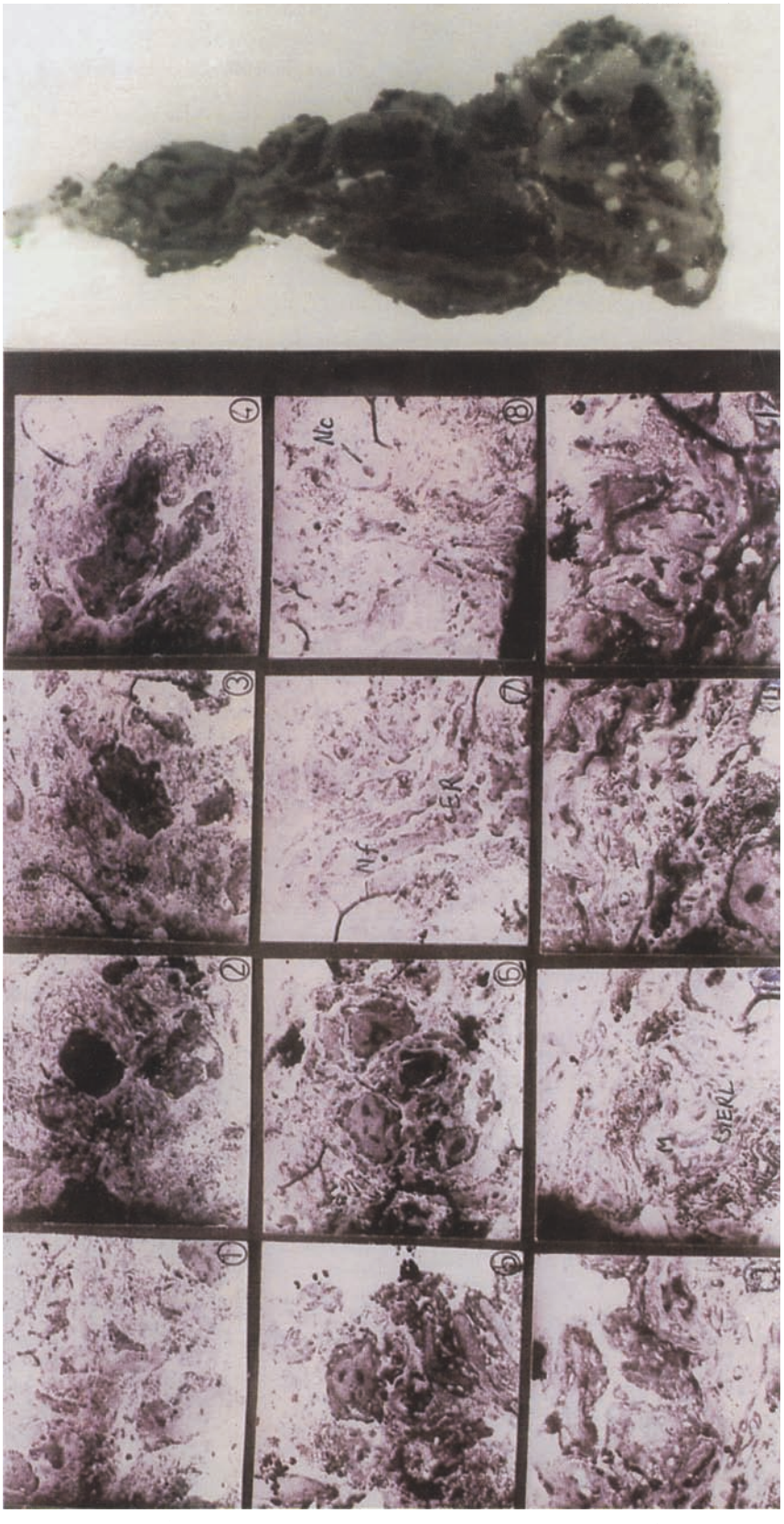


Plate 2 - Showing Electron Micrograph of Breading Adult Female Pineal with habenular nuclei of Rat Tailed bat "Rhinopoma Kinneari" in Joel 100 S electron microschope. Fig. 1 to 8 at x 7000 and fig. 9 to 12 at x 12000 in large format. Unmylinated Nerve cell- having gxon and dendrite, Dense Core vesicle, Mitochonadria, Golgi Body, rough endoplamic reticulumm, Smooth endoplasmic reticulum glycogen granules, Lipid droplets, deviding cell, vocoules gllal cell, melanin pigment, estrocyytes, mast cell, corpora arenacia and skeletal fibers are seen.

## Discussion

The present study highlights the morphological and histochemical characteristics of the pineal gland of an insectivorous micro chiropteran Rhinopoma kinneari during various developmental and reproductive states.

The pineal gland of R. kinneari conformed to type A (4, 31). In the present study increase in width of the pineal gland was inversely proportional to the increase in the length from one stage to the next stage at different developmental and reproductive stagte. The reproductive state is usually reflected in the appearance of the pinealocytes. A feedback system was proposed to be involved between the gonads and pineal gland (28).

Seasonal cycle in the activity of the pinealocytes of bats, Myotis lucifugus and Myotis sodalis. Nuclear diameter of pinealocytes reached a peak in March, which coincided with time of their arousal from hibernation (25, 26). In these bats pinealocytes appeared to become most active during the lenghthening days of early spring. The size of the pineal appeared to be governed by the principle that darkness enhances the pineal activity (15). This principle appears to be applicable to the nocturnal Rhinopoma kinneari used in the present study.

Mercurobromo phenol protein accumulation in case of male was highest in foetus and torpor bats. This staining was localized in the nucleoli and nuclear membrance. In female of this species the concentration of mercurobromphenol protein was found to be highest at breeding and non-breedings stages. While lowest protein concentration was observed in the neonate stage of the male and in the pregnant stage of the female. In pinealocytes of some mammals the proteinaceous component in the pinealocytes may serve as carrier for indoleamines products consisting of combination of peptidergic hormone and indoleamines (14, 16).

In the pinealocytes of cotton tail rat are proteineous nature. Seasonal changes in the number of vesicles that contain protein (19, 20).

Greatest protein synthetic activity in pinealocytes during the early afternoon with a peak at about 4 p.m. Nucleolar volumes of cells are generally largest during or before the increase in nucleolar RNA, which precedes maximum cytoplasm protein synthesis (21, 27). Enhanced protein synthesis probably an indication of altered physiological state of the cell (33) and the daily fluctuations in pineal level of RNA and DNA an immature (21 day old) female and adult male rats. In mature male rats a peak level was reached at about 4 p.m. marked daily fluctuations in pineal RNA were recorded in mature male rats A definite peak occured at noon and sharp nadir at midnight. No significant changes were noted in RNA levels of female animals although a similar pattern in their diurnal rhythm was apperent. This may be attributed to metabolic fluctuation brought by the oestrus cycle and extrinsic factors affecting ovulation. No comparisons are feasible with the data obtained in the present study since all bats were sacrificed in late forenoon.

A significant decrease in the length, but in width of pineal gland of neonate of juvenile stage. Prenatal melatonin comes from the mother by placental transport in the first week of life. Melatonin was acquired by pups through sucking the mother's milk in the second and the third weeks (10). In mammals, the relatioship of the foetal pineal and the mother's interaction appears to be a case of synchronization of circadian rhythmicity.

The Significantly enlarged pineal gland was observed during juvenile stage that is from August and pineal gland of lactating stage that is July. The maximum reduced pineal gland was observed during nulliparous and foetal.

Significant sexual differences have been noticed in the pinealocytes of R. kinneari. The pinalocytes nuclei was larger in female bats than in male bats. In rat pineal gland a significant difference between male and female pinealocytes occured in all age groups. The number of nuclei per unit area was larger in female rat than in male (18).

Thus, the results of the present study on the changes in the morphology, histochemistry of the pineal gland of Rhinopoma kinneari clearly demonstrate stage specific characteristics. Significant alterations have been observed in the morphology of the gland. These differences become sharply defined as the bats transit from one stage to other stage. The present studies clearly indicate that protein, DNA and RNA profile of pinealocytes undergoes profound changes during various developmental and reproductive states.

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## Appendix - 1.1
## Anova of relationship between pineal gland morphology and body weight at male Rhinopoma kineari during various developmental and reproductive states

| Source | df | Bat weight (gm) | | | Brain weight (gm) | | | Width of pineal between 2 HC (um) | | | Length of pineal anter to post. (um) | | | Area of transverse section of pineal galnd (um) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SS | MS | fca | SS | MS | f cal | SS | MS | f cal | SS | MS | f cal | SS | MS | f cal |
| Stages | 5 | 1578.08 | 115.00 | 143.85** | 0.1449 | 0.229 | 3.7920* | 203807.86 | 40761.57 | 9.7796** | 102148.23 | 23429.65 | 14.9125** | 11090933840.4 | 2218186728/08 | 5/9244 |
| Error | 24 | 52.7272 | 2,1969 | | 0.1455 | 0.00606 | | 100034.00 | 4168.0 | | 32879.2 | 1369.96 | | 8985829216.03 | 274409550.66 | |
| Total | 29 | 3630.7672 | | | 0.0604 | | | 303841.86 | | | 135027.47 | | | 2007676856.40 | | |

Significant difference at 15.

Signature differ once at 5 :

## Appendix 1.2
## Anova of relationship between pineal gland morphology and body weight at female Rhinopoma kinneri during various developmental and reproductive states

| Source | df | Bat weight (gm) | | | Brain weight (gm) | | | Width of pineal between 2 HC (um) | | | Length of pineal anter to post. (um) | | | Area of transverse section of pineal galnd (um) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SS | MS | fca | SS | MS | f cal | SS | MS | f cal | SS | MS | f cal | SS | MS | f cal |
| Stages | 6 | 985.26 | 197.052 | 63.7296** | 0.0923 | 0.0153 | 1.6105* | 4818211.50 | 809035.25 | 19.4614** | 2635571.0 | 43875183.0 | 267.25** | 27416249660.38 | 4569374943.39 | 11.995 |
| Error | 28 | 86.582 | 3.092 | | 0.2685 | 0.0095 | | 1155361.5 | 41262.91 | | 45967.00 | 1641.67 | | 10665622322.67 | 360915082.95 | |
| Total | 34 | 1071.8421 | | | 0.3608 | | | 5973573.00 | | | 2678538.00 | 38081871983.066 | | | | |

**Significant difference at 1 t.