CHAPTER 3

Concomitant hypothyroidism prevents the adverse effects on adult testes functions induced by neonatal functional pinealectomy by exposure to light and shows mixed effects of light and hypothyroidism on hormone profiles

Increased reproductive activity in mammals due to pinealectomy has been recorded (Reiter, 1973, 1980; Reiter *et al.*, 1985). The above reported stimulatory influence of pinealectomy on gonads could not be demonstrated in the adult rats (Motta *et al.*, 1967; Pitis and Maya, 1969; Reiter, 1973, 1980; Reiter *et al.*, 1985). Previous study on neonatal surgical pinealectomy had shown increased adult testis 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). The weak photoperiodic responses of most strains of rats coupled with very short critical photoperiods on these effects have lead to characterization of rats as being not photoperiodic (Reiter, 1980; Wallen and Turek, 1981; Bronson, 1989; Nelson *et al.*, 1994). However a reproductive response to short days and/or melatonin treatment can be induced in adult male Sprague-Dawley, Wistar and some other strains of rats

by various procedures (Reiter *et al.*, 1968, 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 Peat, 1971; Kinson and Robinson, 1970) have been shown to delay puberty in some strains of rats, but the effects reported have been slight. Inhibited testes development by constant darkness has been seen in the Wistar strain of rats (Hoffman *et al.*, 1986). In another study involving 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). Previous study involving neonatal functional pinealectomy by light (LFPx) had shown adverse effects on adult testis function marked by increased degeneration of spermatids and spermatozoa and reduced number of germ cells and altered adult hormonal profiles (Chapter 1).

Transient hypothyroidism induced in the neonatal period or hypothyroidism in juvenile period is known to induce precocious puberty and macroorchidism accompanied by increase in sperm production and Sertoli, Leydig and germ cell numbers (Cooke and Meisami, 1991; Cooke *et al.*, 1991; Cooke *et al.*, 1992; Hardy *et al.*, 1993, 1996; Hess *et al.*, 1993; Van Haaster *et al.*, 1993; Anasti *et al.*, 1995; Bruder *et al.*, 1995; Maran, 2003; Krassas and Panitkides, 2004). Previous studies from this laboratory have shown that this influence

of neonatal HPOT on testis size and cell numbers can be influenced by the temperature (Lagu *et al.,* 2004). Apart from the observed testes weight and cell number, the above study also recorded lowered hypothalamic set point for hypothalamo-hypophyseal-thyroid axis and a raised set point for hypothalamo-hypophyseal-adrenal axis.

In the light of above mentioned effects of neonatal light induced functional pinealectomy and hypothyroidism, the present study has been undertaken to evaluate the combined effect of neonatal functional pinealectomy (LFPx) and hypothyroidism (HPOT) on adult testis functions and hormonal profiles.

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.

Group I: Control (C)

Male rat neonates (6) were maintained under normal lighting regimen of LD 8:16 and were provided with food and water *ad libitum.*

Group II Functional Pinealectomy (LFPx) and induced hypothyroidism (HPOT) (LFPx+HPOT):

Male rat neonates (6) were functionally pinealectomised by exposing them to continuous light from day '0' to day '21' and simultaneously subjected to transient hypothyroidism (HPOT) by feeding mothers with 0.1% 6-propyl 2-thiouracil (PTU) in drinking water (PTU was procured from Sigma Chemicals).

Parameters and Methods of Evaluation:

As in chapter one

Histology and Histometry:

As in chapter one

Hormone Assays:

As in chapter one

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 LFPx+HPOT rats showed significantly lower body weights at 35, 45 and 90 days compared to age-matched control. The absolute and relative testes weights were significantly lower at 35 and 45 days but significantly higher at 90 days. These changes in body and testes weight are similar to those recorded in HPOT animals but different from LFPx animals (Tables 3.1 & 3.2; Figs. 3.1a, 3.1b, 3.1c, 3.2a & 3.2b).

Table 3.1: Chronological alterations showing body weight (g), absolute (g) and relative testes weight (g/100g) of control and functionally pinealectomized hypothyroid male 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+HPOT	47[c] ±2.216	103[c] ±1.579	198 ±5.258	287[c] ±0.975	0.24[c] ±0.012	0.95[c] ±0.008	2.69 ±0.268	4.44[c] ±0.025	0.51[c] ±0.011	0.93[c] ±0.008	1.40 ±0.101	1.54[c] ±0.009
LFPx*	108 ±6.396	157[c] ±2.456	268[c] ±7.496	348 ±16.73	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
HPOT@	37.83[c] ±2.242	74.67 ±0.760	164.2[b] ±0.544	287.3[b] ±10.02	0.232[c] ±0.031	0.750[c] ±0.019	2.215 ±0.169	4.765[c] ±0.117	0.613[c] ±0.046	1.011[a] ±0.028	1.340[a] ±0.050	1.522[c] ±0.061

C - Control; **LFPx+HPOT** - Functionally Pinealectomized treated hypothyroid; **LFPx** - Functionally Pinealectomized (Continuous Light); **HPOT** - Hypothyroid

* - Values taken from Chapter 1

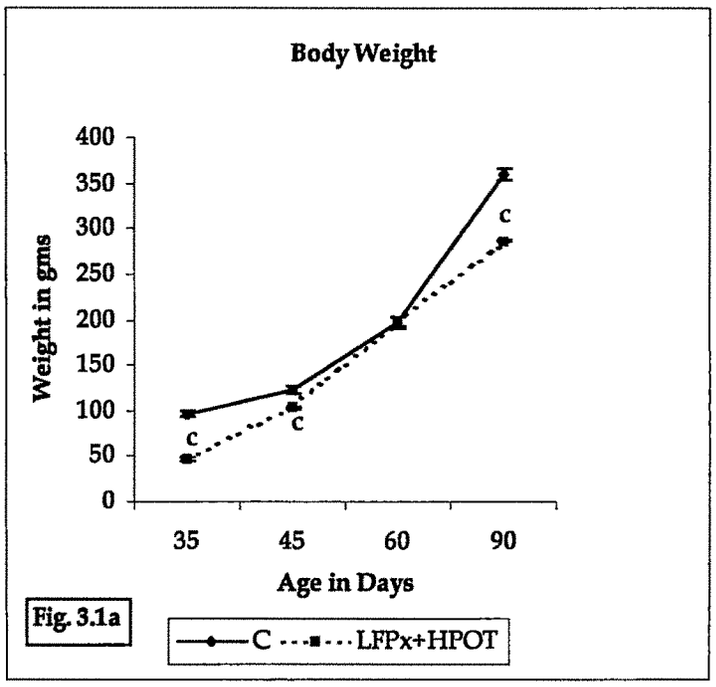
@ - Values taken from Lagu, (2001)

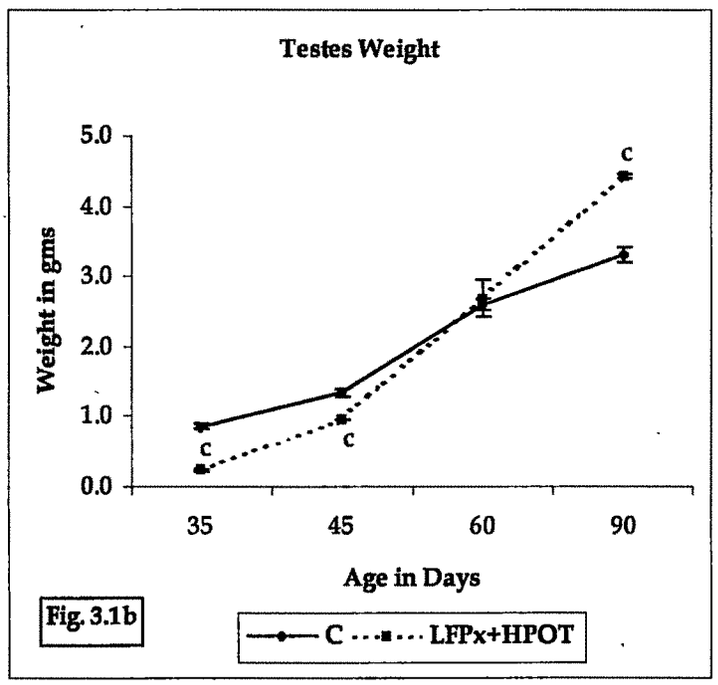
Table 3.2: Per day Body and Testes Growth Rate (g/day) in Control and Functionally pinealectomised Hypothyroid rats

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+HPOT	1.169	1.239	1.578	0.989	0.007	0.016	0.029	0.019
LFPx*	2.934	1.07	1.863	0.888	0.027	0.009	0.022	0.005
HPOT@	0.897	3.68	5.917	4.013	0.011	0.052	0.073	0.080

C – Control; **LFPx+HPOT** – Functionally Pinealectomized treated hypothyroid; **LFPx** – Functionally Pinealectomized (Continuous Light); **HPOT** – Hypothyroid

* - Values taken from Chapter 1
@ - Values taken from Lagu, (2001)

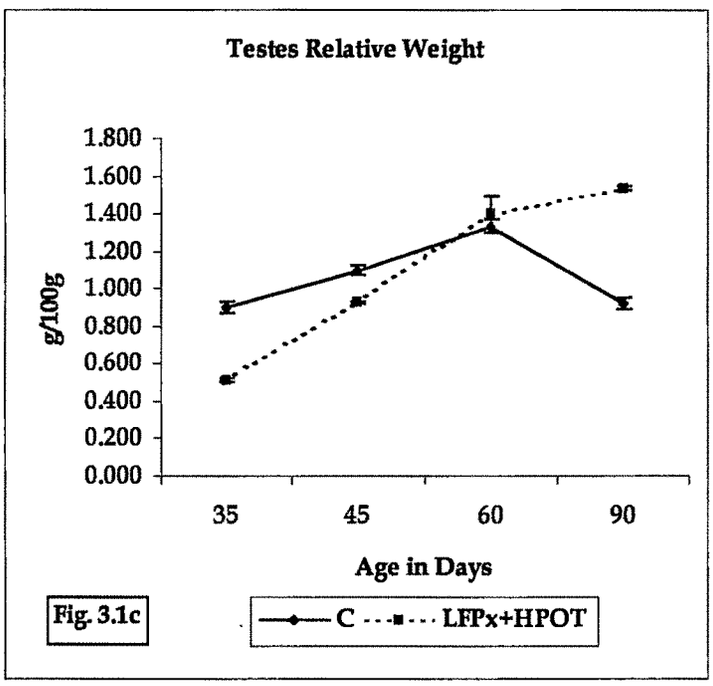




Figures 3.1a&b: Chronological alterations showing body and testes (g) weights in control (C) and functionally pinealectomised hypothyroid (LFPx+HPOT) rats

Values expressed as Mean ± SEM of six animals;

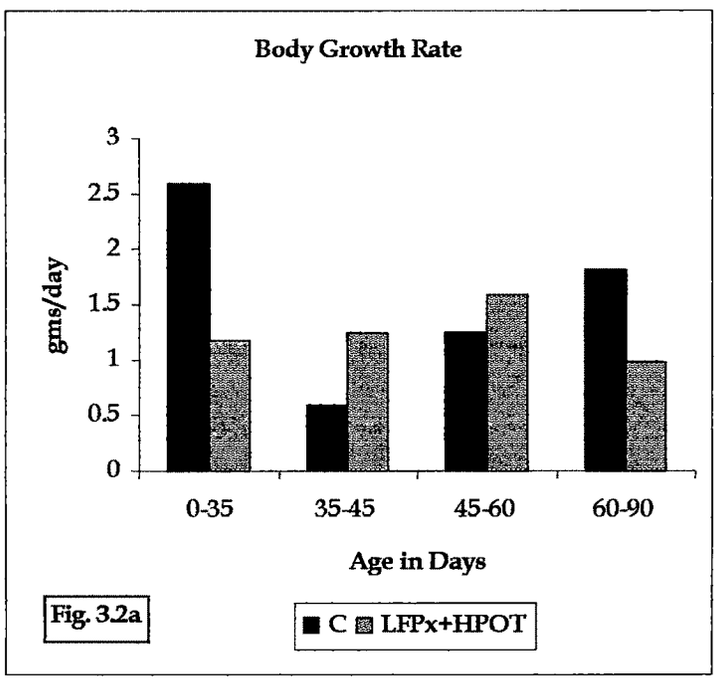
[a]$p<0.01$; [b] $p<0.005$, [c]$p<0.0005$

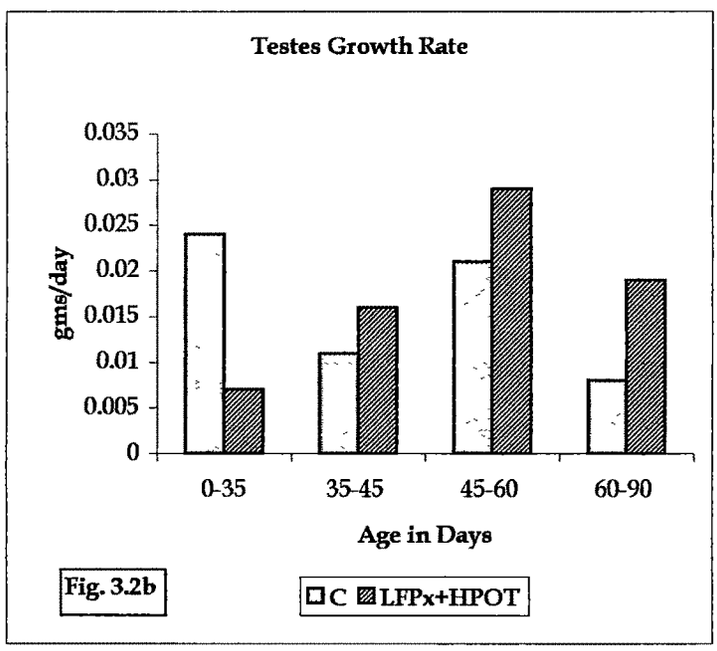


Figures 3.1c: Chronological alterations showing relative (g/100g) testes weight in control (C) and functionally pinealectomised hypothyroid (LFPx+HPOT) rats

Values expressed as Mean ± SEM of six animals;

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





Figures 3.2a&b: Chronological alterations showing body and testes (g/day) growth rate in control (C) and functionally pinealectomised hypothyroid (LFPx+HPOT) 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:

Control:

At 35 days well formed seminiferous tubules with meiotic germ cells and prominent Leydig cells could be seen. Spermatogenesis progressed to elongating spermatids by 45 days. By 60 days, tubules were well formed and spermatogenesis got fully established with sperms seen in most of the tubules. The Leydig cells were well formed. Fully established spermatogenesis in tubules and well formed Leydig cells were the features at 90 days (Plate IA).

LFPx+HPOT:

At 35 days the testis showed shrunken tubules with closely packed germ cells and more degenerating cells with pycnotic nuclei. The basement membrane was greatly thickened. By 45 days the tubules were enlarged, well formed and closely packed. However, the population of germ cells was less and spermatogenesis had progressed up to meiotic stages. The interstitium was characterized by hypertrophic Leydig cells. By 60 days spermatogenesis was fully established with increased number of germ cells and, sperm could be seen in all tubules by 90 days (Plate III).

Histometrics

Except for seminiferous tubules diameter, all other parameter like testicular volume, germinal epithelial thickness, seminiferous tubular volume, seminiferous tubular length, basement membrane area, Sertoli cell number and total number of germ cells were all significantly increased in LFPx+HPOT animals compared to controls. The most prominent increases were with reference to seminiferous tubular length, Sertoli cell number and germ cell number (Table 3.3).

Table 3.3: Histometric Enumeration of Seminiferous Tubules of Control and functionally pinealectomised hypothyroid 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+HPOT	2.027[c] ±0.085	0.0203[c] ±0.0005	0.0092 ±0.0008	1.925[c] ±0.080	5925.3[c] ±85.550	378.73[c] ±6.475	70.89[c] ±1.247	476.7[c] ±5.015	506.0[c] ±6.649	8.47[c] ±0.324	10.09[a] ±0.751	23.83[c] ±1.241
LFPx*	1.419 ±0.055	0.0269 ±0.004	0.0192[c] ±0.0001	1.348 ±0.065	2357.96 ±58.260	199.81 ±4.350	35.22 ±2.60	330.8[a] ±3.405	267.88 ±1.995	14.05 ±0.135	11.79 ±0.172	14.18[c] ±0.119
HPOT@	2.176[c] ±0.100	0.035[c] ±0.0014	0.0110[c] ±0.0003	2.066[c] ±0.075	2197.49 ±65.200	238.89[c] ±2.600	65.42[c] ±1.500	494.0[c] ±8.600	368.0[c] ±1.900	22.48[c] ±0.390	17.00[c] ±0.059	24.37[c] ±0.069

C – Control; **LFPx+HPOT** – Functionally Pinealectomized treated hypothyroid; **LFPx** – Functionally Pinealectomized (Continuous Light); **HPOT** – Hypothyroid

Values expressed as Mean ± SEM of minimum fifteen observations. [a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.0005$
* - Values taken from Chapter 1; @ - Values taken from Lagu, (2001)

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.

PLATE - III

Figures 1 - 8: **Photomicrographs of sections of testis of LFPx rats treated with hypothyroidism.**

Figures 1 and 2: Sections of testis of 35 day old rats showing closely packed shrunken tubules with pyknotic degenerating cells.

Figures 3 and 4: Section of testis of 45 day old rats of showing well formed enlarged tubules, fewer germ cells up to meiotic stage.

Figures 5 and 6: Section of testis of 60 day old rats showing greater population of germ cells and well establishment spermatogenesis.

Figures 7 and 8: 90 day old testis section showing more number of germ cells and hyperplastic interstitium.

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

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

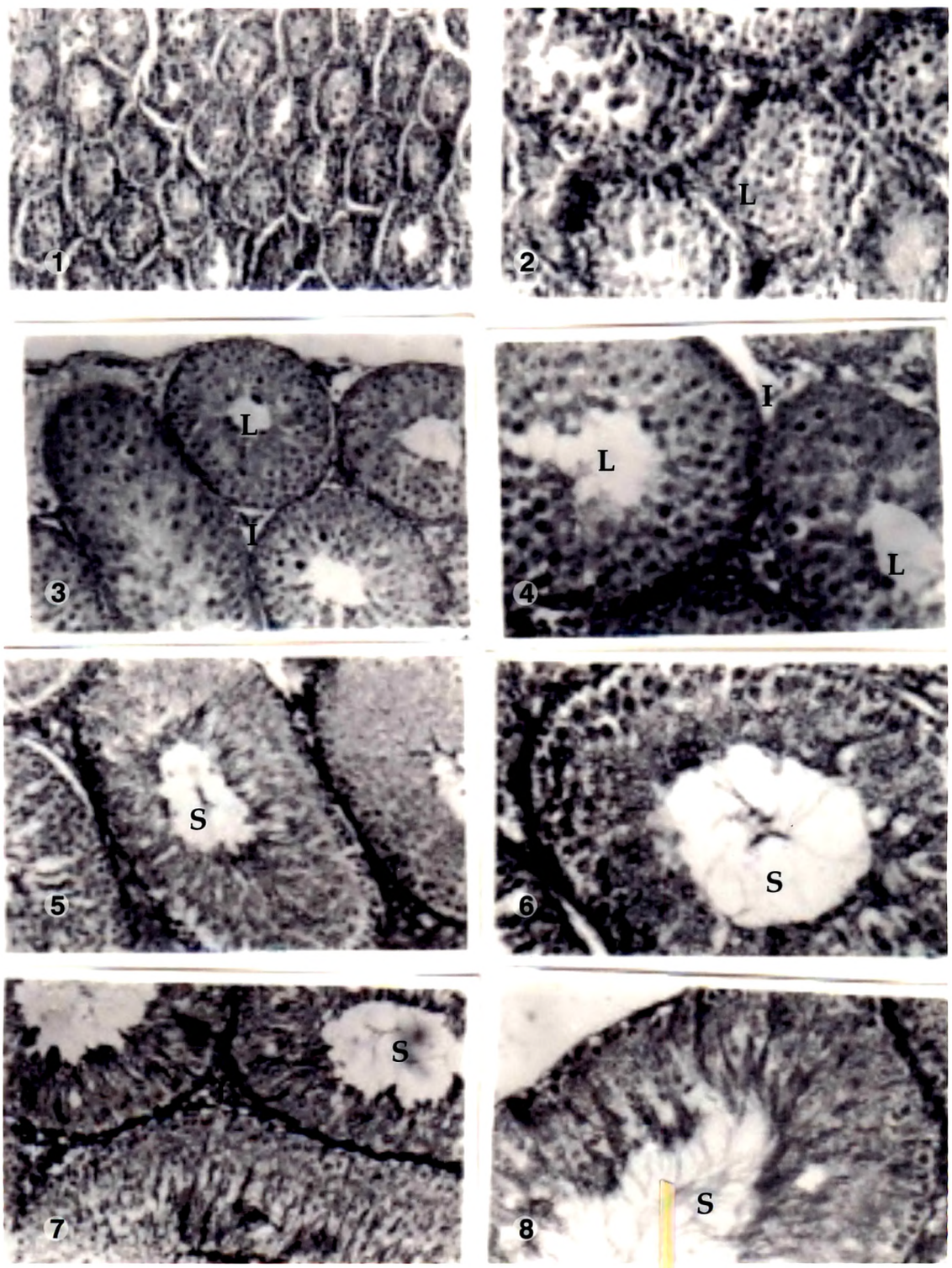


PLATE - III

Photomicrographs of Testis of Control & Functionally Pinealectomised Hypothyroid Rats

Serum Hormone Profile:

TSH and T4 levels were higher at all ages of study but T3 levels were significantly lower compared to controls. Though serum Corticosterone levels were similar to those of controls, at 35 and 45 days, the levels were significantly lower at 60 and 90 days. Both serum LH and Testosterone levels were significantly lower than the controls at all ages except for Testosterone at 35 day, which was significantly higher (Tables 3.4 & 3.5; Figs. 3.4a, 3.4b & 3.4c, 3.5a, 3.5b & 3.5c).

Table 3.4: Serum TSH, T_4 and T_3 (ng/ml) levels of Control and Functionally pinealectomised Hypothyroid 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+HPOT	12.46^{c} ±0.098	10.63^{c} ±0.058	13.96^{c} ±0.124	12.11^{c} ±0.086	0.343^{c} ±0.012	0.492 ±0.88	0.453 ±0.065	0.501^{b} ±0.022	2.94^{c} ±0.112	2.69^{c} ±0.042	4.40^{c} ±0.051	3.83^{b} ±0.132
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
HPOT@	17.0^{c} ±0.844	6.501 ±0.591	9.301^{a} ±0.636	6.103 ±0.528	0.372 ±0.062	0.310 ±0.082	0.493 ±0.084	0.450^{a} ±0.066	0.900^{a} ±0.094	1.020 ±0.073	0.802^{c} ±0.083	1.001^{c} ±0.082

C – Control; **LFPx+HPOT** – Functionally Pinealectomized treated hypothyroid; **LFPx** – Functionally Pinealectomized (Continuous Light); **HPOT** – Hypothyroid

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

* - Values taken from Chapter 1
@ - Values taken from Lagu, (2001)

Table 3.5: Serum levels of LH, Corticosterone and Testosterone (ng/ml) of Control and Functionally pinealectomised Hypothyroid rats

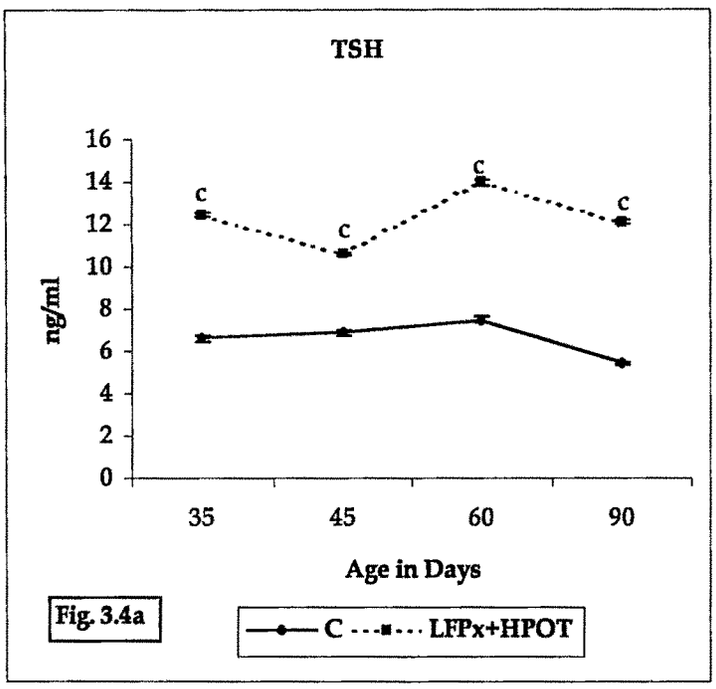
Treatment	LH				Corticosterone				Testosterone			
	Age in days				Age in days				Age in days			
	35	45	60	90	35	45	60	90	35	45	60	90
C	16.45 ±0.634	21.75 ±0.854	48.12 ±1.235	53.25 ±1.031	0.80 ±0.618	1.00 ±0.155	4.80 ±0.705	4.50 ±1.699	0.56 ±0.166	2.23 ±0.278	2.62 ±0.217	4.37 ±0.265
LFPx+HPOT	10.03^{c} ±0.47	17.45^{b} ±0.72	31.54^{c} ±0.02	20.25^{c} ±1.04	8.22^{c} ±0.01	10.19^{c} ±0.29	13.4^{b} ±0.85	10.02^{a} ±1.99	1.50^{b} ±0.08	2.03 ±0.14	2.10^{a} ±0.11	2.30^{b} ±0.62
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
HPOT@	14.81 ±0.821	19.883 ±0.745	21.21^{c} ±1.020	38.84^{c} ±1.030	6.200^{a} ±0.013	5.213^{c} ±0.306	10.201^{c} ±0.987	35.001^{a} ±3.120	0.280^{a} ±0.094	0.562^{c} ±0.099	2.40^{b} ±0.088	1.703^{b} ±0.058

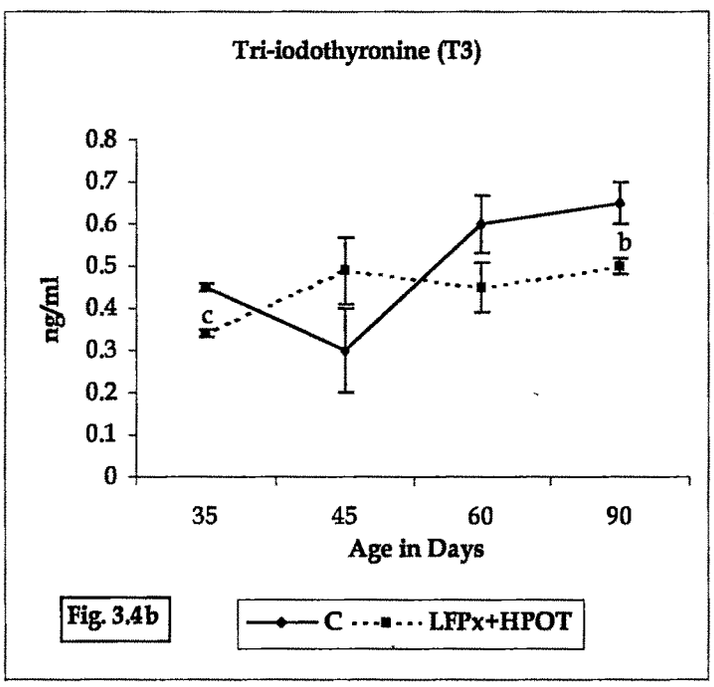
C - Control; **LFPx+HPOT** - Functionally Pinealectomized treated hypothyroid; **LFPx** - Functionally Pinealectomized (Continuous Light); **HPOT** - Hypothyroid

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

* - Values taken from Chapter 1

@ - Values taken from Lagu, (2001)

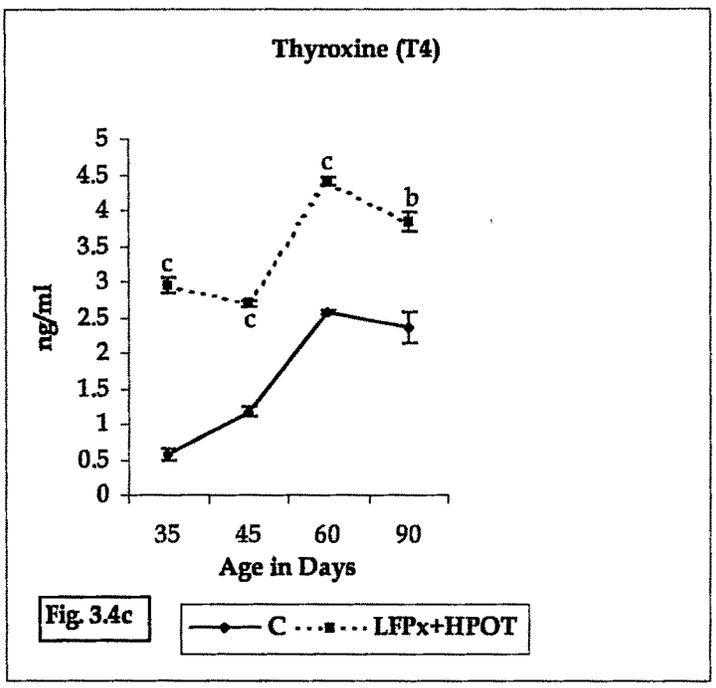


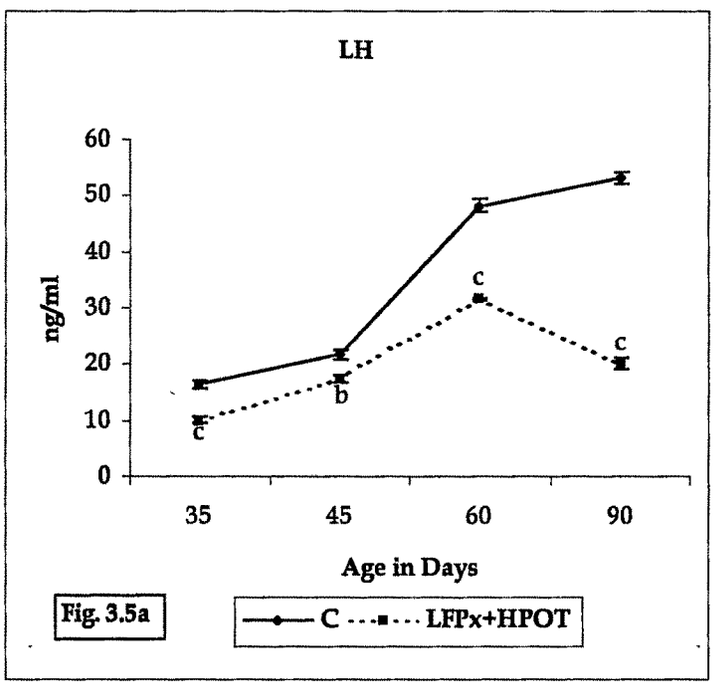


Figures 3.4a&b: Chronological alterations showing serum TSH and T3 (ng/ml) levels in control (C) and functionally pinealectomised hypothyroid (LFPx+HPOT) rats

Values expressed as Mean ± SEM of four samples;

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

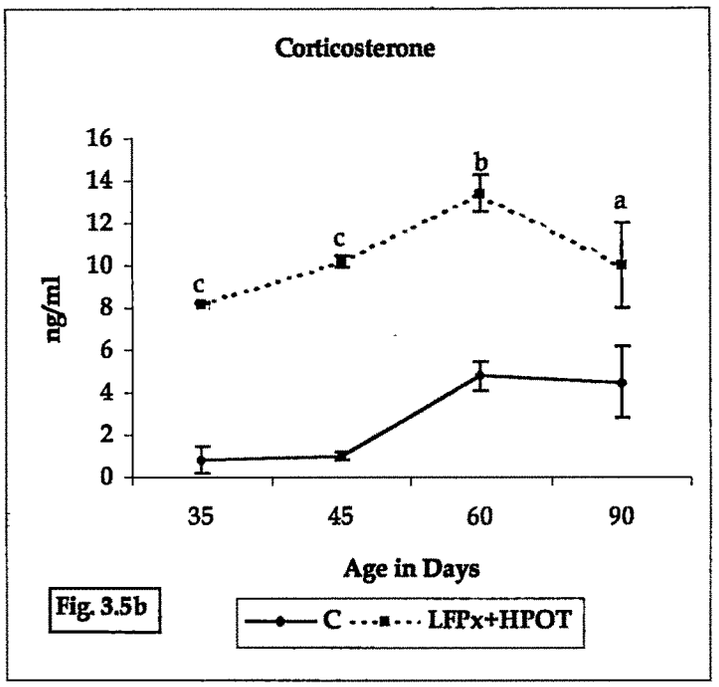


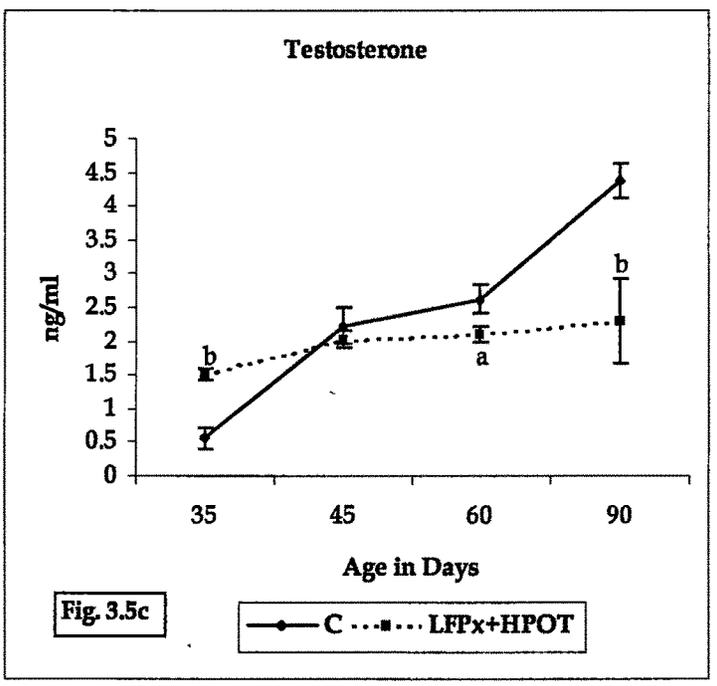


Figures 3.4c&3.5a: Chronological alterations showing serum T4 and LH (ng/ml) levels in control (C) and functionally pinealectomised hypothyroid (LFPx+HPOT) rats

Values expressed as Mean ± SEM of four samples;

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





Figures 3.5b&c: Chronological alterations showing serum Corticosterone and Testosterone (ng/ml) levels in control (C) and functionally pinealectomised hypothyroid (LFPx+HPOT) rats

Values expressed as Mean ± SEM of four samples;

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

DISCUSSION:

The present study on simultaneous neonatal hypothyroidism and functional pinealectomy by light shows a significant reduction in body weight and significant increase in absolute and relative weight of testes compared to age matched controls at 90 days. In the pre-pubertal period, both the body and testes weight are significantly lower. These changes are quite in contrast to the observations made in LFPx alone rats, which showed no difference in body and testes weight at 90 days but increased body weights at 35 and 45 days (Chapter 1). The presently observed changes are more similar to neonatal HPOT alone rats (Lagu *et al.*, 2004). Apparently, neonatal HPOT over-rides the influence of LFPx and the effect of HPOT seems to be more dominant. In the pre-pubertal recovery period (35 days), the body weight of HPOT rats is 51% lighter than controls while, the relative testes weight is 43% less than the control testis weight. Catch up body growth in LFPx+HPOT rats occurs between 35-60 days, as the body weight equals the control weight at 60 days. Subsequently, the growth rate again decreases in experimental animals resulting in 20% deficit at the final body weight at 90 days. Recovery growth is also manifested by testes as the relative weight of testes at 90 days is higher by 67%at 90 days as against 43% deficit at 35 days catch up growth occurs between 35-60 days, as the testis weight got equaled at 60 days. These changes in the LFPx+HPOT rats are comparable to those seen in HPOT rats

(Lagu *et al.*, 2004) and very much in contrast to LFPx rats as, the body weight and testes weight in these rats were like in the control rats (Chapter 1).

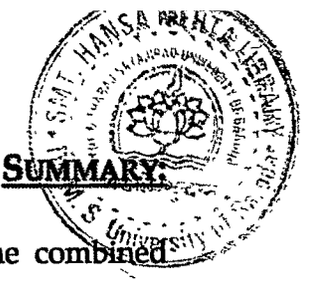
Like in HPOT rats, even in LFPx+HPOT rats spermatogenic process is delayed and sperms fully appear in the tubules and by 90 days as against early onset of spermatogenesis and appearance of sperms in controls by 60 days (Lagu *et al.*, 2004; Chapter 1). Increased tubular diameter and Sertoli cell number seen in the LFPx+HPOT rats with concomitant increase in germ cells corroborate the observed increased testis size and can be related with the neonatal HPOT induced prolonged phase of Sertoli cell proliferation and delayed Sertoli cells differentiation (Lagu, 2001; Maran, 2003; Krassas and Pantikidis, 2004; Lagu *et al.*, 2004).

The above cited studies have shown significant increase in tubular diameter, Sertoli cell number and germ cell population as seen in the present study. However, the significant increase in tubular length more than double that off control and HPOT rats is a novel observation and might suggest a favourable influence of light and HPOT in promoting tubular growth. This remarkable increase in tubular length is well reflected in the 38% increase in the germ cell count seen in LFPx+HPOT testis compared to HPOT alone and nearly 50% increase compared to LFPx alone (Lagu *et al.*, 2004; Chapter 1). The favourable influence of combination of light and HPOT also seems to be

manifested in the histologically observable increase of Leydig cell number. However, this increase in Leydig cell number is not reflected in testosterone output as the circulating testosterone titre at 90 days is significantly lesser than the controls and LFPx rats but significantly more than that of HPOT rats (Lagu *et al.*, 2004; Chapter 1). Almost steady levels of serum testosterone titre right from 35 to 90 days suggest persistently reduced testosterone secretion. Corresponding reduced levels of serum LH in LFPx+HPOT rats probably under scores a down-regulated hypothalamo-hypophyseal-testicular axis. Hypothalamo-hypophyseal-adrenal axis also seems to be down regulated as seen by the significant lower circulatory corticosterone titre relative to control, LFPx and HPOT rats (Lagu *et al.*, 2004; Chapter 1). In contrast, hypothalamo-hypophyseal-thyroid axis is up regulated as seen by the significantly higher TSH and T4 titres relative to control, LFPx and HPOT rats. But the reduced T3 level almost throughout the periods of study (35-90 days) is either indicative of reduced T3 output and/or a reduced peripheral T4 to T3 conversion.

The lowered neuroendocrine-gonadal and adrenal axes are similar to the observations in HPOT rats. Nevertheless, the up regulated thyroid axis seems to be a novel change not shown by HPOT rats. These differential changes in the homeostatic hormonal status are suggestive of varied effects of neonatal LFPx, HPOT and LFPx+HPOT.

Overall the present observations suggest intriguing interactions between light and HPOT in the neonatal period and consequent long-term effects on adult testis structure and function and neuroendocrine homeostasis. It is also presumable that the favourable influences are either a consequence of HPOT in a low melatonin background or else interaction of HPOT with as yet unknown effects of light in the neonatal period.



SUMMARY:

The present study has been undertaken to evaluate the combined effects of neonatal functional pinealectomy and hypothyroidism on adult testis functions and hormonal profiles. The male rat neonates exposed to continuous light were simultaneously subjected to transient hypothyroidism (HPOT) by feeding mothers with 0.1% 6-propyl 2-thiouracil (PTU) in drinking water from day 0 to day 21. The LFPx+HPOT rats showed significantly lowered body weight whereas the absolute and relative testes weights were significantly greater at 90 days compared to controls. Seminiferous tubules were enlarged and interstitium was characterized by hypertrophic Leydig cells at 45 days compared to age-matched controls. Testicular histometry features like testicular volume, germinal epithelial thickness, seminiferous tubule volume, seminiferous tubule length, basement membrane area, Sertoli cell number and total number of germ cells were all significantly increased in LFPx+HPOT animals compared to controls. Serum LH and T levels were significantly lower at all ages while corticosterone levels were significantly lower only at 90 days. The TSH and T4 levels were higher at all ages and the T3 levels were lower in age-matched controls. Overall, the present investigation suggests intriguing interaction between light and hypothyroidism in neonatal period on adult testes structure and function. The favourable influence seen n testis histoarchitechture is related with the effect of HPOT in a low

melatonin background or an interaction of HPOT with as yet unknown effects of light in neonatal period.