CHAPTER 1

Neonatal functional pinealectomy by light shows adverse effect on adult testis functions and alters neuroendocrine homeostasis

Form the time pineal was recognized as an endocrine gland, its relation with gonads has been under investigation (Theibolt, 1960; Motta *et al.*, 1967; Reiter, 1981). Pinealectomy has been shown to increase reproductive activity in mammals (Reiter, 1973; Reiter, 1980; Reiter *et al.*, 1985). Nevertheless, pinealectomy of the adult rat seems to have little or no stimulatory influence on the gonads (Motta *et al.*, 1967; Pitis and Maya, 1969; Reiter, 1973, 1980; Reiter *et al.*, 1985). A previous study on neonatal pinealectomy had shown increased adult testes size and germ cell number (Sharma, 1996). Reproductive functions in many species are known to be responsive to changes in environmental photoperiodicity (Berndtson and Desjardins, 1974; Wurtman, 1975; Lincoln and Short, 1980). Different laboratory strains of *Rattus norvegicus* also differ in their responses to photoperiod with most strains tested having either no response or slight to moderate reproductive inhibition and/or reduced growth rate (Cohen and Mann, 1979; Vanecek and Illnerova, 1982; Rivest *et al.*, 1986; Aubert *et al.*, 1989; Boon *et al.*,

1997). The weak photoperiodic responses of most strains coupled with the very short critical photoperiods for those effects have lead to the characterization of rats as being functionally non-photoperiodic (Reiter, 1980; Wallen and Turek, 1981; Bronson, 1989; Nelson *et al.*, 1994). However, a reproductive response to short days (SD) and / or melatonin treatment can be induced in adult male Sprague-Dawley, Wistar, and some other strains of rats by several procedures; food deprivation, neonatal androgen treatment, chronic exposure to exogenous testosterone or olfactory bulbectomy (Reiter *et al.*, 1968; Reiter *et al.*, 1969, 1971; Sorrentino *et al.*, 1971; Wallen and Turek, 1981; Peiper *et al.*, 1990). Constant darkness (Fiske, 1941), blinding (Reiter *et al.*, 1968) or very short days (Kinson and Robinson, 1970; Kinson and Peat, 1971) have been shown to delay puberty in some strains of rats, but the effects reported have been slight on the order of 1 to 2 week delay in vaginal opening or a 0-2% smaller testis size relative to controls. In the Wistar strain, there was inhibited testes development by constant dark (Hoffman *et al.*, 1986). In another study of short photoperiod, testes development of Wistar or Sprague-Dawley rats was either not affected (Vanecek and Illnerova, 1982; Heideman and Sylvester, 1997) or moderately inhibited (Rivest *et al.*, 1986). However, the effect of exposure of neonates to a long photoperiod on adult testis functions has not been studied. Hence in the present study, rat neonates have been

exposed to continuous light from day 0 to day 21 (light induced functional pinealectomy - LFPx) and body and testes weight, histomorphology and hormonal titres have been assessed at 35, 45, 60 and 90 days of age.

MATERIALS AND METHODS:

Animals and Maintenance:

Healthy male laboratory rat neonates (Charles Foster strain) were used for the present study. The animals were maintained in Sarabhai Research Center, with a constant temperature range of 21 ±2°C and under a lighting regimen of LD 8:16 or LD 24:0 throughout the experimental period of study. The animals were fed with standard diet (Amrut Rat Feed) and water *ad libitum*. The treatment was initiated on day '0' (day of birth) and terminated on day 21 postpartum.

Experimental Protocol:

The experimental setup was divided into two groups of study consisting of 6 animals each.

Group I: Control (C)

Male rat neonates were maintained under normal lighting regimen of LD 8:16 maintained as such.

Group II: Functional Pinealectomy by continuous light (LFPx)

Male Rat neonates were functionally pinealectomised by exposing them to continuous light of 250 lx intensity (LFPx) from day '0' to day '21'.

Lighting schedules

Controls rats were exposed to a photoschedule of LD 8:16 with lights on at 09.00 hrs and lights off at 17.00 hrs. The experimental rats were exposed to continuous light (LD 24:0) with lights on fully in a separate enclosure. Lighting was from white fluorescent tubes and with an intensity of 250 lx.

Parameters and Methods of Evaluation:

The treatment was discontinued from day 22 and the animals were sacrificed at 35, 45, 60 and 90 days of age and various morphometric, gravimetric and histocytometric studies were carried out. The animals were sacrificed under mild anaesthesia and blood was collected by brachial venipuncture in epindorff tubes. They were centrifuged at 4000 rpm and serum was collected and stored at -4°C. Later, these serum samples were utilized for assay of various hormones. The viscera was cut open and testes were excised, blotted free of tissue fluids and weighed accurately in a Mettler balance. The absolute weights so obtained were converted to

relative weights and expressed as percentage of body weight. The testes were then fixed in Bouin's fluid and processed for paraffin wax histology.

Histology and Histometry:

Testes were fixed immediately in Bouin's fluid and processed for histological studies. Paraffin sections of 5μm thickness were cut on a microtome and stained with Haematoxylin-Eosin (HE). For morphometry and enumeration of seminiferous tubules, homologous cross-sections from the middle part of testis showing the largest cross-sectional diameters were chosen. The diameter of seminiferous tubules and germinal epithelial thickness were measured using an ocular micrometer. Total count of Sertoli cells was done in 20 tubular cross sections. The total Sertoli and germ cell number were estimated using morphometric methods based on the count of round objects in sections of known thickness, modified from the reports of Wing and Christensen (1982) and Russell *et al.* (1990). Inherent error was corrected using Floderus equation (Floderus, 1944). The entire enumeration procedure was done as discussed earlier (Ramachandran *et al.*, 2001). The following parameters were determined:

Testicular volume (T_V) in cubic cm

$$T_V = \frac{\text{Absolute weight of single testis}}{\text{Sp. gravity of water (1.04)} \times \text{Fixation shrinkage (1.053)}}$$

Testis sectional area in cm^2 (T_A)

$T_A = \pi r^2$ (r = radius of the cross section of testis)

Seminiferous Tubule diameter in cm (S_D)

$S_D = 2 r_s$ (r_s = mean radius of seminiferous tubules)

Seminiferous Tubule area in cm^2 (S_A)

$S_A = \pi r_s^2$

Effective Tubular area in a testis cross section (E_A)

$E_A = T_A - I$ (I = interstitial area) (I is 5% of T_A)

Number of tubules per section (N_T)

$$N_T = \frac{E_A}{S_A}$$

Relative area of tubule in cm^2 (R_T)

$$R_T = \frac{E_A}{T_A}$$

Seminiferous tubule volume in cm^3 (S_V)

$$S_V = R_T \times T_V \qquad \text{(Hess } et\ al.,\ 1993\text{)}$$

Seminiferous tubular length in cm (S_L)

$$S_L = \frac{S_V}{S_A}$$

Total basement membrane area in cm^2 (bm)

$$bm = 2\,\pi\, r_s\, S_L$$

Total number of Sertoli cells in testis (SC_N)

$$SC_N = \left[\frac{SC_T}{\text{thickness of section}}\right] \times S_L$$

(SC_T = number of Sertoli cells per seminiferous tubule)
(thickness of section in cm, 0.0005)

Total number of Sertoli cells per unit basement membrane area in (cm^2) (SC_{bm})

$$SC_{bm} = \frac{SC_T}{bm}$$

Theoretical Germ cell number (GC_N) in a tubule section

$$GC_N = \frac{GC_a}{GC_{a1}}$$

(GC_a = effective area – area occupied by Sertoli cells)

1. effective area of tubule section containing cells – ea
2. Sertoli cell area – Sca
3. Area occupied by one germ cell – GC_{a1}

ea = Area of tubule – Area of lumen

Note: Area of tubule is calculated by subtracting the area of lumen from area of tubule section (the diameter of lumen is calculated by subtracting the germinal epithelial height (GE) × 2, from the diameter of tubule)

$GC_a = GC_N$ with Correction of area occupied by Sertoli cells

$(GC_a = ea - SC_a)$

SC_a = Sertoli cell area in tubule section

$SC_a = SC_N \times$ area of one SC

$(SC - 2\pi rh)$

* r = 0.000075 cm radius of Sertoli cell

* h = 0.009 cm is the Sertoli cell height

= 0.00004239 cm^2 is average Sertoli cell area based on standard Sertoli cell dimension known

GC_{a1} = Area of one germ cell

$(GC_{a1} = \pi r^2)$ (r- radius of germ cell i.e. 0.000675)

∴ Average GC_{a1} = 0.000014313882 cm^2

$= 1.4314 \times 10^{-5}$ cm^2

Theoretical Germ cell number per testis (TGC_T)

$$TGC_T = \frac{GC_N \times S_L}{\text{thickness of section}}$$

(section thickness = 0.0005 cm)

TGC_T = Final theoretical count with correction of inherent error using Floderus equation

$$TGC_T = \frac{\text{Total germ cell count}}{D + T - 2h}$$

D – Diameter (2.5×10^{-7})
T – Section thickness (0.0005 cm)
h – height of smallest recognisable nucleus, which is assumed to be 0.00001 cm
i.e. D + T – 2h = 5.002

Theoretical Germ cell number per meter length of seminiferous tubule (TGC_M)

$$TGC_M = \frac{TGC_T}{S_L}$$

True germ cell count per section (TGCs) (with correction of inherent error using Floderus equation)

$$TGCs = \frac{\text{Raw count}}{D + T - 2h}$$

Raw count is the number of germ cells counted in terms of nuclei at stages V and VI under the microscope and D + T – 2h = 5.002, as calculated above

Actual Germ cell number per Testis (AGC_T)

$$AGC_T = TGCs \times S_L$$

Actual Germ cell number per meter length of seminiferous tubule (AGC_M)

$$AGC_M = \frac{AGC_T}{S_L}$$

Degeneration of germ cells

Theoretical germ cell count – True germ cell count

Hormone Assays:

The blood for hormone assays was collected from the brachial vein under mild anesthesia before sacrificing the animals. T_3 and T_4 were assayed by ELISA using kit purchased from Glaxo (product code H - T_3H-0010 and H-T_4H-0010) and expressed in ng/ml of serum.

TSH, LH, corticosterone and testosterone were assayed by RIA. Rat TSH (NIDDK-rTSH-I-9) and LH (NIDDK-rLH-I-7), were iodinated by the chloramine T method with carrier free ^{125}I obtained from Amersham International Plc as described by Greenwood *et al.* (1963). Pure rat hormones (2.5 μg) were incubated with specific concentrations of chloramine T (5 μg for TSH; 7 μg for FSH; 4 μg for LH) for 45s. The reaction was stopped with 20 μl sodium metabisulphite (80 μg), and purified in a PD-10 column pre-saturated with barbitone buffer (0.07 M) and pre-coated with BSA (1%) (Pharmacia LKB Biotechnology, Bromma, Sweden), eluted in

barbitone buffer and tubes with peak specific activity of 60-80 μCi/μg were used for RIA.

☒ RIA of Peptide hormones:

Peptide hormones were measured by liquid-phase RIA using specific antibodies and reference preparations from NIDDK. Antisera were anti-rTSH-S-5, anti-rFSH-S-11, anti-rLH-S-10, rFSH-RP2 and rLH-RP-3. Hormones and antisera were dissolved in peptide assay buffer (pH 7.4) containing sodium dihydrogen phosphate (3.05 g/l), sodium chloride (8.8 g/l), sodium azide (100 mg/l) and BSA (5 g/l). EDTA (0.025 M) was also added. The tracer was diluted in the peptide assay buffer containing 0.5 % normal rabbit serum to have approximately 20,000 - 30,000 c.p.m./100 μl tracers. Anti-rabbit γ-globulin (ARGG) in peptide assay buffer (1:26) and 8 % polyethylene glycol (PEG) in saline were used for separation of bound and free hormones.

Two hundred micro litres each of antiserum, sample and tracer in 600 μl assay buffer were incubated for 24 h at 4°C, followed by the addition of 100 μl ARGG and the incubation was continued for another 24 h. At the end of the second incubation, 1 ml PEG was added and centrifuged at 1500 g for 45 min. The radioactivity in the pellet was counted for 1 min in a microprocessor-based LKB gamma counter. The sensitivity of the assays was as follows: TSH 0.01

ng/ml, FSH 0.2 ng/ml, and LH 0.14 ng/ml. Inter-assay variations were TSH 8.2 %, FSH 12.2 %, and LH 9.9 %. Intra-assay variations were TSH 4.7-6.9 %, FSH 5.7-8.9 % and LH 4.9-8.4 %. The antibodies were highly specific to the respective rat antigens with very minimal cross-reactivity with other peptides as per NIDDK specifications. The maximum binding of the antibodies recorded by us was TSH 39 %, FSH 30 %, and LH 32 %.

☒ RIA of Testosterone:

Testosterone was assayed by liquid-phase RIA using the protocol and reagents from World Health Organisation. Maximum binding of the testosterone antiserum was 40 % and its cross-reactivity to cortisol, dehydrotestosterone, androstenedione and Δ^5-androstenediol was 0.001 %, 14 %, 0.8 % and 6 % respectively. The inter-assay and intra-assay variations were 6 % and 4 % respectively. The sensitivity of the assay was 0.3 pg/ml.

☒ RIA of Corticosterone

The RIA for corticosterone was carried out by RIA kits, procured from Amersham International Plc. and were expressed as ng/ml.

ng/ml, FSH 0.2 ng/ml, and LH 0.14 ng/ml. Inter-assay variations were TSH 8.2 %, FSH 12.2 %, and LH 9.9 %. Intra-assay variations were TSH 4.7-6.9 %, FSH 5.7-8.9 % and LH 4.9-8.4 %. The antibodies were highly specific to the respective rat antigens with very minimal cross-reactivity with other peptides as per NIDDK specifications. The maximum binding of the antibodies recorded by us was TSH 39 %, FSH 30 %, and LH 32 %.

☒ RIA of Testosterone:

Testosterone was assayed by liquid-phase RIA using the protocol and reagents from World Health Organisation. Maximum binding of the testosterone antiserum was 40 % and its cross-reactivity to cortisol, dehydrotestosterone, androstenedione and Δ^5-androstenediol was 0.001 %, 14 %, 0.8 % and 6 % respectively. The inter-assay and intra-assay variations were 6 % and 4 % respectively. The sensitivity of the assay was 0.3 pg/ml.

☒ RIA of Corticosterone

The RIA for corticosterone was carried out by RIA kits, procured from Amersham International Plc. and were expressed as ng/ml.

STATISTICAL ANALYSIS:

All data are expressed as mean ± SEM. The data were analysed by student's 't' test and analysis of variance (ANOVA) wherever applicable, at 95% confidence limit.

RESULTS:

Body and Testes weight

The body weight gain of LFPx rats was significantly higher at 45 and 60 days while at 90 days the weight was identical in both control and LFPx rats. Though there was no difference in the absolute and relative weights of testes between control and LFPx rats at 90 days, the relative weight of testes of LFPx rats was significantly lower at 45 and 60 days (Tables 1.1 & 1.2; Figs. 1.1a, 1.1b, 1.1c, 1.2a & 1.2b).

Table 1.1: **Chronological alterations showing body weight (g) and absolute (g) and relative (g/100) testes weight of control and functionally pinealectomised rats**

Treatment	Body Weight				Absolute Testes Weight				Testes Relative Weight			
	Age in Days				Age in Days				Age in Days			
	35	45	60	90	35	45	60	90	35	45	60	90
C	96.00 ±3.804	123.00 ±3.677	197.00 ±6.396	361.00 ±6.280	0.860 ±0.050	1.350 ±0.058	2.600 ±0.085	3.301 ±0.104	0.900 ±0.033	1.10 ±0.027	1.33 ±0.033	0.92 ±0.030
LFPx	108 ±6.396	157[c] ±2.456	268[c] ±7.496	348 ±16.730	0.94 ±0.051	1.36 ±0.030	2.67 ±0.085	3.11 ±0.140	0.87 ±0.038	0.87[c] ±0.006	1.00[c] ±0.017	0.90 ±0.024

C – Control; LFPx – Functionally Pinealectomized (Continuous Light)
Values expressed as Mean ± SEM of six animals. [a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.0005$

Table 1.2: Per day Body and Testes Growth Rate (g/day) in Control and Functionally pinealectomised rats

Treatment	Per Day Body Growth Rate				Per Day Testes Growth Rate			
	Age in days				Age in days			
	0-35	35-45	45-60	60-90	0-35	35-45	45-60	60-90
C	2.583	0.589	1.247	1.815	0.024	0.011	0.021	0.008
LFPx	2.934	1.07	1.863	0.888	0.027	0.009	0.022	0.005

C – Control; **LFPx** – Functionally Pinealectomized (Continuous Light)

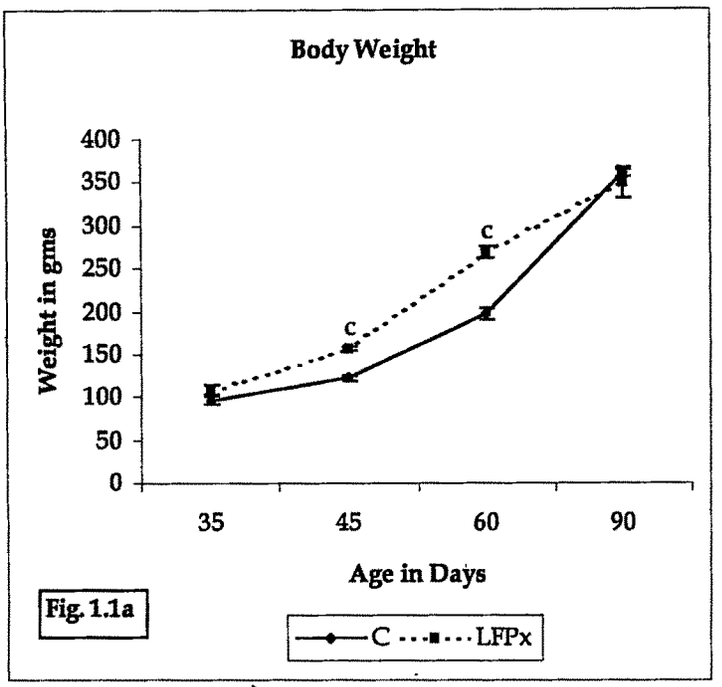


Fig. 1.1a

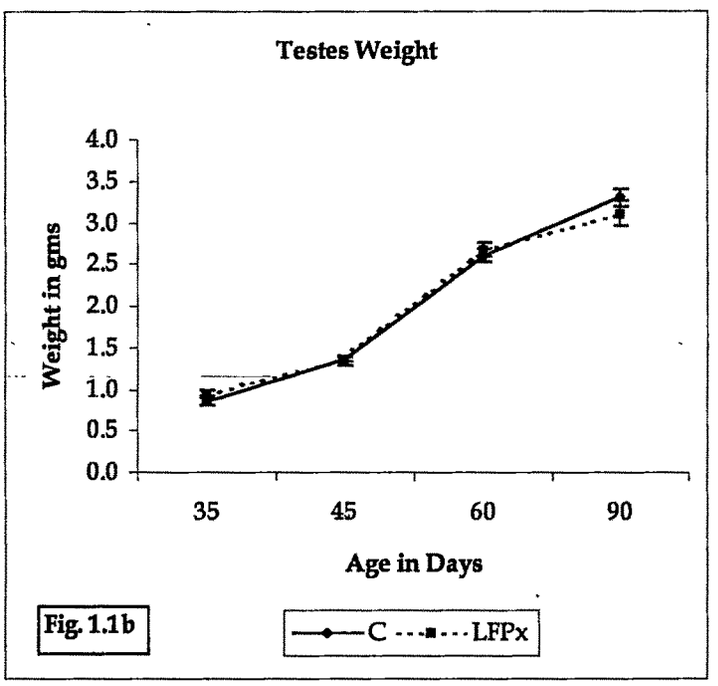
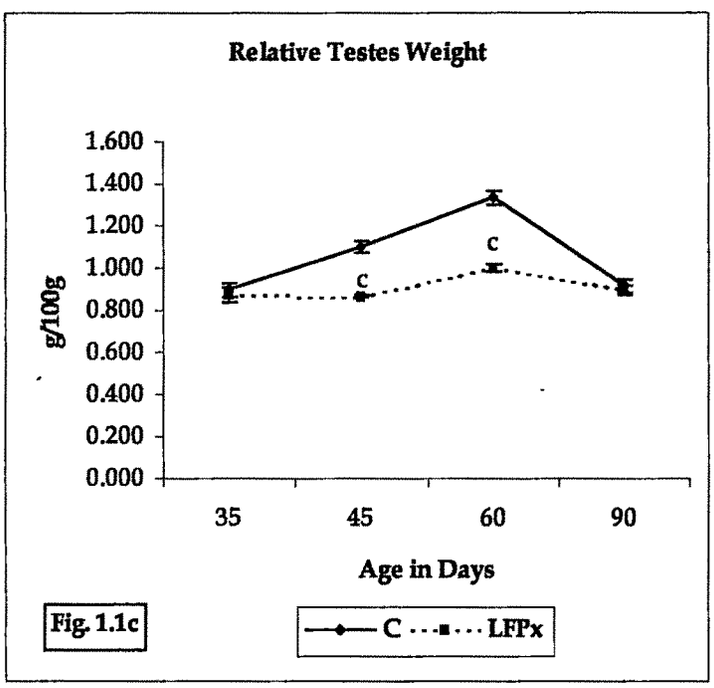


Fig. 1.1b

Figures 1.1a &b: Chronological alterations showing body and testes (g) weights in control (C) and functionally pinealectomised (LFPx) rats

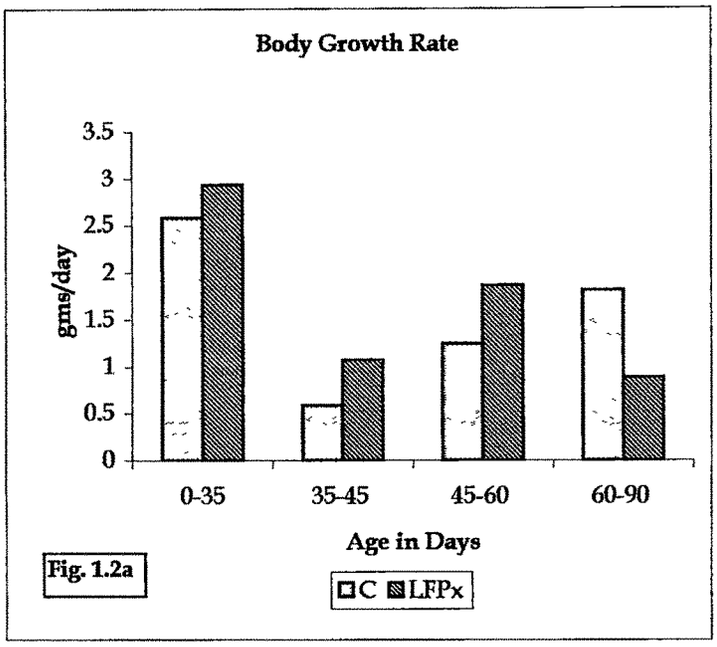
Values expressed as Mean ± SEM of six animals; [a]$p<0.01$; [b] $p<0.005$, [c]$p<0.0005$

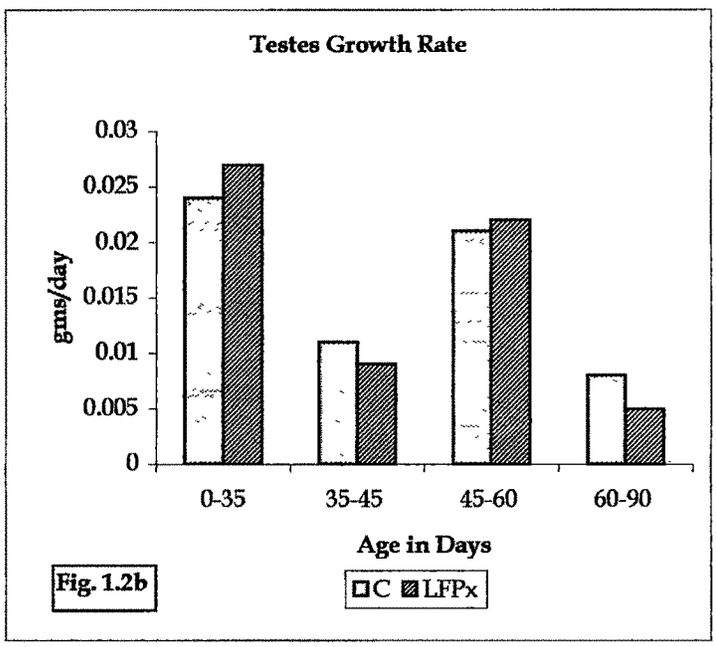


Figures 1.c: Chronological alterations showing relative (g/100g) testes weight in control (C) and functionally pinealectomised (LFPx) rats

Values expressed as Mean ± SEM of six animals;

[a]$p<0.01$; [b] $p<0.005$, [c]$p<0.0005$





Figures 1.2a &b: Chronological alterations showing body and testes (g/day) growth rate in control (C) and functionally pinealectomised (LFPx) rats

Values expressed as Mean ± SEM of six animals;

[a]$p<0.01$; [b] $p<0.005$, [c]$p<0.0005$

Histology and Histometrics of Testis

The sections of testis of LFPx animals at 35 days showed no significant difference from those of control animals except for a slightly smaller tubular diameter. Germ cells were compactly packed and spermatogenesis had progressed to post-meiotic spermatids. By 45 days, apart from reduced diameter of the tubules, germ cells were loosely packed compared to controls and elongating spermatids could be seen. At 60 days, both control and LFPx testis showed presence of sperm in the tubules but increasing degenerative changes with thinner population of sperm were the feature of LFPx testis. At 90 days, the tubules of LFPx animals showed increasing germ cell degeneration with loosely packed germ cells. The loss of post-meiotic germ cells (spermatids and sperm) was prominent (Plates IA and IB).

Histometrics

Histometric evaluations have showed no significant difference with respect to testis volume, seminiferous tubule diameter, tubular volume, tubular length, basement membrane area or Sertoli cell number between control and LFPx rats. However, there was an increase in tubular length. Decreased germinal epithelial thickness with reduced germ cell count due to increased percentage of germ cell loss was the only changes seen in the LFPx testis (Table 1.3).

Table 1.3: Histometric Enumeration of Seminiferous Tubules of Control and Functionally pinealectomised rats at 90 days

Treatment	T_V in cc	S_D in cm	GE in cm	S_V in cc	S_L in cm	bm in cm^2	SC_N $\times 10^6$	TGC_T $\times 10^6$	AGC_T $\times 10^6$	TGC_M $\times 10^6$	AGC_M $\times 10^6$	% Loss
C	1.503 ±0.030	0.0279 ±0.0006	0.0074 ±0.0003	1.427 ±0.050	2321.03 ±94.200	204.045 ±5.230	32.49 ±1.800	311 ±6.300	280.84 ±5.600	13.39 ±0.260	12.1 ±0.150	10.00 ±0.0002
LFPx	1.419 ±0.055	0.0269 ±0.004	0.0192^c ±0.0001	1.348 ±0.065	2753.96 ±58.260	199.81 ±4.350	35.22^c ±2.60	330.8^a ±3.405	267.88 ±1.995	14.05 ±0.135	11.79 ±0.172	14.18^c ±0.119

C – Control; LFPx – Functional pinealectomy (continuous light)

Values expressed as Mean ± SEM of minimum fifteen observations. $^a p < 0.05$, $^b p < 0.005$, $^c p < 0.0005$

T_V - Volume of Testis, S_D - Seminiferous tubule diameter, GE - Germinal epithelial thickness, S_V - Volume of Seminiferous tubule, S_L - Length of seminiferous tubule, **bm** - basement membrane area of the seminiferous tubule, SC_N - Total Sertoli cell number in testis, TGC_T - Theoretical germ cell number per testis, AGC_T - Actual germ cell number per testis, TGC_M - Theoretical germ cell number per meter of seminiferous tubule, AGC_M - Actual germ cell number per meter of seminiferous tubule.

Note: % loss of germ cells is calculated by taking into consideration the difference between the possible theoretical total number and the actual counted total number.

PLATE - IA

Figures 1 - 8: **Photomicrographs of sections of testis of Control rats**

Figures 1 and 2: Sections of testis of 35 day old control rats showing well formed tubules with meiotic stages and well developed interstitium.

Figures 3 and 4: Section of testis of 45 day of showing advanced stages of spermatogenesis and appearance of sperms in few tubules.

Figures 5 and 6: Section of testis of 60 day old rats showing well-established spermatogenesis and sperms in lumen.

Figures 7 and 8: Section of testis of 90 day old rats showing prominent interstitium and fully established spermatogenesis.

Figures: 1, 3, 5, & 7 - 250 x

Figures: 2, 4, 6, & 8 - 400 x

L - Lumen, S - Sperms, St - Spermatids, I - Interstitium

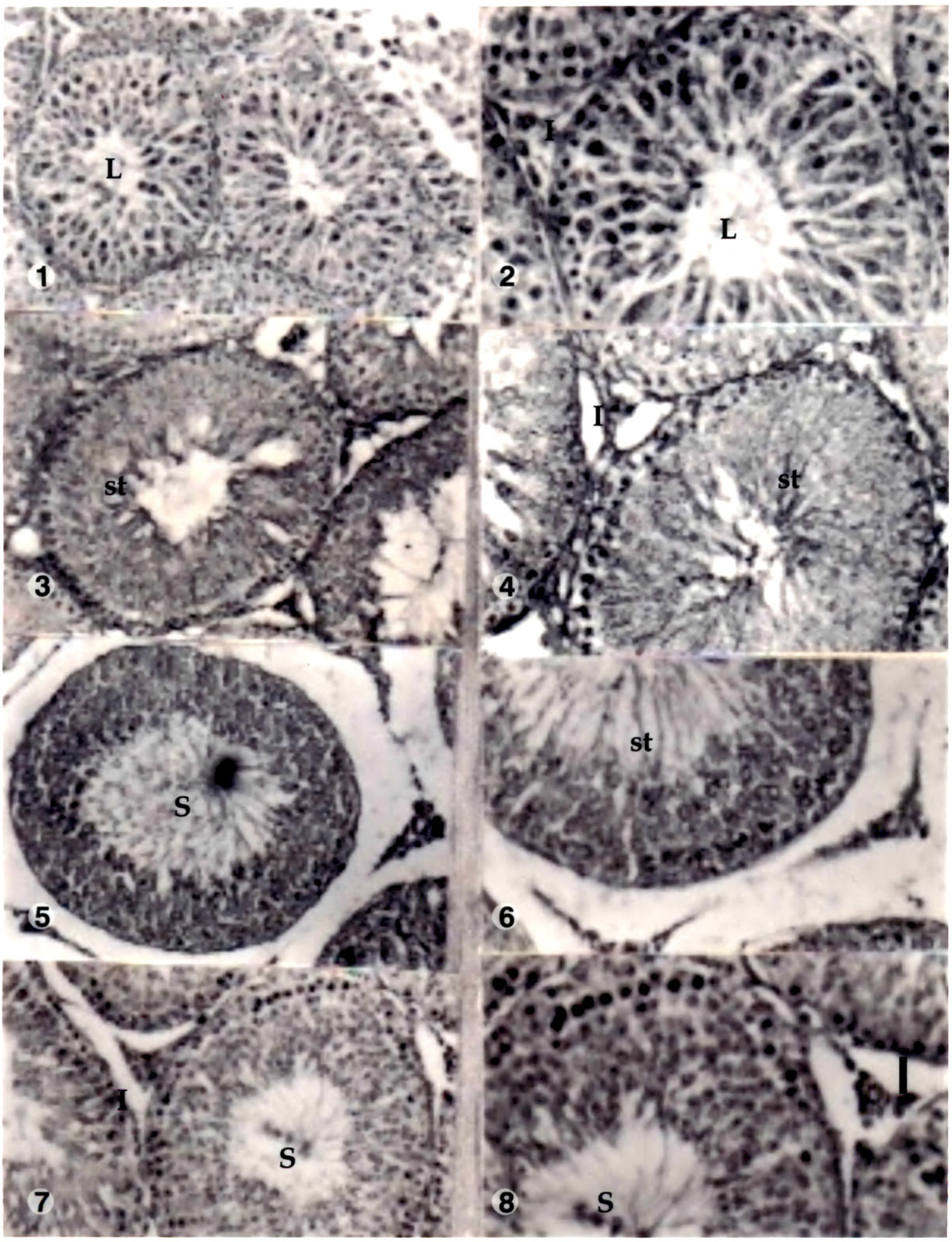


PLATE - IA

Photomicrographs of Testis of Control Rats

PLATE - IB

Figures 1 - 8: Photomicrographs of sections of testis of Functionally pinealectomised (Continuous Light - LFPx) rats.

Figures 1 and 2: Sections of testis of 35 day old rats showing smaller tubules compactly packed, spermatogenesis proceeded up to meiosis. Some tubules show elongating spermatids with less germ cell number.

Figures 3 and 4: Section of testis of 45 day old rats of showing spermatogenesis up to elongating spermatids, thinner population of germ cells and moderate interstitium. Some have loosely and some are compactly packed germ cells.

Figures 5 and 6: Section of testis of 60 day old rats showing smaller population of germ cells, late spermatids getting sloughed off with greater degeneration in germ cells.

Figures 7 and 8: 90 day old testis section showing more degeneration of germ cells and thinner density of sperms with post-meiotic spermatogenesis.

Figures: 1, 3, 5, & 7 - 250 x
Figures: 2, 4, 6, & 8 - 400 x

L - Lumen, S - Sperms, St - Spermatids, I - Interstitium

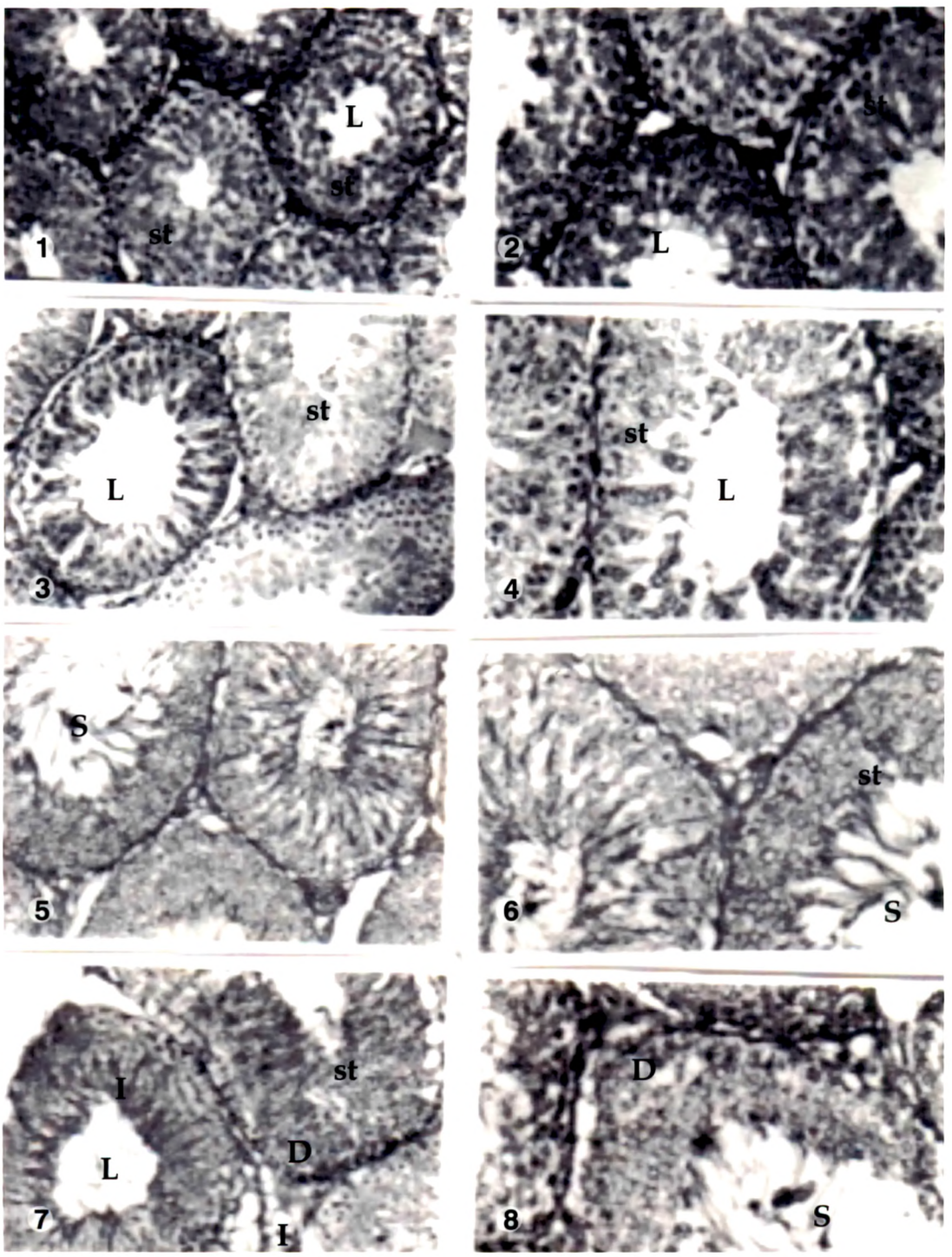


PLATE - IB

Photomicrographs of Testis of Functionally Pinealectomised Rats

Serum Hormone Profile:

Serum luteinizing hormone (LH) and testosterone (T) levels were significantly higher at all ages of study in LFPx animals. Serum corticosterone level was higher at 35 and 45 days but significantly lower at 60 and 90 days of age. Similar was case with reference to thyroid stimulating hormone (TSH) as its level was higher at 35 and 45 days and lower at 60 and 90 days. However serum T3 and T4 levels were consistently higher throughout (Tables 1.4 & 1.5; Figs. 1.4a, 1.4b, 1.4c, 1.5a, 1.5b & 1.5c).

Table 1.4: Serum levels of TSH, T_4 and T_3 (ng/ml) of Control and Functionally pinealectomised rats.

Treatment	TSH				T_3				T_4			
	Age in days				Age in days				Age in days			
	35	45	60	90	35	45	60	90	35	45	60	90
C	6.60 ±0.12	6.87 ±0.11	7.49 ±0.14	5.44 ±0.06	0.45 ±0.01	0.30 ±0.10	0.60 ±0.08	0.65 ±0.05	0.58 ±0.08	1.17 ±0.06	2.56 ±0.02	2.36 ±0.22
LFPx	13.3^{c} ±0.06	11.6^{c} ±0.06	9.2^{c} ±0.13	6.81^{c} ±0.04	0.91^{c} ±0.01	0.55^{a} ±0.02	0.71 ±0.02	0.79^{b} ±0.01	3.00^{c} ±0.07	2.72^{c} ±0.02	3.09^{c} ±0.01	2.90^{a} ±0.02

C – Control; **LFPx** – Functional pinealectomy (continuous light)

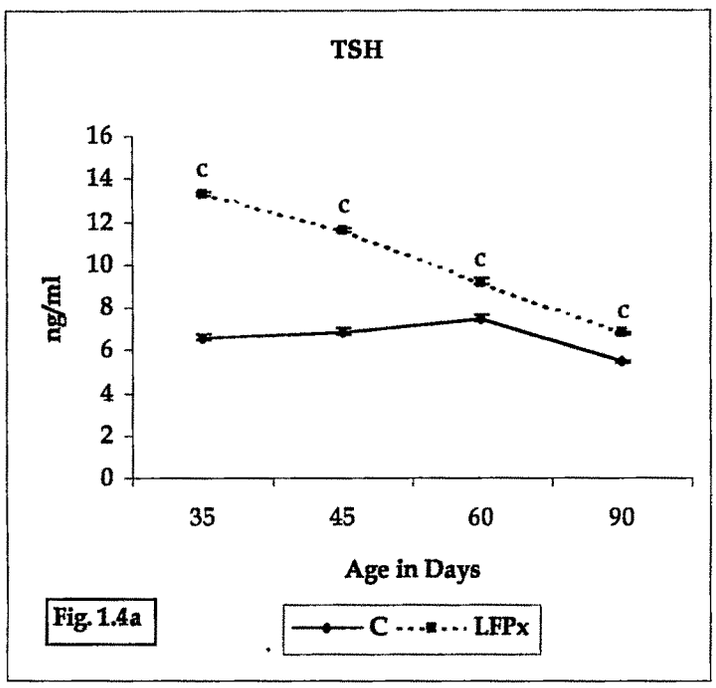
Values expressed as Mean ± SEM of four samples. $^{a}p < 0.05$, $^{b}p < 0.005$, $^{c}p < 0.0005$

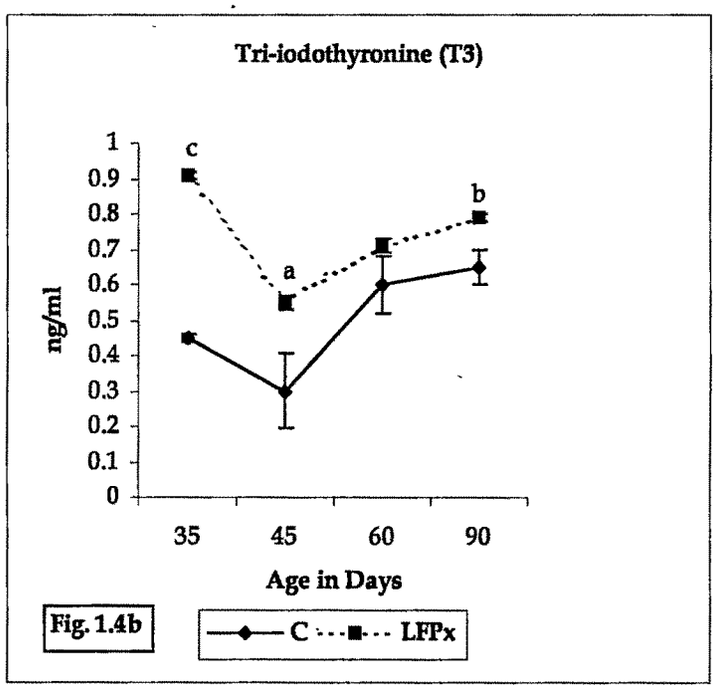
Table 1.5: Serum LH, Corticosterone and Testosterone (ng/ml) levels of Control and Functionally pinealectomised rats

Treatment	LH				Corticosterone				Testosterone			
	Age in days				Age in days				Age in days			
	35	45	60	90	35	45	60	90	35	45	60	90
C	16.45 ±0.63	21.75 ±0.85	48.12 ±1.23	53.25 ±1.03	0.80 ±0.61	1.00 ±0.15	4.80 ±0.70	4.50 ±1.69	0.56 ±0.16	2.23 ±0.27	2.62 ±0.21	4.37 ±0.26
LFPx	5.48[c] ±0.02	20.69 ±0.01	39.51[b] ±0.01	23.02[c] ±0.07	2.08[a] ±0.02	3.50[c] ±0.03	2.17[b] ±0.01	1.48 ±0.01	6.60[c] ±0.01	6.20[c] ±0.02	5.90[c] ±0.04	5.60[b] ±0.03

C – Control; LFPx – Functional pinealectomy (continuous light)

Values expressed as Mean ± SEM of four samples. [a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.0005$

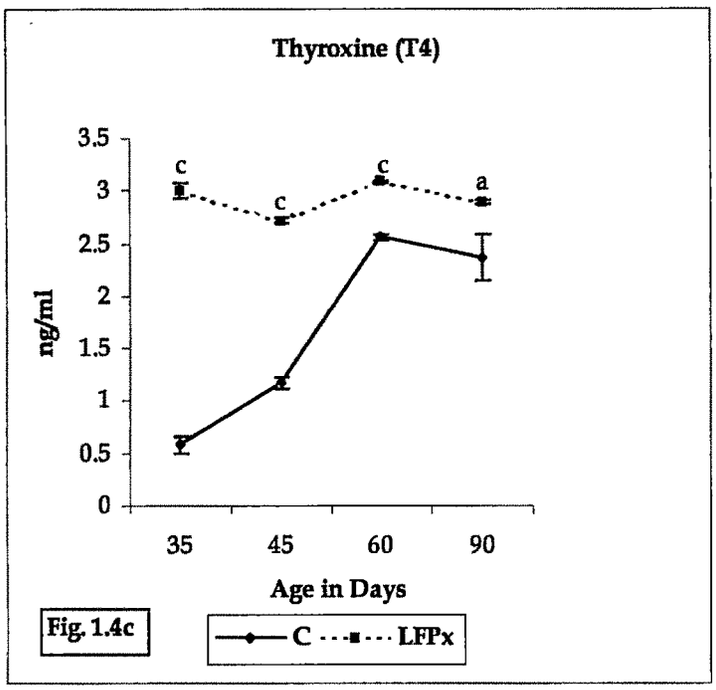


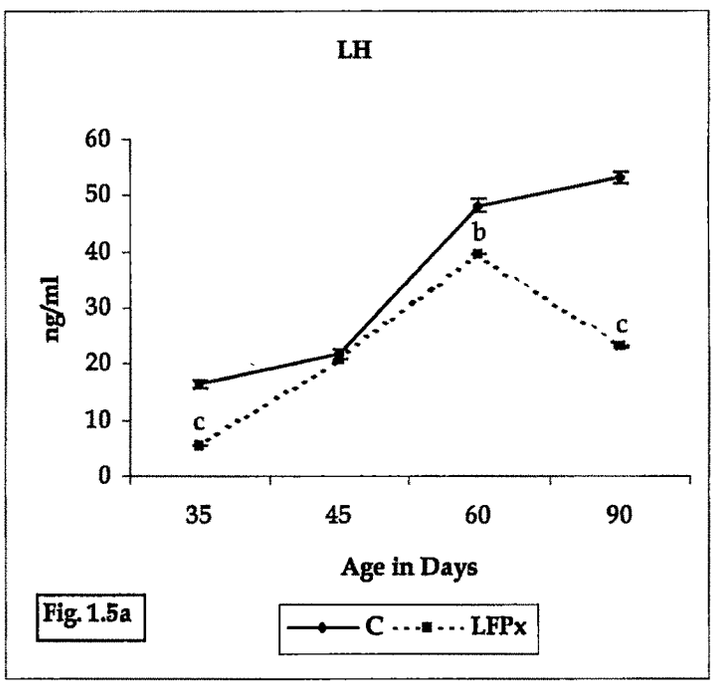


Figures 1.4a &b: Chronological alterations showing serum TSH and T3 (ng/ml) levels in control (C) and functionally pinealectomised (LFPx) rats

Values expressed as Mean ± SEM of four samples;

$^{a}p<0.01$; $^{b}p<0.005$, $^{c}p<0.0005$





Figures 1.4c & 1.5a: Chronological alterations showing serum T4 and LH (ng/ml) levels in control (C) and functionally pinealectomised (LFPx) rats

Values expressed as Mean ± SEM of four samples;

$^{a}p<0.01$; $^{b}p<0.005$, $^{c}p<0.0005$

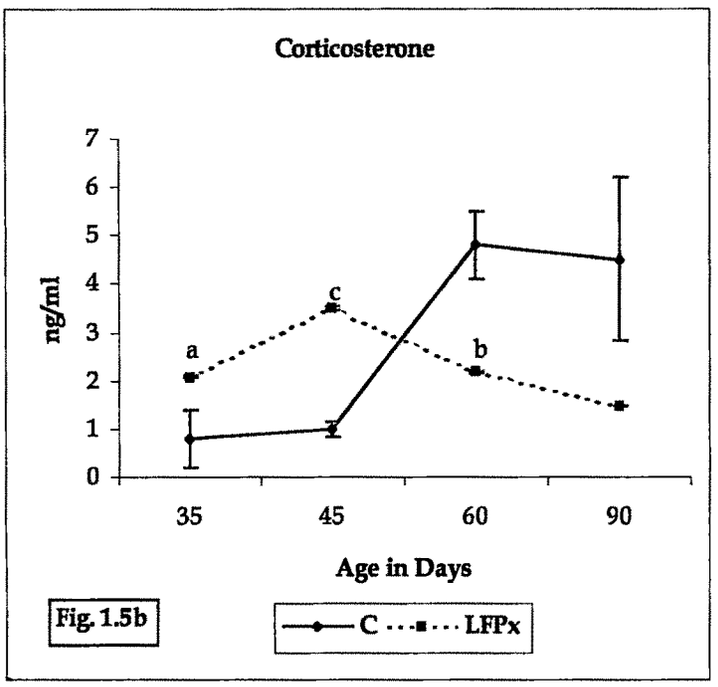


Fig. 1.5b

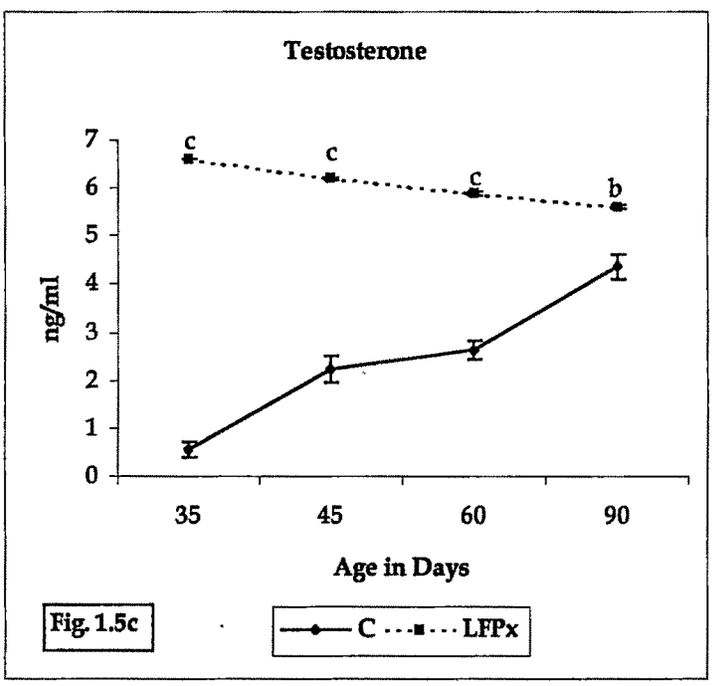


Fig. 1.5c

Figures 1.5b & 1.5c: Chronological alterations showing serum Corticosterone and Testosterone (ng/ml) levels in control (C) and functionally pinealectomised (LFPx) rats

Values expressed as Mean ± SEM of four samples;

$^{a}p<0.01$; $^{b}p<0.005$, $^{c}p<0.0005$

DISCUSSION:

Different laboratory strains of rat differ in their responses to photoperiod, with most strains studied having either no response or slight to moderate reproductive inhibition and/or reduced growth rate (Cohen and Mann, 1979; Vanecek and Illnerova, 1982; Rivest *et al., 1986;* Aubert *et al., 1989;* Boon *et al., 1997*). In the recent past, strains of laboratory rats have been shown to have neuro-anatomical connections that mediate reproductive sensitivity to photoperiod (Wallen and Turek, 1981; Nelson *et al.,* 1982). But these connections have been thought to range from non-functional in adults to functional in young rats of some strains (Sizonenko *et al., 1985;* Heideman and Sylvester, 1997). Peripubertal rodents are considered to be theoretically more likely to be responsive to photoperiod than are adults (Donham *et al.,* 1989; Horton and Rowsemitt, 1992). Higher sensitivity of younger animals to photoperiods has been reported in rats and some other rodents (Sorrentino *et al., 1971;* Johnston and Zucker, 1979; Donham *et al.,* 1989; Stanfield and Horton, 1996).

The present study shows that LFPx form day 0 to day21, neither alters the adult body weight nor the absolute or relative testes weight at 90 days. However, a previous study form this laboratory had shown increased adult body weight in rats subjected to surgical pinealectomy at neonatal day 5 (Sharma, 1996). Apparently

functional pinealectomy has a differential effect on long-term body and testes weight as complete lack of pineal abolishes melatonin rhythm permanently against LFPx, where melatonin rhythm gets re-established on return back to ambient photoperiod. The unchanged testes weight and early onset of spermatogenesis can be correlated with the increased thyroid hormone titres in the prepubertal periods, which favour Sertoli cell differentiation (Maran and Aruldhas, 2002; Lagu *et al.,* 2004). Comparison of the histometric enumeration of seminiferous tubules of control and LFPx rats reveals that except for an increased germinal epithelial thickness and tubular length in LFPx testis, there is no other favourable change. This increase in tubular lentght could be due to an increased FSH level due to hypermelatonemia and which in turn promotes tubular growth as inferred earlier in a study on surgical pinealectomy (Sharma, 1996). However, the total number of germ cells was significantly less in LFPx testis compared to control testis despite the fact that the theoretical total number of germ cells was significantly higher in the former (calculated taking into consideration the average area occupied by germ cells and the total area available subtracting the area occupied by Sertoli cells). Further, there is also increased degeneration/apoptotic loss of advanced germ cells like spermatids and sperms in LFPx exposed rats. The LFPx rats also show increased serum corticosterone levels

in the pre-pubertal period, which is detrimental to germ cell survival especially for the most mature classes probably due to altered adhesive properties between Sertoli cells and germ cells. Interestingly, both neonatal melatonin administration as well as corticosterone administration in earlier studies had shown increased germ cell survival by preventing apoptosis (Ramachandran *et al.*, 2004; Bhavsar, 2001). These apparently contradictory inferences may have to be considered on two major points of differences inherent in the two studies.

1. The increment in corticosterone level found in the prepubertal periods is significantly higher in the present study compared to the above studies.
2. Whereas the present increment occurs in a melatonin less background the increment in the above studies occur in a hypermelatonemic status.

Inferably, milder prepubertal hypercorticalism on a normal melatonin background is favourable for Germ cell survival with reduced apoptosis while, higher corticosterone levels especially on a melatonin less background is unfavourble with increased germ cell apoptosis/reduced adhesional properties and reduced germ cell survival. Support to this concept stems from the observations that elevation of circulating glucocorticoids affects Leydig cell functions (Rabin *et al.*, 1988), that prolonged stress suppresses reproductive

functions (Orr and Mann, 1990) and that male rats immobilized for a short period results in complete disappearance of spermatogenic cells (Meitner, 1976). These effects have been considered the consequence of apoptosis directly triggered by glucocorticoids in testicular tubules (Yazawa *et al., 1999,* 2000). Further, apoptosis induced by glucocorticoids has been shown to be localized to the basal zone of the tubule, where increased glucocorticoid receptor immunoreactivity after corticosterone administration to rat pups has been observable (Biagini and Pich, 2002).

Neonatal LFPx exposure seems to have a favourable influence on the hypothalamo-hypophyseal-gonadal (HHG) axis, which is marked by increased secretions of LH and testosterone. This would suggest a negative regulatory influence of melatonin on the central HHG axis. Confirmation comes from the observed effects of exogenous melatonin administration decreasing serum LH levels and testosterone in the adult rat. Further, in the same study, pinealectomy was shown to increase serum LH level without changing the testosterone titre lending credence to the central inhibitory action of melatonin (Yilmaz *et al.,* 2000). In an earlier study they had also demonstrated pinealectomy induced increase in testis weight and hyperactivity of the Leydig cells (Sarsilmaz *et al.,* 1998). An earlier study from this laboratory has shown decreased LH and T levels in adult rats due to neonatal melatonin excess

(Ramachandran *et al.,* 2004). Clearly, neonatal LFPx exposure resulting in functional pinealectomy has pronounced effects in up-regulating the HHG axis.

Finally, it can be concluded from the present observations that neonatal light induced functional pinealectomy has up-regulatory influence on the HHG axis and also induces greater germ cell loss, especially of the most mature classes probably by greater apoptosis due to increased prepubertal corticosterone level. Apparently, though laboratory rat may not be wholly photoperiodic, exposure to continuous light in the immature stages has subtle long-lasting effects in the adults.

SUMMARY:

The present study deals with the long-term effects of neonatal functional pinealectomy induced by light (LFPx) on adult testis structure and functions and serum hormone profiles. Rat neonates were divided in two groups of study, group 1 consisting of control animals and group 2 of animals which were functionally pinealectomized by exposing them to continuous light of 250 lx intensity from day 0 to day 21 and were assessed for testis histomorphology, gravimetry and hormone profiles at 35, 45, 60 and 90 days of age. The body weight of LFPx rats was significantly higher at 45 & 60 days but similar to controls at 90 days. The absolute and relative testes weights were significantly lower at 45 & 60 days but then became similar to control weights at 90 days. Histologically, the testis of LFPx rats showed prominent germ cell degeneration with loosely packed germ cells and thinner population of sperms compared to fully established spermatogenesis in controls. There were no significant changes with respect to testis volume, seminiferous tubule diameter, testicular volume, tubular length, basal membrane area or Sertoli cell number, Serum LH, T, T_3 and T_4 levels were significantly higher in LFPx rats at all ages of study while the levels of corticosterone and TSH were higher at 35 and 45 days and lower at 60 and 90 days. Form the above observations it is concluded that LFPx has up regulatory influence

on hypothalamic-hypophyseal-gonadal axis and above induce greater germ cell loss due to pre-pubertal corticosterone. Though laboratory rats may not be wholly photoperiodic exposure to continuous light in the immature stages has subtle long lasting effects in the adults.