EFFECT OF CONTINUOUS DARKNESS ON THE PINEAL GLAND, GONADS AND BODY WEIGHT OF A TROPICAL MAMMAL, FUNAMBULUS PENNANTI.

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ABSTRACT
In the present communication it is reported that continuous darkness for 60 days stimulates the pineal gland of sexually active male Indian palm squirrel, Funambulus pennanti. A corresponding inhibitory effect is visible on the gonads.

INTRODUCTION
Literature related to the effects of environmental lighting condition on the pineal and gonadal function of tropical animals is very limited. A number of studies have been performed on temperate animals and the function of the pineal gland has received increasing attention. It is involved in the regulation of photoperiodic responses in a number of species (Czyba et al., 1964; Reiter, 1978 and Everard and Clark, 1985). The function of pineal gland in tropical animals, however, is still unclear. This fact initiated interest in the present investigation on the Indian palm squirrel, Funambulus pennanti. It has been demonstrated that it presents a pineal dependent seasonal testicular cycle (Haldar and Saxena, 1990). Thus, the objective of the present study has been to observe the effects of constant darkness on the gonads and pineal gland of the sexually active male squirrel.

MATERIALS AND METHODS
Sixty adult males of F. pennanti (100-120 g body wt.) were obtained from local suppliers and acclimatized, for two weeks, in a room fully exposed to normal environmental conditions. The animals were housed in wire net cages throughout the experimentation and had an easy access to food consisting of soaked gram seeds and water ad libitum. They were divided into the following two groups:

GROUP I : Thirty animals were subjected to continuous dark condition.

GROUP II : Thirty animals were maintained under natural daylength (NDL).

GROUP II : Served as controls for group I

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Ten animals, from each group, were sacrificed by cervical dislocation on day 15, 30 and 60 after the onset of the experiment and their body weights noted. Testes were removed and fixed in Bouin's fluid for routine histological observations. The pineal glands were dissected out, weighted quickly on a microelectrical balance and kept frozen till the biochemical assay of protein by the method of Lowry et al. (1953). The data was analysed with the help of student's 't' test (Bruning and Kintz, 1977).

RESULTS AND DISCUSSION

The results are presented in Table-1 and Figs. 1-2.

**FIG. 1**  T. S. Testes of control F. pennanti under natural environmental condition. x 400.

**FIG. 2**  T. S. Testes of F. Pennanti exposed to continuous darkness for sixty days. Note inhibition of spermatogenesis. x 400.

15 DAYS

Table-1 depicts that the testicular and pineal gland weights of animals exposed to continuous darkness were not significantly different from those of control animals kept under NDL. No significant difference was observed between the pineal protein levels of the two groups. Histologically, the testes of both groups exhibited an active state.

30 DAYS

Exposure for 30 days resulted in a significant decrease in the testes weight when compared to the controls. A significant corresponding increase was noticed in the pineal gland weight and its protein content. However, no significant difference in the testicular histology between the dark exposed and normal squirrels was observed. Both showed an active testicular condition.

60 DAYS

After 60 days the testes of animals under constant darkness exhibited a significantly lower weight than that of controls. When compared with the controls, the animals under darkness revealed a significantly higher pineal weight and protein level. Histology of the testes indicated spermatogenic activity of squirrels under dark in comparison to testes of control squirrels (Figs. 1-2).
**TABLE 1**

Effect of continuous darkness on the pineal protein, pineal, testicular and body weight of Indian palm squirrel, Funambulus pennantii.

<table>
<thead>
<tr>
<th></th>
<th>Pineal Protein (g/100 mg tissue)</th>
<th>Pineal Weight (mg/100 g body wt.)</th>
<th>Testes Weight (g/100 g body wt.)</th>
<th>Body Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>15 DAYS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>125.62 ± 3.24</td>
<td>3.01 ± 0.02</td>
<td>0.99 ± 0.05</td>
<td>124.00</td>
</tr>
<tr>
<td>Dark</td>
<td>129.00 ± 6.74</td>
<td>2.90 ± 0.09</td>
<td>0.97 ± 0.02</td>
<td>120.00</td>
</tr>
<tr>
<td><strong>30 DAYS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>100.21 ± 2.64</td>
<td>2.54 ± 0.06</td>
<td>0.96 ± 0.04</td>
<td>126.00</td>
</tr>
<tr>
<td>Dark</td>
<td>119.76 ± 5.20**</td>
<td>3.06 ± 0.02**</td>
<td>0.81 ± 0.03</td>
<td>112.00</td>
</tr>
<tr>
<td><strong>60 DAYS</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>86.02 ± 6.26</td>
<td>2.00 ± 0.07</td>
<td>0.88 ± 0.02</td>
<td>121.00</td>
</tr>
<tr>
<td>Dark</td>
<td>115.50 ± 3.76***</td>
<td>3.22 ± 0.01***</td>
<td>0.74 ± 0.01***</td>
<td>108.00</td>
</tr>
</tbody>
</table>

Significance of difference from control -

* p < 0.01;

** P < 0.005;

*** P < 0.0001.
Animals under continuous darkness showed significantly lower body weights than controls, after 30 and 60 days of exposure.

Animals are exposed to several environmental variables such as daylength, temperature, rainfall, food availability, etc. In order to ensure the survival of the species a synchronization of their reproductive activity with the period of the year when the conditions are most conducive for the offspring is a must. Therefore, the animals need to adapt themselves to the changing environment. Variations in the environmental factors act as signals on which the animals rely to cue changes in their sexual activity by making the required adaptive physiological alteration in anticipation of the coming season (Pevet, 1987). Thus, the mediation of the environmental message to the hypothalamic-hypophysial-gonadal axis of the seasonal breeders is of utmost importance. It has been well documented that the pineal gland is implicated in this phenomenon in temperate and arctic zone mammals (Reiter, 1980; Vivien-Roels and Pevet, 1983; Brainard et al., 1985). The present study provides evidence, for the first time in the case of *F. pennanti*, that continuous darkness leads to alterations in the activity of the pineal gland and consequently testicular activity of the animals. The tropical mammalian species experience an entirely different climatic condition. It was interesting to note that squirrels maintained in dark showed an active testes even after a period of 30 days. This suggests that in this animal a duration of 30 days is not sufficient for the expression of effect of darkness on its gonads. However, the increase in pineal weight and protein level indicates an effect on its pineal activity. But this has not been able to affect the gonadal activity. Thus, though darkness affects pineal function after 30 days, it is unable to produce a significant effect on testes and the animals still maintain active spermatogenesis. This probably indicates that the pineal gland of *F. pennanti*, at this stage, is unable to exert any significant influence on its gonads. However, it cannot be denied that a close link between the pineal gland and the photoperiodic control of the neuroendocrine gonadal axis in mammals exists as pinealectomy alters the ability of a number of animals to respond appropriately to the changes in the light dark environment (Thorpe and Herbert, 1976; Turek and Campbell, 1979).

Further, after 60 days the dark exposed squirrels showed a significant decrease in testicular weight and also inhibition of spermatogenic activity. A concomitant stimulatory effect was also visible on the pineal gland weight and its activity as evident by the increase in the protein level. This confirms the antagonadotropic activity of the pineal gland of this tropical mammal (Haldar and Saxena, 1989).

The mammalian pineal gland is generally assumed to produce a substance which inhibits neuroendocrine gonadal function during short days, since pinealectomy can block the inhibitory effects of short days (Turek and Campbell, 1979). Therefore, it may be possible that after prolonged exposure to darkness, the pineal gland of *F. pennanti* produces some antagonadotropic substance which inhibits testicular function. This can be opined on the basis of the present study. Further investigations involving receptor studies of pineal as well as gonads are required to resolve this phenomenon.

When considering the effects on body weight it was again observed that the animals maintained a steady body weight after 15 days. A significant decline in the body weight was evident after 30 days of exposure to darkness. This further decreased after 60 days of exposure. It appears that the animals in dark are under stress which might be affecting their feeding habits resulting into change in body weight. It can also be suggested that darkness produces an inhibitory influence on the body weight of
squirrels. This finding corroborates the previous study on *Djungarian hamster* (Hoffmann, 1973) which shows that the seasonal rise in body weight is enhanced by exposing them to long photoperiods in mid winter and that the stimulating effect of light is suppressed by melatonin. However, in adult golden hamsters no effect of photoperiod on body weight was observed (Gaston and Menaker, 1967; Eichler and Moore, 1971). In some other rodents a cyclicity of body weight with maximum at the time of maximal gonadal activity and minimal at the time of gonadal quiescence has been reported (Khateeb and Johnson, 1971 a; Evans, 1973). Photoperiodic effects on both functions have been demonstrated (Khateeb and Johnson, 1971 b). In *F. Pennantii* constant darkness has a similar effect on its gonad and body weight but an opposite one on its pineal.

The results suggest that darkness inhibits gonadal activity of this tropical mammal and the pineal gland appears to be involved in this phenomenon.

ACKNOWLEDGEMENTS

Grateful acknowledgements are made to CSIR, New Delhi for the award of a research associateship to the first author.

REFERENCES


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