ADDENDUM

Scientific papers published and manuscripts submitted during my tenure of research for the degree of doctor of philosophy in Zoology.

Papers published	-	3
Papers in press	-	3
Manuscripts submitted	-	6
TOTAL		12

A. A LIST OF PUBLISHED SCIENTIFIC PAPERS

(XEROXED COPIES ATTACHED)

SERIAL NO.	TITLE OF PAPER	JOURNAL	YEAR
1.	Extraretinal photoreception in lacertilian tail regeneration : the lateral Eyes are not involved in photoreception in the gekkonid lizard, Hemidactylus flaviviridis.	Journal of Experimental Zoology, U. S. A.	1988
2.	Preliminary evidence for pineal mediated extraretinal photoreception in relation to tail regeneration in the gekkonid lizard, Hemidactylus flaviviridis.	Journal of Pineal Research, U. S. A.	1989
3.	Parachlorophenylalanine retards tail regeneration in the gekkonid lizard, Hemidactylus flaviviridis exposed to continuous light.	Journal of Experimental Biology, Great Britain	1989

B. A LIST OF SCIENTIFIC PAPERS IN PRESS

SERIAL NO	TITLE OF PAPER	JOURNAL	YEAR
1.	Tail regeneration in normal blinded and pinealectomized gekkonid lizard, Hemidactylus flaviviridis exposed to four light conditions under three seasons (temperatures).	Acta Zoologica, Sweden.	1989
2.	Is the pineal involved in the stimulatory influence of prolactin on tail regeneration in lizards ? Studies with exogenous prolactin in lizards exposed to continuous darkness.	General and Comparative Endocrinology Great Britain	1989
3.	Dopamine antagonist speeds up tail regeneration in lizards exposed to continuous darkness : Evidence for prolactin involvement.	Proceedings of the Society for Experimental Biology and Medicine, U.S.A.	1989

C. A LIST OF MANUSCRIPTS SUBMITTED FOR PUBLICATION

SERIAL NO	TITLE OF PAPER	JOURNAL	YEAR
1.	Evidence showing that the time of administration of the indoleamine, melatonin determines its proregenerative or anti-regenerative effect in the gekkonid lizard, Hemidactylus flaviviridis.	General and Comparative Endocrinology, Great Britain.	1989
2.	Bromocriptine retards tail regeneration in 12L : 12D but not 24L : OD exposed lizards : Evidence for photoperiodic control of prolactin release mechanisms in lizards.	Journal of Experimental Zoology, U.S.A.	1989
3.	Melatonin in lacertilian tail regeneration : Duration, Phase specificity and Synchronization of the daily light - dark cycle.	Journal of Pineal Research, U.S.A.	1989
4.	Effect of different photoperiodic lengths on tail regeneration in the gekkonid lizard, Hemidactylus flaviviridis.	Indian Journal of Experimental Biology, New Delhi.	1989

Cont...

SERIAL NO	TITLE OF PAPER	JOURNAL	YEAR
5.	Effects of photoperiodism, pinealectomy and seasonal variation in temperature on tail regeneration in the gekkonid lizard, Hemidactylus flaviviridis.	Indian Journal of Experimental Biology, New Delhi.	1989
6.	Failure of the dopamine agonist, bromocriptine, to retard tail regeneration in the gekkonid lizard, Hemidactylus flaviviridis exposed to continuous light and continuous darkness.	Indian Journal of Experimental Biology, New Delhi.	1989

THE JOURNAL OF EXPERIMENTAL ZOOLOGY 248:73-80 (1988)

Extraretinal Photoreception in Lacertilian Tail Regeneration: The Lateral Eyes Are Not Involved in Photoperiodic Photoreception in the Gekkonid Lizard, *Hemidactylus flaviviridis*

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ABSTRACT The tail of the Gekkonid lizard, *Hemidactylus flaviviridis* was autotomized and subjected to eight different photoperiodic lengths during the period of tail regeneration, namely, 1) continuous light (LL: LD 24:0) of high intensity; 2) continuous light (LL: LD 24:0) of low intensity; 3) continuous (total) darkness (DD: LD 0:24); 4) normal light and darkness (NLD: LD 12:12); 5) 18 hours light and 6 hours darkness (LD 18:6); 6) 6 hours light and 18 hours darkness (LD 6:18); 7) 16 hours light and 8 hours darkness (LD 16:8); and 8) 8 hours light and 16 hours darkness (LD 8:16). In an attempt to determine the potential contribution of the lateral eyes, or vision, on photoperiodic photoreception in *H. flaviviridis* during the process of tail regeneration, some animals had both the lateral eyes surgically removed (bilateral orbital enucleation) and the enucleated animals were exposed, along with the normal (unoperated) ones, to the various photoperiodic regimes. Our observations demonstrate that blinded *Hemidactylus* regenerate their lost (autotomized) tails similar to their sighted (unoperated) counterparts and under LL, LD 18:6, and LD 16:8 better than sighted (unoperated) animals exposed to DD and NLD experimental lighting regimens. We, therefore, conclude that photoperiodic control of regeneration in the Gekkonid lizard, *H. flaviviridis* is mediated entirely by extraretinal photoreceptor(s) situated in the brain region of the head. Having established that the lateral eyes, or retinae, do not participate in photoperiodically significant photoreception and under the presumption that an extraretinal light receptor(s) may be involved, we discuss the pineal organ as the possible transmitter of the photic stimulus in these animals.

Previous studies with lizards demonstrated that extraretinal photoreceptors are involved in the photoperiodic response in *Anolis carolinensis* (Underwood, '75). Accordingly, long stimulatory photoperiods have been shown to induce testicular recrudescence and maturation in blinded Anoles Similar studies have been conducted on a variety of vertebrate species such as fishes and birds where testicular growth could be induced in blinded animals by exposure to stimulatory photoperiods (Underwood, '79). The first demonstration that extraretinal photoreceptors could participate in the photoperiodic responses of vertebrates occurred more than 50 years ago when Benoit showed that light could induce testicular growth in blinded ducks (Benoit, '35) One of the techniques developed to ascertain the potential contribution of eyes and extraretinal receptors in birds involved opaquing the heads of birds while leaving the eyes exposed (McMillan et al., '75) In the house sparrow so treated, no response occurred when the birds were maintained under long stimulatory photoperiods, despite the fact that the eyes were exposed showing that extraretinal receptors were solely involved in mediating photoperiodic photoreception in these birds.

Other studies have indicated that the eyes, or vision, are not necessary for photoperiodic induction of gonadal growth in the chicken, *Gallus domesticus* (Ookawa, '70), the common coturnix or Japanese quail, *Coturnix coturnix japonica* (Homma and Sakakibara, '71), and the house sparrow, *Passer domesticus* (Menaker, '71)

According to Maier and Singer ('77) and Turner and Tipton ('72), long-length photoperiod can speed up the rate of forelimb regeneration in the newt and tail regeneration in lizards, respectively. This effect is not mediated by the optic system as blinded newts kept in continuous light (LL) regenerated their fore-

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limbs more rapidly than their sighted counterparts kept in total darkness (DD) (Maier and Singer, '77). With the above-mentioned literature in view and owing to the fact that no investigation has yet been carried out on extraretinal photoreception in relation to tail regeneration in lizards in general and *Hemidactylus* in particular, the present investigation was deemed appropriate and timely To demonstrate the potential contribution of the lateral eyes, or vision, on photoperiodic photoreception in *H. flaviviridis*, both the lateral eyes were surgically removed (bilateral orbital enucleation) and the enucleated animals were exposed, along with their sighted (unoperated) counterparts, to different photoperiodic lengths during the process of tail regeneration.

MATERIALS AND METHODS

Adult *Hemidactylus flaviviridis* of both sexes weighing 10 ± 1 gm and measuring 145 ± 5 mm snout–vent length were obtained from a local commercial supplier (M/s. Zoophyton, Baroda, India) and maintained on a diet of cockroaches for a period of 7 days for acclimation to laboratory conditions.

Two groups (normal, NL; blinded, BL) of 40 lizards for each of the eight experimental photoregimens were employed in this investigation. One group of 40 lizards in each setup, which served as experimentals, was blinded by surgical removal of both the lateral eyes (bilateral orbital enucleation). The enucleated animals were allowed 5 days recovery period to eliminate any traumatic side effects due to surgery A second group of 40 lizards in each setup, which served as the controls, remained sighted without any operations performed.

Eight photoperiodic lengths were investigated, namely, 1) continuous light (LL: LD 24:0) of high intensity—2,500 lux units; 2) continuous light (LL: LD 24:0) of low intensity—638 lux units; 3) 18 hours of light and 6 hours of darkness (LD 18:6); 4) 16 hours of light and 8 hours of darkness (LD 16:8); 5) 12 hours of light and 12 hours of darkness (LD 12:12); 6) 8 hours of light and 16 hours of darkness (LD 8:16); and 7) 6 hours of light and 18 hours of darkness (LD 6:18); and 8) continuous (total) darkness (DD: LD 0:24) Photoregimens 3–7 were of high intensity—2,500 lux units

The cages housing the animals measured 18 in × 15 in × 10 in with one side made of transparent glass and ventilated on three sides. Each cage housed a total of 20 lizards balanced for size and sex. Food and water were provided ad libitum. The cages housing animals for the light experiments were placed (glass surface up) under suspended 40-W fluorescent lamps, thereby facing the source of illumination. The inside of the wooden cages were lined with aluminum foil so that lighting was direct as well as reflected The distance from the fluorescent lamp to the glass surface of the cage was 15 in and to the floor level was 25 in. The light intensity was measured at the floor level and reflecting surfaces of the cage using a lux meter (Weston Electrical Instrument Corporation, NJ). To obtain a high light intensity of 2,500 lux units, four fluorescent lamps were fixed and beamed together, and for the low light intensity of 638 lux units only one fluorescent lamp was utilized. The cages housing animals for the continuous (total) darkness (DD: LD 0:24) experiment were placed in a dark chamber completely shielded from light with opaque papers. Except for about 2 minutes daily exposure to dim light for taking measurements, animals in this experimental photoperiodic regimen were completely deprived of light. The source of the dim light used for taking measurements of animals in DD was a small electric bulb completely wrapped with a red transparent paper. The animals of LD 18:6, LD 16:8, LD 12:12, LD 8:16 and LD 6:18 were kept in the lighted chamber at 7:00 A.M. and were shifted into the dark chamber at the end of the respective lengths of exposure.

Tail autonomy, in NL and BL groups of lizards, was carried out by pinching off the tails at the third segment from the vent and the animals exposed to the eight different experimental photoregimes during the entire process of tail regeneration. The length of new growth (regenerate), in mm, was measured with a graduated meter rule and recorded at fixed time intervals of 5, 10, 20, 30, 40, 50, and 60 days postcaudal autotomy. This investigation was conducted during the post-breeding monsoon months (August–October) and the recorded average monthly ambient, room and cage temperatures are given in Table 2. The recorded average cage temperature in the lighted and dark chambers did not vary by more than 2°C at any stage (Table 2). The data on the length of tail regenerated and the percentage replacement were subjected to an analysis of variance and further to Duncan's multiple range test with an alpha level of both 0.05 and 0.01 (Duncan, '55).

RESULTS

The results are depicted clearly in Table 1 and Figures 1–3.

Growth rate and total length regenerated and percentage replacement

A measurable growth occurred in normal (NL) and blinded (BL) groups of animals exposed to LL and LD 18:6 photoperiodic schedules by day 5, while in NLD, LD 16:8, and LD 8:16 groups of animals it occurred between days 5 and 10 However, in lizards

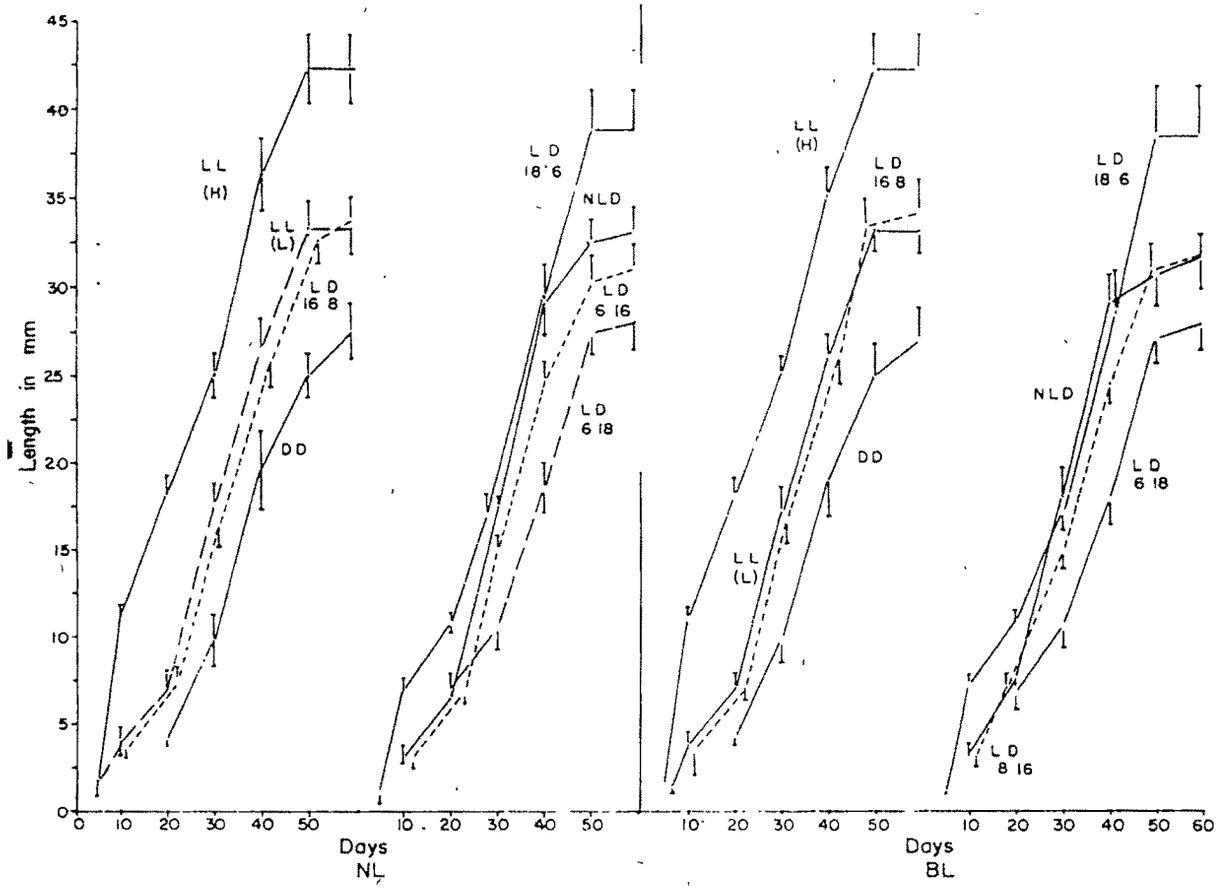

Fig 1 Regenerative tail elongation in sighted and blinded *Hemidactylus* under different photoperiodic regimens

TABLE 1 Approximate number of days taken to reach the various arbitrary stages of tail regeneration in both normal and blinded H. flaviviridis[1]

	Wound healing	Blastema	Early differentiation	Differentiation	Late differentiation	Growth	Fully regenerated tail
LL (H)	3	5–7	7–9	10	20	30	50
LL (L)	3	5–7	7–9	10	30	40	50
LD 18 6	3	5–7	7–9	10	25	35	50
LD 16 8	5	8–10	12–14	20	30	40	60
LD 12 12	5	8–10	12–14	20	30	40	60
LD 8 16	5	10–12	14–16	22	32	42	60
LD 6 18	8	12–14	16–18	25	35	45	60
LD 0 24	8	12–14	16–18	25	40	45	60

LL (H) – continuous light (high intensity), LL (L) – continuous light (low intensity), LD 18 6 – 18 hours (high intensity) light and 6 hours darkness, LD 16 8 – 16 hours (high intensity) light and 8 hours darkness, LD 12 12 – 12 hours (high intensity) light and 12 hours darkness, LD 8 16 – 8 hours (high intensity) light and 16 hours darkness, LD 6 18 – 6 hours (high intensity) light and 18 hours darkness, LD 0 24 – continuous (total) darkness

TABLE 2 Average ambient, room, and cage temperatures and humidity during the period of study[1]

	Temperature measurement							
	Ambient		Room		Cage		Humidity (%)	
Months	Max.	Min	Max	Min	Max	Min	Max	Min
August[1] 1986	29 0	23 9	28 0	21 0	28 0	22 0	99	42
September 1986	35 4	24 6	32 0	22 0	33 0	21 0	94	40
October 1986	37 7	22 0	34 0	20 0	35 0	20 0	93	13
Average daily temperature Lighted chamber: 27°C Dark chamber 25°C								

[1]Postbreeding monsoon season

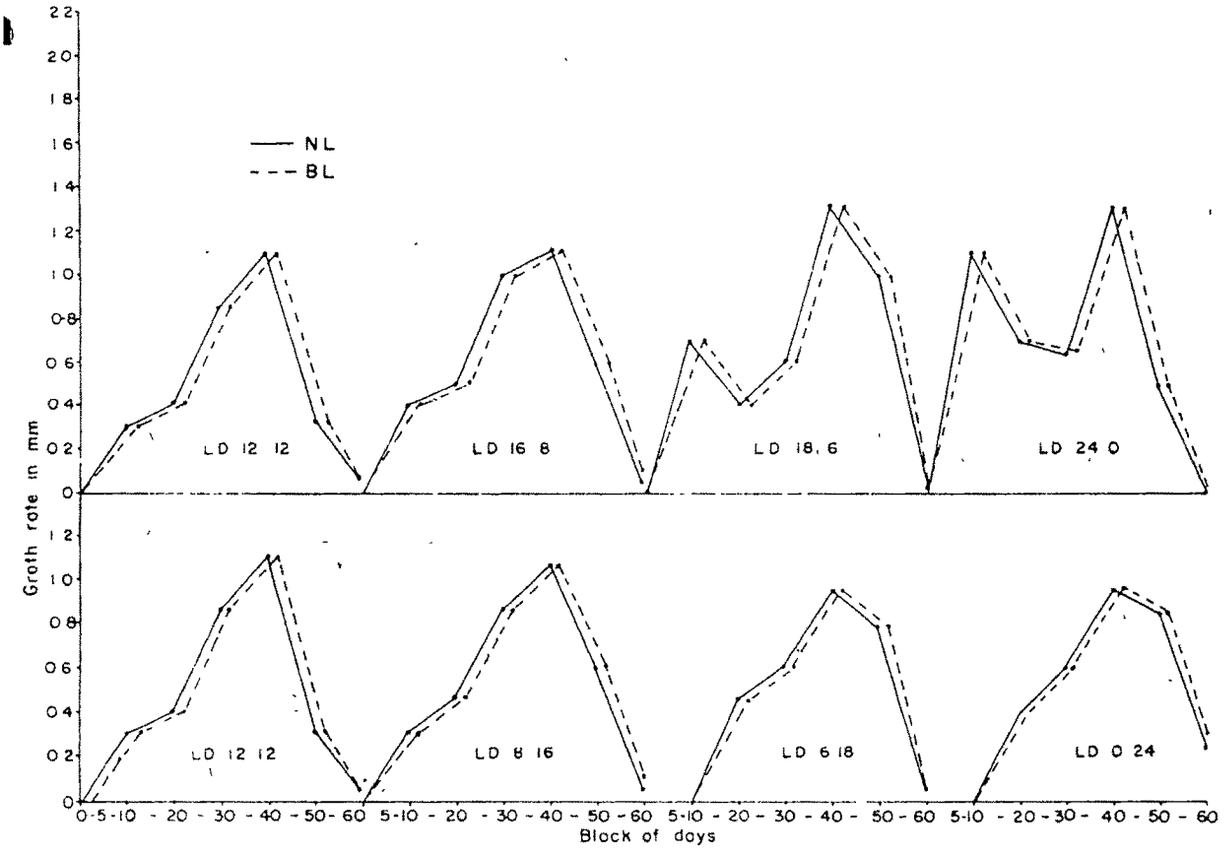

Fig 2 Per day rate of regenerative growth in sighted and blinded *Hemidactylus* under different photoperiodic conditions

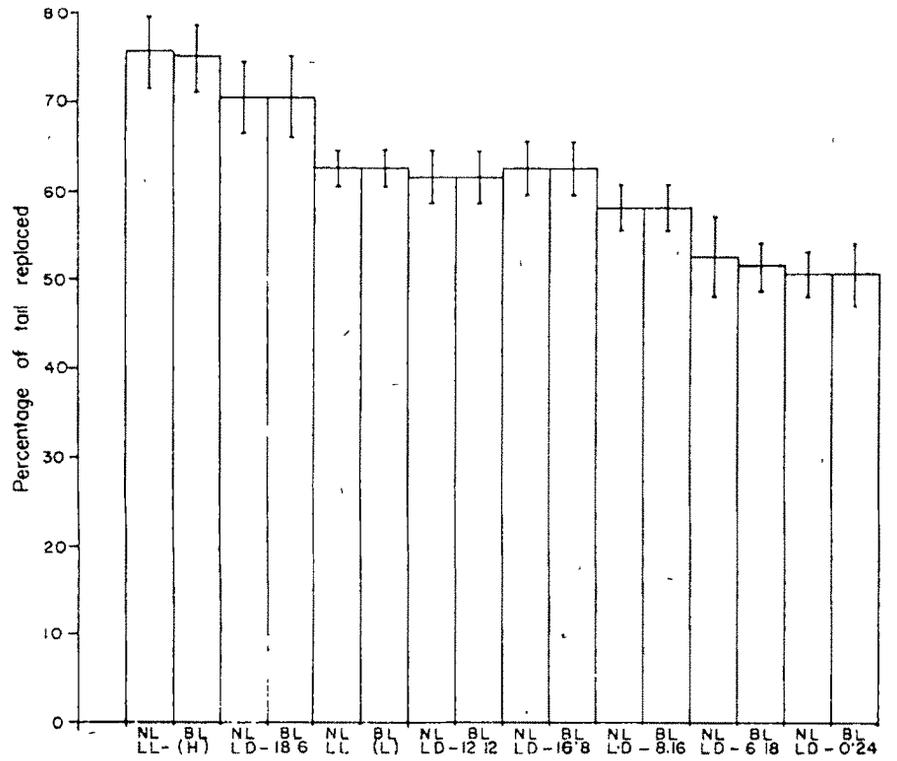

Fig. 3. Percentage replacement during tail regeneration in sighted and blinded *Hemidactylus* under different photoperiodic regimens. LL(H), continuous light, high intensity; LL(L), continuous light, low intensity; DD, continuous (total) darkness; NLD, normal light and darkness; 18LL 6DD, 18 h light, 6 h darkness; 6LL 18DD, 6 h light, 18 h darkness, 16LL 8DD, 16 h light, 8 h darkness; 8LL 16DD, 8 h light, 16 h darkness

exposed to LD 6:18 and DD a measurable growth occurred only between days 10 and 15 postcaudal autotomy The regeneration process was completed in LL(H), LL(L), and LD 18:6 groups of animals by the 50th day, while in the other groups of lizards, the regenerative growth ceased by day 60. The total length of tail regenerated was maximal under LL(H)—41.7 mm (NL), 42 0 mm (BL)—and minimal under DD—27.4 mm (NL), 27.0 mm (BL)—which was a replacement of 75.3% (NL), 75 1% (BL), and 50 5% (NL), 50 4% (BL), respectively, with the total length of tail regenerated and percentage replacement in the other groups of animals in between.

The pattern of growth rate depicted in Figure 2 indicates a linear increase peaking at 30–40 days in both NL and BL groups of animals exposed to all photoperiodic regimes from DD to LD 16 8 However, LD 18 6 and LL schedules induced a very significant initial growth spurt which rendered the growth rate curve a biphasic one with increasing photoperiodism beyond 16 hours having a definite positive influence on this initial spurt. The positive influence of photoperiodism was further revealed by the gradually decreasing peak growth rate from LD 16.8 to DD.

All possible comparisons between the eight experimental setups (Duncan's multiple range test) revealed no statistical significance between NL and BL groups of animals, and those exposed to DD and LD 6.18 on the one hand and between NLD, LL(L) and LD 16·8 on the other. However, all other comparisons other than these were statistically significant at both 5% and 1% levels

DISCUSSION

Long-day photoperiod stimulates tail regeneration in the Gekkonid lizard, *Hemidactylus flaviviridis*, whereas short-day photoperiod has no effect (Ndukuba and Ramachandran '88) We now report that this stimulatory photic effect is not mediated by the lateral eyes, or retinae, as blinded *Hemidactylus* re-

generated their lost (autotomized) tails similar to their sighted (unoperated) counterparts. We, therefore, presume that an extraretinal photoreceptor(s) situated in the brain region of the lizard head mediate in photoperiodic photoreception during the process of tail regeneration.

Most investigations on a potential role for retinal receptors have been conducted with birds. A long series of investigations by Benoit on the domestic duck demonstrated the participation of extraretinal receptors in the photoperiodic response in ducks (Benoit, '35). Many other different combinations of experiments led Benoit to conclude that both retinal and extraretinal photoreceptors are involved (Benoit, '70). However, a careful reconsideration of the published work of Benoit by McMillan et al. ('75) led them to conclude that a retinal participation in photoperiodism in ducks has not been conclusively demonstrated. The participation of extraretinal receptors in testicular responses in a second avian species, the house sparrow, *Passer domesticus*, was shown by Menaker and Keatts ('68). A series of experiments with house sparrows, utilizing several different experimental approaches, demonstrated that the eyes are not involved in photoperiodic photoreception, extraretinal receptors located in the brain are fully capable of mediating this response (McMillan et al., '75) Subsequently, other avian species (chickens, Japanese quail, and white-crowned and golden-crowned sparrows) have been investigated (Turek, '75) and no clear demonstration of a retinal involvement in the stimulation of gonadal recrudescence in birds has been shown with blinded birds responding as well as intact birds to stimulatory photoperiods.

In our study of extraretinal photoreception in relation to tail regeneration in the lizard, *H. flaviviridis*, we first applied a technique of shielding the lateral eyes with a piece of dark cloth while leaving the head region exposed to light. This technique proved unsuccessful because the lizards frequently removed the coverings by scratching their heads against the walls of the wooden cages. We then proceeded surgically to extirpate both the lateral eyes (bilateral orbital enucleation) and our observations showed that *Hemidactylus* accepts enucleation very well and the mortality rate was negligible. From the time of autotomy till the completion of the regenerative growth, there was no significant alteration either in the initiation and onset of regeneration, the daily growth rate, the total new growth (regenerate) produced at the end of regeneration or the total percentage replacement of the lost (autotomized) tails in blinded lizards as compared to their sighted (unoperated) counterparts exposed to similar experimental photoperiodic schedules.

We used two different light intensities in this investigation, a high light intensity of 2,500 lux units and a low intensity of 638 lux units. Although the higher intensity produced a better regenerative performance, the lateral eyes did not play any significant role in photoperiodic photoreception since enucleated animals regenerated their autotomized tails similar to their sighted counterparts. Underwood ('80) demonstrated that the eyes were not involved in testicular recrudescence in *Anolis carolinensis* exposed to light of 40 lux units intensity and doubted whether they could be involved at higher intensities. Although we were unaware of Underwood's work at the time we began our investigation on extraretinal photoreception in relation to tail regeneration in the lizard, *Hemidactylus*, we now feel that our observations in this report can satisfactorily erase that doubt since at either 638 or 2,500 lux units of light intensity, the eyes did not participate in photoperiodically significant photoreception.

In mammals, intact retinae appear to be required for lighting information to influence most endocrine systems (Hollwich, '64) and circadian rhythmicity (Snyder et al., '64). However, Ganong et al. ('63) have found that measurable amounts of light can penetrate the skull to the brain of mammals without the intervention of the eyes. Other workers have obtained evidence suggesting that light can directly affect hypothalamic neurons in the duck (Benoit, '64) and rat (Liske and Kannwischer, '64). The effects of light exposure on pineal were reportedly abolished by bilateral orbital enucleation (Snyder et al., '64; Zweig et al., '66).

In the present investigation, blinded (BL) and sighted (NL) *Hemidactylus* responded similarly to continuous illumination as well as to the other experimental lighting regimens. From Figures 1 and 3, it becomes obvious that both the total length of tail regenerated and percentage replacement are maximal under LL(H) and minimal under DD in NL as well as BL groups of animals. Though the values with regards to these two parameters were quite similar in LL (L), NLD and LD 16:8 on the one hand and LD 6:18 and DD on the other, a definite linear correlation between the length of tail regenerated and total percentage replacement can be inferred. This fact is confirmed by the observed values under LD 18:6 which were significantly more than all the other groups except for LL(H). Another striking inference that could be drawn by careful study of Figure 2 is the biphasic growth spurt, in both NL and BL groups of animals, during the course of tail regeneration, under LL (H), LL (L), and LD 18:6 photoregimens and the significant linear positive influence of increasing intensity and duration of light

exposure on the initial growth spurt. The observable effect of decreasing light schedules on the initiation of the regeneration process is a delayed temporal shift by 5 and 10 days, respectively, in LD 6:18 and DD groups of animals. Moreover, there is no remarkable effect of increasing photoperiodism on the normal regenerative growth spurt which occurs between 30 and 40 days. It is, however, difficult at this juncture to give any interpretive explanation on the observed initial growth spurt in LD 18:6 and LL regimes and the resultant biphasic growth curve.

Our results demonstrate that the lacertilian lateral eyes, or retinae, do not participate in photoperiodically significant photoreception. On the basis of current knowledge, the most likely photoreceptor is the pineal organ. Over the years, a body of information, based largely upon indirect morphological evidence, has accumulated to the effect that the epiphyseal complex of the lower vertebrates is responsive to light and darkness. A few electrophysiological studies now lend direct evidence of such activity in fishes (Hangri et al., '69) and reptiles (Miller and Wolbarsht, '62)

How the pineal may respond to affect the rate of tail regeneration can only be speculated upon. Melatonin is produced by the pineal gland and is a mitotic inhibitor (Banerjee and Margulis, '73) Melatonin levels are lowest during the day and can be suppressed by extended exposure to light (Brownstein, '75). Litwiller ('40) demonstrated that the mitotic rate of blastemal cells peaks during the light phase of the diurnal cycle Our results demonstrate that it is during the preblastemic, blastemic, and early differentiation stages of regeneration, characteristized by high mitotic potential, that the positive influence of increasing photoperiodism on the regenerative performance is essentially exerted. Apparently, photic input is being transduced and translated into hormonal and or physiological responses favouring growth potential, though the exact action at the cellular level remains speculative. It may be that the increased mitotic rate during the daylight hours and its subsequent decline during the dark phase bears a causal relation to the melatonin cycle. Alternatively, increased or decreased lengths of light may affect the production of prolactin which is a known growth promoter (Crim, '75). Bourne and Tucker ('75) had, in fact, demonstrated the positive influence of increasing lengths of light on the level of serum prolactin. Serotonin could, in this respect, mediate the light effect since it is enhanced by light (Brownstein, '75). Moreover, serotonin and its precursors have been shown to elevate serum prolactin levels (Lu and Meites, '73) and, therefore, could operate as a mitotic stimulator by way of its ability to induce prolactin release.

ACKNOWLEDGMENTS

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Journal of Pineal Research 6:121–134 (1989)

Preliminary Evidence for Pineal-Mediated Extraretinal Photoreception in Relation to Tail Regeneration in the Gekkonid Lizard, *Hemidactylus flaviviridis*

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The tail of the Gekkonid lizard *Hemidactylus flaviviridis* was autotomized and the animals were subjected to eight different photoperiodic schedules during the process of tail regeneration. Our previous observation had shown that long-day photoperiods stimulate the regeneration process, whereas short-day photoperiods depress it. Furthermore, it has also been demonstrated that the lateral eyes, or retinae, do not participate in photoperiodically significant photoreception in *H. flaviviridis*, as blinded *Hemidactylus* regenerated their autotomized tails like their sighted counterparts exposed to similar experimental photoregimes. In a further attempt to localize the site(s) of photoreception in these animals, one group of lizards had their heads painted with a mixture of Indian ink and Nile blue sulphate (II-NBS) [NL (HP)] in order to prevent light from penetrating to the pineal gland, and another group had their pineal glands surgically removed (pinealectomy, Px), the regenerative potentials were compared with their normal (NL) counterparts. Our results showed that the initiation and onset of regeneration, the daily growth rate, the total new growth (regenerate) produced at the end of regeneration and the total percentage replacement of the lost (autotomized) tails were significantly retarded in Px and NL (HP) animals, compared with the NI (unoperated and nonpainted) ones. Since pinealectomy as well as light deprivation to the pineal abolished the stimulatory influence of long-length photoperiods, the pineal gland is discussed here as a major transmitter of photic stimulus in lacertilian tail regeneration. It is presumed that in the lizard, as in mammals and some birds, the pineal gland acts by way of the neuroendocrine complex and/or the hypothalamohypophyseal axis.

Key words: photoperiod, pinealectomy, reptilia

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INTRODUCTION

It is well established that among fishes, amphibians, and reptiles the pineal gland, a small structure embedded in the top of the brain, and such associated structures as the parietal "eye" are sensitive to light. The pineal system (pineal organ and parietal eye) is light sensitive on the basis of neurophysiological and cytological evidence [Wurtman et al., 1968]. Ultrastructural and neurophysiological studies have convincingly shown that the lizard's parietal eye is a functional photoreceptor [Eakin, 1973]. The parietal eye often contains a well-defined cornea, a lens, and a retina; the retina contains photosensory cells similar in appearance to those found in lateral eyes [Hamasaki and Eder, 1977]. These photosensory cells synapse with ganglion cells that send axons to the rest of the brain. In some studies, removal or shielding of the parietal eyes of lizards has affected photoperiodic responses and activity of individuals exposed to field conditions or to photothermal gradients in the laboratory [Stebbins, 1963, 1970; Stebbins and Eakin, 1958]. The obvious photoreceptive capabilities of lizard parietal eyes have prompted studies on the role this organ may play in mediating such light-dependent processes as activity, reproduction, metabolism, and thermoregulation [Eakin, 1973; Ralph et al., 1979]. These studies suggest that the parietal eye has an inhibitory role, since removal or shielding the parietal eye in some cases causes increased exposure of the lizards to photothermal stimuli. In such cases, however, it is not clear whether the parietal eye is directly involved as a photoreceptor organ or indirectly involved via the role it plays in thermoregulation [Ralph et al., 1979].

The pineal gland is present in all vertebrates and generally appears to be glandular in nature. In lower forms, cells are present that are not unlike rod and cone cells in the retinae of normal eyes but are not organized as such. It is possible, however, that in the course of evolution there has been a change from a primitive photoreceptive type of organ, which can translate photic stimuli into physiological controls of different types, to a sensory structure, which can carry out similar functions in response to stimuli affecting normal optic pathways. Many species of fish, reptiles, and amphibians monitor the light-dark cycle by way of a third eye (parietal or parapineal eye) or via the pineal itself [Eakin, 1973; Adler, 1976]. Photosensory cells, ependymal (supportive) cells, and certain other types of cells have been recognized and described in the pineal organs of lacertilians [Steyn, 1960; Eakin et al., 1961; Collin, 1967; Wartenberg and Baumgarten, 1968; Hamasaki and Dodt, 1969] and chelonians [Vivien-Roels, 1969]. The consensus of opinion seems to be that the particular cells having outer segments containing laminated cells are photosensory.

The perception of light provides important information for the organism on its environment. For this purpose most animals possess well-developed photoreceptors and neuronal networks in the retina of their lateral eyes. Interestingly, even in species with highly organized ocular photoreceptors, additional photoreceptive structures—extraocular photoreceptors—are utilized in the transmission of photic information about the day-night schedule and seasonal photoperiod changes. Considerable evidence supports the view that the pineal gland is the principal site of extraocular photoreception in lower vertebrates [cf. Meissl and Dodt, 1981]. Recent radioimmunoassay studies have

revealed the presence of circadian oscillators in the isolated pineal organs of the lizard *Anolis carolinensis* [Menaker and Wisner, 1983].

Previous studies with lizards demonstrated that extraretinal photoreceptors are involved in the photoperiodic response in *A. carolinensis* [Underwood, 1975]. Accordingly, long-stimulatory photoperiods have been shown to induce testicular recrudescence and maturation in blinded *Anoles*. Similar studies have been conducted on a variety of vertebrate species such as fishes and birds in which testicular growth could be induced in blinded animals by exposure to stimulatory photoperiods [Underwood, 1979]. According to Maier and Singer [1977] and Turner and Tipton [1972], long-length photoperiod can speed up the rate of forelimb regeneration in the newt and tail regeneration in lizards, respectively. This effect is not mediated by the optic system, as blinded newts kept in continuous light regenerated their forelimbs more rapidly than their sighted counterparts kept in total darkness [Maier and Singer, 1977].

The response of homeothermic animals to light is known to be influenced by pineal activity [see reviews by Kappers, 1971; Sorrentino and Benson, 1970; Relkin, 1975; Oksche, 1976]. In reptiles, the effects of pinealectomy have been studied mainly with regard to gonadal development and behavioral activity [Stebbins, 1970; Levey, 1973; Haldar and Thapliyal, 1977; Thapliyal and Haldar, 1979; Underwood, 1981] To our knowledge, no investigation has yet been carried out on pineal-mediated extraretinal photoreception in relation to tail regeneration in lizards in general and *Hemidactylus* in particular. Hence the present preliminary investigation was designed to elucidate the role of the pineal gland in photoperiodic photoreception during the process of tail regeneration. One group of *H. flaviviridis* had their heads painted with a mixture of Indian ink and Nile blue sulphate (II-NBS) in order to prevent light from penetrating to the pineal gland, another group had their pineal glands surgically removed (pinealectomy), and the regenerative potentials were compared with the normal (unoperated and nonpainted) counterparts exposed to similar experimental photoperiodic schedules.

MATERIALS AND METHODS

Freshly collected adult *H. flaviviridis* of both sexes weighing 10 ± 1 gm and measuring 80 ± 5 mm snout-vent length were obtained from a commercial supplier (M/s.Zoophyton, Baroda, India) and maintained on a diet of cockroaches for a period of 7 days for acclimation to laboratory conditions. A total of 760 lizards was used in this investigation, and they were divided into four groups.

Group 1—Experimental

The first group, of 320 lizards that served as the experimentals, had their pineal glands surgically removed (Px). The group was then divided into eight batches of 40 lizards each and exposed to eight lighting schedules, as detailed later.

Group 2—Experimental

The second group, of 80 lizards, had the brain region of the head painted with a mixture of equal parts of Indian ink and 10^{-3} Nile blue sulphate, in order

to prevent light from penetrating to the pineal organ (NL-HP). Since *H. flaviviridis* sheds its skin epithelium periodically, it was necessary to reapply the painting mixture every alternate day. The mixture was applied, using a thin brush, to an area extending rostrocaudally from the snout to the base of the skull and laterally to an area between the ears. Two batches of forty lizards each from the group were exposed to continuous light and 12-hour light regimes.

Group 3—Controls

The third group, of 320 lizards, had intact pineals without any head paint (NL). Forty lizards each were then exposed to the eight photoregimes.

Group 4—Controls

To be certain that the results observed contained no toxicity artefact from the application of Indian ink-Nile blue sulphate mixture, a group of forty lizards had their dorsal pelvic region painted with the mixture. Twenty lizards each from this group were then exposed to continuous light and 12-hour light schedules.

The eight photoperiodic schedules investigated were: 1) continuous light (LL:LD 24:0) of high intensity—2,500 lux units, 2) continuous light (LL:LD 24:0) of low intensity—638 lux units; 3) 18 hours of light and 6 hours of dark (LD 18:6); 4) 16 hours of light and 8 hours of dark (LD 16:8); 5) 12 hours of light and 12 hours of dark (LD 12:12); 6) 8 hours of light and 16 hours of dark (LD 8:16); 7) 6 hours of light and 18 hours of dark (LD 6:18); and 8) continuous (total) darkness (DD:LD 0.24). Photoregimes three to seven were of high intensity—2,500 lux units.

The cages housing the animals measured 18 in × 15 in × 10 in, with one side made of transparent glass and ventilated on three sides. Each cage housed a total of 20 lizards, and they were balanced for size and sex. Food and water were provided ad libitum. The cages housing animals for the light experiments were placed (glass surface up) under suspended 40-W fluorescent lamps, thereby facing the source of illumination. The inside of the wooden cages was lined with aluminum foil so that lighting was direct as well as reflected. The distance from the fluorescent lamp to the glass surface of the cage was 15 in and to the floor level, 25 in. The light intensity was measured at the floor level and reflecting surfaces of the cage using a lux meter (Weston Electrical Corporation, NJ). To obtain a high light intensity of 2,500 lux units, four fluorescent lamps were fixed and beamed together; for the low intensity of 638 lux units, only one fluorescent lamp was utilized. The cages housing animals for the continuous (total) darkness (DD:LD 0.24) experiment were placed in a dark chamber completely shielded from light with opaque papers. Except for a period of about 2 minutes' exposure to dim red light for taking measurements, animals in this experimental photoperiodic regime were maintained in complete darkness. The source of the dim light used in taking the measurement of animals exposed to DD was a small electric bulb completely wrapped with a red transparent paper. The animals of LD 6:18, LD 8:16, LD 12:12, LD 16:8, and LD 18:6 were kept in the lighted chamber at 7:00 A.M. and were shifted into the dark chamber at the end of the respective lengths of exposure.

TABLE 1. Average Ambient and Room and Cage Temperatures (°C) During the Period of Study

	Temperature (°C)							
	Ambient		Room		Cage		Humidity (%)	
Months[1]	Max	Min	Max	Min	Max	Min	Max	Min
August, 1986	29.0	23.9	28.0	21.0	28.0	22.0	99	42
September, 1986	35.4	21.6	32.0	22.0	33.0	21.0	94	40
October, 1986	37.7	22.0	34.0	20.0	35.0	20.0	93	13

[1]Postbreeding monsoon season. Average daily temperatures: lighted chamber, 27°C., dark chamber, 25°C.

Tail autotomy was performed by pinching off the tail at the third segment from the vent. The length of new growth (regenerate) in mm was measured with a graduate meter rule and recorded at fixed time intervals of 5, 10, 20, 30, 40, 50, and 60 days postcaudal autotomy. At the end of the experimentation, the pinealectomized animals were sacrificed, and microscopic examination of the head region and histologic examination of the brain were performed to ensure complete removal of the pineal without any damage to the brain. This investigation was conducted during the postbreeding monsoon months (August–October); the recorded average monthly ambient and room and cage temperatures are given in Table 1. The average daily temperatures at the level of the animals in the lighted and dark chambers did not differ by more than 2°C. Data on the length of tail regenerated and the percentage of replacement were subjected to an analysis of variance and to Duncan's multiple range test with an alpha level of both 0.05 and 0.01 [Duncan, 1955].

RESULTS

The results are depicted in Table 2 and Figures 1–3. The blastemic stage appeared in LL and LD 18:6 exposed animals by day 5 to day 7 and in DD and LD 6:18 exposed animals by day 12 to day 14 postcaudal autotomy. In Px lizards, the blastemic stage occurred by day 8 to day 10 and day 18 to day 20, respectively. In the intermediate photoperiod of NLD:LD 12:12 and LD 16:8, the regeneration process started by day 8 to day 10 in NL and by day 15 in Px, while in the LD 8:16 exposed animals, it occurred by day 10 to day 12 in NL and day 16 to day 18 in Px (Table 2). This temporal difference in regenerative outgrowth persisted and was amplified until the early differentiation phase, after which it was minimized during the late differentiation and growth phases (Table 2).

Growth Rate and Total Length Regenerated

A measurable growth occurred in LL and LD 18:6 groups of animals by day 5 in the case of NL and by day 8 in the case of Px, while in NLD, LD 16:8 and LD 8:16 groups of animals it occurred between day 8 and 12 and days 15 and 18, respectively. However, in lizards exposed to DD and LD 6:18, a measurable growth occurred only between days 12 and 14 in the case of NL and days 18–20 in the case of Px (Table 2). The regeneration process was completed in LL (H), LL (L), and LD 18:6 photoperiodic schedules by the 50th day in both groups of

TABLE 2. Approximate Number of Days Taken to Reach the Various Arbitrary Stages of Tail Regeneration in Normal and Pinealectomized *H. flaviviridis*

Photoregime	Wound healing		Blastema		Early differentiation		Differentiation		Late differentiation		Growth		Fully regenerated tail	
	NL/BL	Px	NL/BL	Px	NL/BL	Px	NL/BL	Px	NL/BL	Px	NL/BL	Px	NL/BL	Px
LL (H)	3	5	5–7	8–10	7–9	10–12	10	15	20	25	30	32	50	50
LL (L)	3	5	5–7	8–10	7–9	10–12	10	15	30	35	40	45	50	50
LD 18 6	3	5	5–7	8–10	7–9	10–12	10	15	25	30	35	40	50	50
LD 16 8	5	7	8–10	15–17	12–14	18–20	20	25	30	35	40	42	60	60
LD 12 12	5	7	8–10	15–17	12–14	18–20	20	25	30	35	40	42	60	60
LD 8 16	5	7	10–12	16–18	14–16	20–22	22	27	32	37	42	44	60	60
LD 6 18	8	10	12–14	18–20	16–18	22–24	25	36	35	40	45	46	60	60
LD 0 24	8	10	12–14	18–20	16–18	24–26	25	38	40	42	45	47	60	60
LD (HP)	8	10	12–14	18–20	16–18	24–26	25	38	40	42	45	47	60	60
NLD (HP)	8	10	12–14	18–20	16–18	24–26	25	38	40	42	45	47	60	60

LL (H) continuous light (high intensity), LL (L), continuous light (low intensity), LD 18 6, 18 hours of light (high intensity) and 6 hours of dark, LD 16 8, 16 hours of light (high intensity) and 8 hours of dark, LD 12 12, 12 hours of light (high intensity) and 12 hours of dark, LD 8 16, 8 hours of light (high intensity) and 16 hours of dark, LD 6 18, 6 hours of light (high intensity) and 18 hours of dark, LD 0 24 continuous (total) darkness LL (HP), head-painted lizards exposed to continuous light (high intensity), NLD (HP), head-painted lizards kept in normal light and dark (LD 12 12) NL normal lizards, BL blinded lizards, Px, pinealectomized lizards

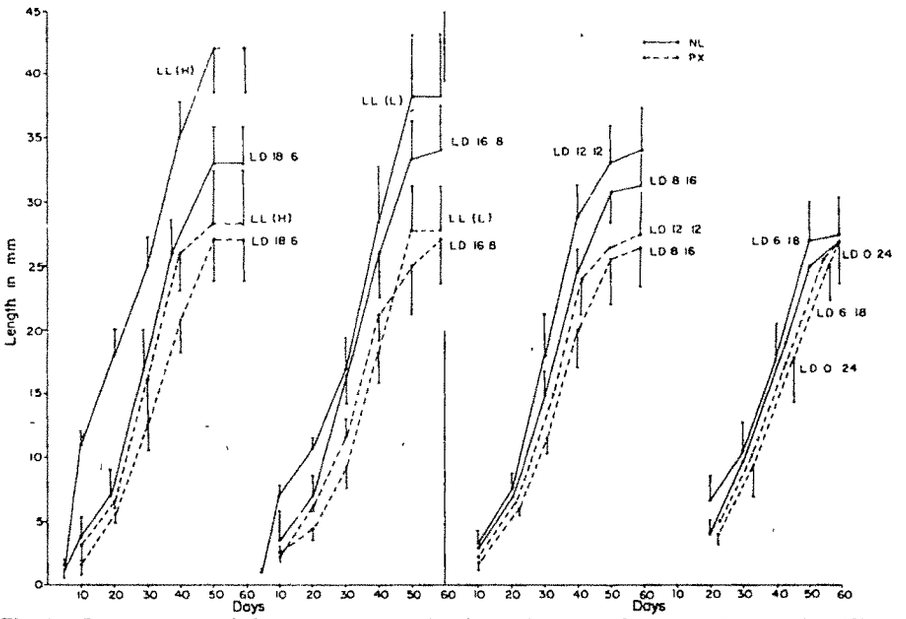

Fig. 1. Regenerative tail elongation in normal and pinealectomized *Hemidactylus* under different photoperiodic regimens

animals, at which time the total length of tail regenerated was 41.7 mm, 33.3 mm, and 38.7 mm, respectively, in NL and 28 3 mm, 27 8 mm, and 27.1 mm, respectively, in Px (Fig 1 and Table 2). In the other groups of lizards, the regenerative growth ceased by day 60, and the least lengths regenerated (28.2 mm—NL, 27.2 mm—Px, and 27.4 mm—NL, 26 7 mm—Px) were in the LD 6:18 and DD groups. The total lengths of tail regenerated in the remaining groups of animals were nearly similar and were 33.6 mm (NL) and 27.9 mm (Px) in LD 16·8, 33.0 mm (NL) and 27 5 mm (Px) in LD 12.12, and 31.0 mm (NL) and 26.6 mm (Px) in LD 8.16 (Fig 1 and Table 2).

From Figures 1 and 3, it is obvious that in NL, both the total length of tail regenerated and the percentage of replacement are maximal under LL (H) and minimal under DD. Although the values with regard to these two parameters were quite similar in LL (L), NLD, and LD 16:8 on the one hand and LD 6:18 and DD on the other, a definite linear correlation between the length of photoillumination and the ultimate length of tail regenerated and total percentage replacement can be inferred This fact is confirmed by the observed values under LD 18:6, which were significantly more than all the groups except LL (H) (Figs. 1 and 3) In Px animals, the stimulatory influence of long-length photoperiods is abolished, as can be deduced from the significant retardation in their regenerative potential when compared with their NL counterparts (Figs. 1 and 3) Secondly, there is no significant alteration either in the initiation and onset of regeneration, the daily growth rate, the final length of tail replaced at the end of regeneration, or the percentage of replacement of the autotomized

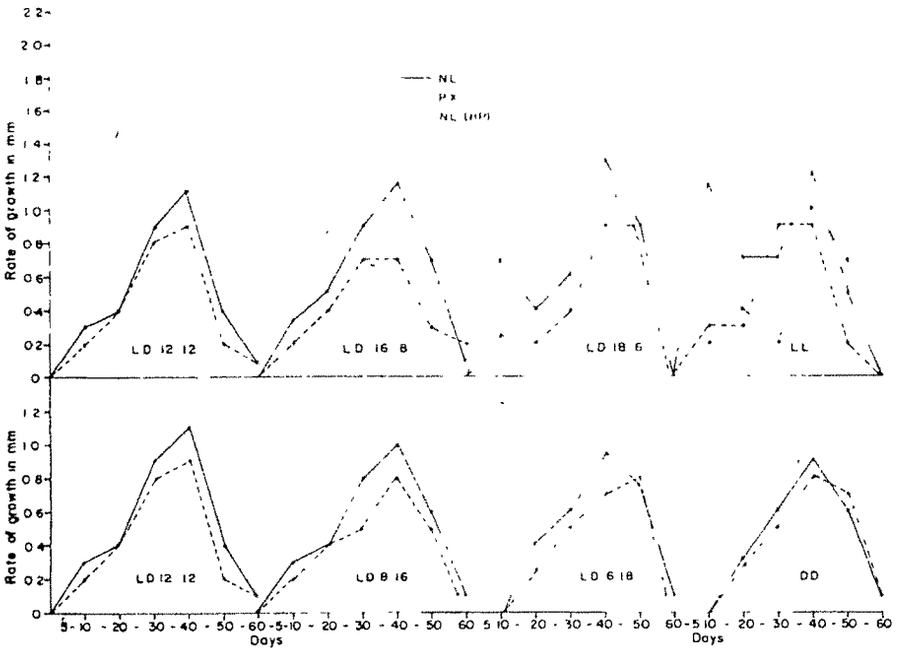

Fig. 2. Per day rate of regenerative growth in normal and pinealectomized *Hemidactylus* under different photoperiodic regimens

tail in Px lizards exposed to the eight experimental photoperiodic regimens under investigation (Figs 1–3 and Table 2)

The pattern of growth rate depicted in Figure 2 indicates a linear increase peaking at 30–40 days in NL lizards exposed to all photoperiodic schedules from DD to LD 16 8 However, LD 18 6 and LL photoregimes induced a very significant initial growth spurt, which rendered the growth rate curve a biphasic one with increasing lengths of light beyond 16 hours having a definite stimulatory influence on this initial spurt The stimulatory influence of long-day photoperiods was further revealed by the gradually decreasing peak growth rate from LD 16·8 to DD (Fig. 2) A biphasic growth pattern, although quantitatively attenuated, was also discernible in Px and NL (HP) lizards under LD 18 6 and LL photoperiodic schedules, while the animals exposed to the other lighting regimens showed a linear increase peaking at 30–40 days postcaudal autotomy (Fig 2)

Total Percentage of Replacement

Percentage of replacement in NL lizards, calculated in terms of total length of tail regenerated and total length of tail autotomized, was a minimum of 50.5 in DD exposed lizards and a maximum of 75 3 in LL (H) exposed animals, followed by 70 4 in animals exposed to LD 18 6 photoperiodic schedule (Fig. 3). Lizards exposed to LL (L), NLD, and LD 16 8 recorded nearly similar replacements of 62 5%, 61 7%, and 62 7%, respectively Lizards exposed to 8 hours of

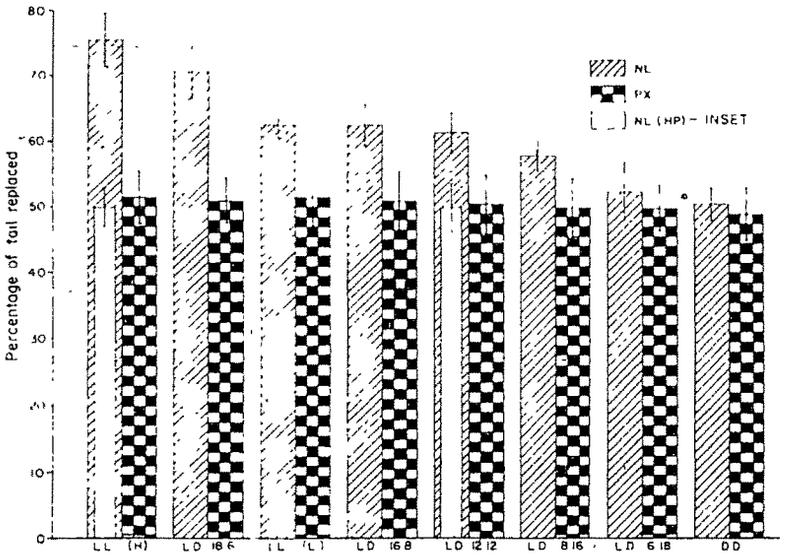

Fig. 3. Percentage replacement during tail regeneration in normal and pinealectomized *Hemidactylus* under different photoperiodic regimens LL(H) continuous light (high intensity), LL(L) continuous light (low intensity), DD continuous (total) darkness, NLD normal light and darkness LD 18 6 18 hours light and 6 hours dark, LD 6 18 6 hours light and 16 hours dark, LD16 8 16 hours light and 8 hours dark, LD 8 16 8 hours light and 16 hours dark NL normal lizards, PX pinealectomized lizards, NL (HP) normal lizards with head painted

light showed a slightly reduced percentage replacement of 57 8%, while those exposed to 6 hours of light produced a replacement of 52 7%, more like the DD exposed animals (Fig 3) Pinealectomy as well as head paint in general nullified the stimulatory effects of light and produced a nearly similar replacement of 49–51% (Fig 3)

All possible comparisons among the eight experimental setups in NL, NL (HP), and Px were made with reference to Duncan's multiple range test [Duncan, 1955] No statistical significance was found among NLD, LL (L), and LD 16 8 in the NL groups and among all Px and NL (HP) groups of animals However, all other comparisons other than these among NL, as well as among NL, Px, and NL (HP) groups, were statistically significant at both 5% and 1% levels

DISCUSSION

Previous studies in our laboratory have demonstrated that the duration of photic input as well as its intensity have a definite stimulatory influence on lacertilian tail regeneration [Ndukuba and Ramachandran 1988a] Furthermore, it has also been shown that the lateral eyes, or retinae, do not participate in photoperiodically significant photoreception in the Gekkonid lizard *H flaviviridis*, since blinded lizards regenerated their lost (autotomized) tails like their sighted counterparts exposed to similar experimental photoperiodic regimens

[Ndukuba and Ramachandran, 1988b] This study, aimed at evaluating the possible role of the pineal gland in mediating the photic influence on tail regeneration in lacertilians, has revealed that in *H flaviviridis*, the pineal is the principal photoreceptor organ, since both pinealectomy as well as light deprivation to the pineal abolished the stimulatory influence of long-length photoperiods on tail regeneration A comparative assessment of the new growth (regenerate) shows that the initiation and onset of regeneration, the daily growth rate, the final length of tail replaced at the end of regeneration, and the percentage of replacement of the autotomized tail are all significantly retarded in Px *Hemidactylus* and also in NL(HP) lizards, compared with their NL counterparts exposed to similar experimental photoregimes

Most investigations on a potential role for retinal receptors have been conducted with birds. A long series of investigations by Benoit on the domestic duck demonstrated the participation of extraretinal receptors in the photoperiodic response of ducks [Benoit, 1935] Many other different combinations of experiments led Benoit to conclude that both retinal and extraretinal photoreceptors are involved [Benoit, 1970]. However, a careful reconsideration of the published work of Benoit by McMillan et al [1975] led them to conclude that a retinal participation in photoperiodism in ducks has not been conclusively demonstrated. The participation of extraretinal receptors in testicular responses in a second avian species, the house sparrow, *Passer domesticus*, was shown by Menaker and Keatts [1968] A series of experiments with house sparrows, utilizing several different experimental approaches, demonstrated that the eyes are not involved in photoperiodic photoreception and that extraretinal receptors located in the brain are fully capable of mediating this response [Underwood and Menaker, 1970; McMillan et al , 1975] Previous studies with lizards demonstrated that extraretinal photoreceptors are involved in photoperiodic response in *A. carolinensis* [Underwood, 1975] Accordingly, long stimulatory photoperiods have been shown to induce testicular recrudescence and maturation in blinded *Anoles* Similar studies have been conducted on a variety of vertebrate species, such as fishes and birds, in which testicular growth could be induced in blinded animals by exposure to stimulatory photoperiods [Underwood, 1979].

In our study of pineal-mediated extraretinal photoreception in *H flaviviridis* during the process of tail regeneration, NL lizards replaced a minimum of 50.5% in LD 0:24 and a maximum of 75 3% in LL (H) In contrast, Px lizards replaced about 50% of the autotomized tail in all photoperiodic schedules investigated. Obviously, a 50% replacement can be considered as a basal or innate level of regenerative ability that is independent of any photoperiodic influence and can occur irrespective of the presence or absence of light. However, replacements beyond 50% are positively correlatable with increasing photoperiodism, which could be nullified by pinealectomy as well as light deprivation to the pineal organ. Presumably, an intact pineal is the essential receptor cum synchronizer of the photostimulatory response in *Hemidactylus* during its tail regeneration However, a biphasic growth pattern, although quantitatively attenuated, was discernible in both Px and NL (HP) lizards under LD 18:6 and LL photoregimes. It is difficult to give a sound interpretative explanation to this observation. Nevertheless, it could be speculated that in the

absence of the pineal—the principal photoreceptor organ in lacertilians—the lateral eyes, or retinae, may indeed, be able to absorb and transmit some amount of photic information under long stimulatory photoperiods of higher light intensities, which could be responsible for the expression of the biphasic growth pattern in Px lizards. It is known that the hypothalamus controls and integrates many of the neuroendocrine functions in vertebrates and that the suprachiasmatic nucleus serves as a circadian pacemaker [Rusak and Zucker, 1979]. In this context, it may be presumed that under long photoperiods of higher light intensities, a small amount of retinally transmitted light impinges upon the suprachiasmatic nucleus to produce the biphasic growth spurt observed in Px lizards.

On the basis of current knowledge, the most likely photoreceptor in lower vertebrates is the pineal organ. Over the years, a body of information, based largely upon indirect morphological evidence, has accumulated to the effect that the epiphyseal complex of the lower vertebrates is responsive to light and darkness. A few electrophysiological studies now lend direct evidence of such activity in fishes [Doft, 1963, Morita, 1966, Hangri et al., 1969, Falcon and Meissl, 1981] and reptiles [Miller and Wolbarsht, 1962]. The pineal complex of lizards, the most extensively studied group of reptiles, is photosensory. Many lizard species have as part of the pineal complex a superficial parietal or "third" eye [Gundy and Wurst, 1976]. The eye, an excellent wavelength discriminator, is more highly organized than the homologous frontal organ of amphibians [Dodt and Scherer, 1968]. In *Crotaphytus collaris*, the parietal eye and the intracranial pineal organ have a feedback relationship wherein the parietal eye sends afferent impulses to the pineal body, and the pineal body sends efferent signals to the parietal eye [Engbretson and Lent, 1976]. A parietal nerve in *Lacerta viridis* projects into the habenular region, and a nerve from the pineal body reaches the subcommisural organ with some fibers traversing the posterior commisure [Kappers, 1967].

Recent radioimmunoassay studies have revealed the presence of circadian oscillators in the isolated pineal organ of the lizard *A. carolinensis* [Menaker and Wisner, 1983]. Circadian rhythms are characterized by three major properties: they oscillate under constant conditions (free-run), they can be synchronized by environmental light-dark cycles (entrainment), and their periods vary only slightly with changes in ambient temperature (temperature-compensation). The pineal organ of *A. carolinensis* must contain one or more temperature-compensated circadian oscillators coupled with photoreceptors on the input side and to melatonin synthetic pathways on the output side. In *A. carolinensis*, some of the photoreceptors are coupled with the circadian oscillators that regulate the synthesis of melatonin, since the rhythm in isolated *Anolis* pineals can be entrained by LD cycles [Menaker and Wisner, 1983].

We may only speculate on how the pineal may respond to affect the rate of tail regeneration in *H. flaviviridis*. Melatonin is produced by the pineal gland and is a mitotic inhibitor [Banerjee and Margulis, 1973]. Melatonin can be suppressed by extended exposure to light [Brownstein, 1975]. Latwiller [1940] demonstrated that the mitotic rate of blastemal cells peaks during the light phase of the diurnal cycle. Our results demonstrate that it is during the preblastemic, blastemic, and early differentiation stages of regeneration, char-

acterized by high mitotic potential, that the stimulatory influence of increasing lengths of light is essentially exerted in NL animals. Apparently photic input is being transduced and translated into hormonal and or physiological responses favouring growth potential, although the exact action at the cellular level remains speculative. It may be that the increased mitotic rate during the daylight hours and its subsequent decline during the dark phase bears a causal relation to the melatonin cycle. Alternatively, increased or decreased lengths of light may affect the production of prolactin, which is a known growth promoter [Crim, 1975]. Bourne and Tucker [1975] have, in fact, demonstrated the positive influence of increasing lengths of light on the level of serum prolactin. Serotonin could, in this respect, mediate the light effect, since it is enhanced by light [Brownstein, 1975]. Moreover, serotonin and its precursors have been shown to elevate serum prolactin levels [Lu and Meites, 1973] and therefore could operate as a mitotic stimulator by way of ability to induce prolactin release. These modulatory effects of light in NL animals are abolished by pinealectomy as well as light deprivation to the pineal, since the regeneration process in Px and NL(HP) groups of lizards was not affected by either increased or decreased lengths of exposure to light.

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PARACHLOROPHENYLALANINE RETARDS TAIL REGENERATION IN THE GEKKONID LIZARD *HEMIDACTYLUS FLAVIVIRIDIS* EXPOSED TO CONTINUOUS LIGHT

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Summary

Parachlorophenylalanine (*p*-CPA) was used for chemical pinealectomy in a study of tail regeneration in the gekkonid lizard, *Hemidactylus flaviviridis*. Two doses of *p*-CPA (200 or 400 $\mu g\,kg^{-1}$ body mass) were injected into two groups of lizards (5 days prior to and 30 days after caudal autotomy) exposed to continuous light of 2500 lx intensity during the summer season (March–May). Our observations show that the initiation of regeneration, the daily growth rate, the total length of new growth (regenerate) produced, and the total percentage replacement of the lost (autotomized) tails 30 days after autotomy were all significantly less with 400 $\mu g\,kg^{-1}$ and insignificantly less with 200 $\mu g\,kg^{-1}$ of *p*-CPA than in the control group of animals. The results may indicate that the effect of the drug *p*-CPA, an agent employed for chemical pinealectomy, on tail regeneration in *H. flaviviridis* is dose-dependent and that *p*-CPA at the high dose of 400 $\mu g\,kg^{-1}$ has a similar retardation effect to that of complete pineal ablation. The role of the pineal in photoperiodic photoreception, and the effect of *p*-CPA on serotonin-melatonin biosynthesis and the consequent effects on tail regeneration, are discussed.

Introduction

A physiological role for serotonin (5-HT) in the regulation of gonadotrophin secretion in vertebrates has frequently been suggested (see Vitale *et al.* 1986). The distribution of serotonergic fibres in the median eminence (Villar *et al.* 1984) and their spatial relationship to luteinizing hormone-releasing hormone (LHRH) fibres (Jennes *et al.* 1982) provide neuroanatomical support for the conclusion that 5-HT can be involved physiologically in the release of LHRH from the median eminence through an action on axon terminals (Vitale *et al.* 1984).

The large number of studies supporting a neurohormonal role for 5-HT in the central nervous system accounts for the continuing interest in drugs capable of selectively depleting brain 5-HT, either by a selective release mechanism or by

Key words: light, lizard, *p*-CPA, regeneration, tail.

inhibiting 5-HT biosynthesis (see Costa *et al.* 1962*a,b*). *p*-CPA is reported to deplete the 5-HT stores in the brain, peripheral tissues and blood in rats and dogs. The 5-HT content of the brain, in particular, is reduced to very low levels, although brain norepinephrine and dopamine concentrations are only slightly decreased (Sloviter *et al* 1978). The injection of *p*-CPA, an inhibitor of tryptophan hydroxylase (Koe & Weisman, 1966; Walker, 1982), is reported to increase luteinizing hormone (LH) levels and suppress prolactin (PRL) levels of broody turkeys, resulting in ovarian growth (El Halawani *et al.* 1983). Blockage of 5-HT synthesis by *p*-CPA completely inhibits the rise in PRL that is normally associated with the return of broody turkeys from cages to the nest (El Halawani *et al.* 1980). *p*-CPA, as well as the 5-HT antagonists methysergide, SQ10631 and cyptoheptadine, have been shown to decrease basal PRL levels in male chickens (Rabii *et al.* 1981)

There are reports indicating the influence of the pineal and PRL in the regeneration of amphibian appendages (see Maier & Singer, 1981) Our recent observations have shown that exogenous PRL improves tail regeneration in lizards exposed to continuous darkness (P. I. Ndukuba & A. V Ramachandran, in preparation). The aim of the present investigation was to determine the effect, if any, on the regenerative performance of lizards exposed to continuous light with physically intact pineals, but deprived of their ability to synthesize 5-HT by the injection of *p*-CPA.

Materials and methods

Experimental animals

Adult *Hemidactylus flaviviridis* of both sexes weighing 10 ± 1 g (± S.D) and measuring 80 ± 5 mm (±S.D., snout–vent length) were obtained from a commercial supplier (M/S Zoophyton, Baroda, India) and maintained on a diet of cockroaches *ad libitum* for a period 7 days prior to experimentation, for acclimation to the laboratory conditions. 30 lizards were used for the investigation, and they were divided into three groups of 10 lizards each and exposed to continuous light (24 h:0 h L:D) of 2500 lx intensity.

Experimental methods

Group 1. p-CPA treated (200 μg kg^{-1} body mass)

The first group of 10 lizards received a daily intraperitoneal injection of 200 μg kg^{-1} *p*-CPA (low dose) 5 days before and 30 days after tail autotomy. Food and water were provided *ad libitum*

Group 2. p-CPA treated (400 μg kg^{-1} body mass)

A second group of 10 lizards received daily intraperitoneal injection of 400 μg kg^{-1} *p*-CPA (high dose) 5 days before and 30 days after tail autotomy. Food and water were provided *ad libitum*.

Group 3. Saline-treated (0·6 % sterile saline)

The third group of 10 lizards, which served as the control, received a daily intraperitoneal injection of 0·6 % sterile saline 5 days before and 30 days after tail autotomy. Food and water were provided *ad libitum*.

Preparation of solutions

Parachlorophenylalanine, *p*-CPA (Sigma chemical company, St Louis, USA), was dissolved in 0·6 % (w/v) NaCl and brought to pH 6·0 by the addition of 5 mol l^{-1} Na_2HPO_4. 0·6 g of reagent grade sodium chloride (NaCl) was dissolved in 100 ml of redistilled water and stored in a refrigerator for daily use.

Experimental set-up

All the experimental animals were exposed to continuous light of 2500 lx intensity. The cages housing the animals measured 46 cm × 38 cm × 25 cm with one side made of transparent glass and ventilation on three sides. Each cage housed 10 lizards, five males and five females, and the animals selected were of similar size to eliminate any possible error in the comparative analysis of the regeneration process due to sex and size differences. The three cages housing the animals were placed (glass surface up) under suspended 40 W fluorescent lamps, facing the source of illumination. The inside of the wooden cage was lined with aluminium foil so that lighting was direct as well as reflected. The distance from the fluorescent lamp to the glass surface of the cage was 38 cm and to the floor level 63 cm. The light intensity was measured at the floor level using a luxmeter (Weston Electrical Instrument Corporation, New Jersey, USA). To obtain the high light intensity of 2500 lx needed for the experiment, four fluorescent lamps were fixed and beamed together. We employed a high light intensity in this investigation because we have earlier demonstrated that the regeneration process is markedly enhanced by the length of photoillumination as well as its intensity (P. I. Ndukuba & A. V. Ramachandran, in preparation).

Tail autotomy was performed by pinching off the tail at the third segment from the vent. The length of tail removed from the animals varied from 50 to 60 mm. The length of new growth (regenerate), in mm, was measured daily with a meter rule and recorded at fixed intervals of 10, 15, 20, 25 and 30 days after caudal autotomy. The recorded readings were used later for morphometric calculations and Student's *t*-tests were used in determining the statistical significance. This investigation was conducted during the summer month of May and the average daily temperature at the level of the animals was 30 °C. Differences at the $P < 0·05$ level were considered to be statistically significant.

Results

Growth rate, total length of tail regenerated and total percentage replacement

The regeneration blastema appeared in saline-treated animals and those treated

Table 1 *Approximate number of days taken to reach the various arbitrary stages of tail regeneration in p-CPA-treated and control lizards,* Hemidactylus flaviviridis, *exposed to continuous light during the summer*

	Days after tail autotomy					
Experimental animals ($N = 10$)	Wound healing	Blastema	Early differentiation	Mid differentiation	Late differentiation	Growth
Controls	1	3–5	5–7	8	14	20
200 $\mu g\,kg^{-1}$ *p*-CPA	1	3–5	5–7	8	14	20
400 $\mu g\,kg^{-1}$ *p*-CPA	5	8–10	12–14	16	18	24

p-CPA, parachlorophenylalanine

with 200 $\mu g\,kg^{-1}$ *p*-CPA by day 5 and in those injected with 400 $\mu g\,kg^{-1}$ *p*-CPA by the tenth day after tail autotomy (Table 1). The high dose of *p*-CPA retarded the regeneration process more than the low dose. The total lengths of tail regenerated by day 30 in control lizards and lizards injected with 200 and 400 $\mu g\,kg^{-1}$ *p*-CPA were 27·7 mm, 26·3 mm and 13·2 mm, respectively, which corresponded to a replacement of 52·8 %, 50·5 % and 25·7 % (Figs 1, 3). The pattern of growth rate (Fig. 2) indicates a linear increase up to 15–20 days in animals treated with 400 $\mu g\,kg^{-1}$ *p*-CPA. The saline-injected lizards showed a biphasic growth rate curve, with the first phase lasting up to 10 days and the second occurring between

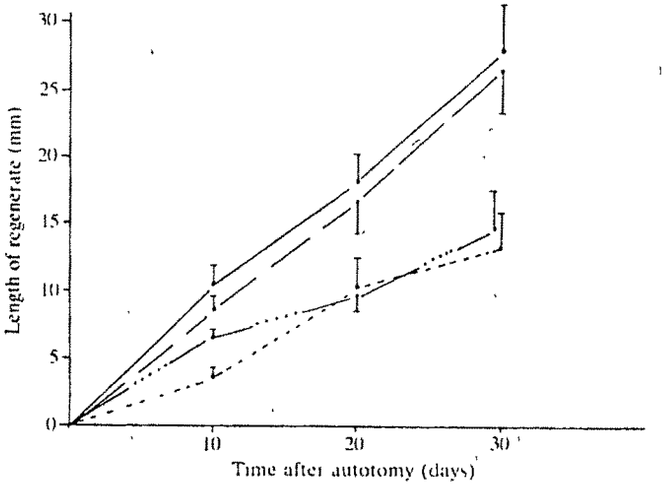

Fig. 1. Length of tail regenerated at the end of 30 days in control (●——●) and *p*-CPA-treated (●— —● 200 $\mu g\,kg^{-1}$, ●---● 400 $\mu g\,kg^{-1}$) lizards exposed to continuous light. Vertical lines are ± S.D. $N = 10$. ●— —●, pinealectomized and exposed to continuous light (data from Ramachandran & Ndukuba, 1988).

20 and 30 days, whereas the lizards treated with 200 $\mu g\,kg^{-1}$ *p*-CPA did not show the second phase.

Comparisons (total length of tail regenerated and total percentage replacement) between the three groups of animals (Student's *t*-test) revealed no statistically

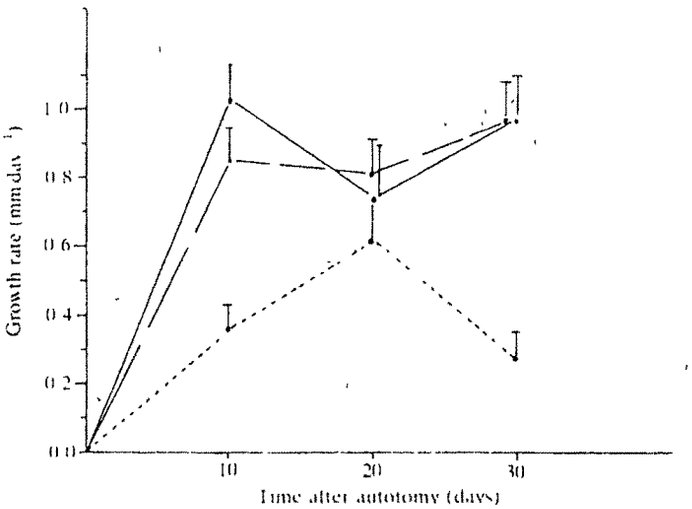

Fig. 2 Growth rate in blocks of 10 days in control (●——●) and *p*-CPA-treated (●— —●, 200 $\mu g\,kg^{-1}$, ●---●, 400 $\mu g\,kg^{-1}$) lizards exposed to continuous light. Mean ± S.D ($N = 10$)

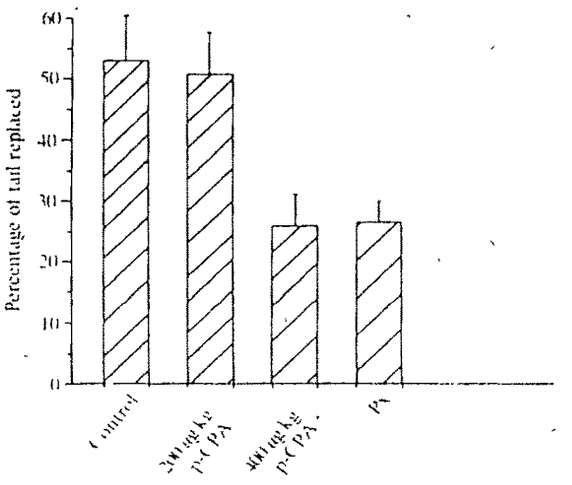

Fig. 3 Percentage of tail replaced at the end of 30 days in control and *p*-CPA-treated lizards exposed to continuous light. PX pinealectomized and exposed to continuous light (taken from Ramachandran & Ndukuba 1988)

significant difference between the saline and 200 $\mu g\,kg^{-1}$ *p*-CPA groups. However, comparisons between the control and 400 $\mu g\,kg^{-1}$ *p*-CPA groups and between 200 $\mu g\,kg^{-1}$ *p*-CPA and 400 $\mu g\,kg^{-1}$ *p*-CPA groups were statistically significant at the 5 % level (Student's *t*-test).

Discussion

These results show that tail regeneration in the gekkonid lizard *Hemidactylus flaviviridis* was significantly retarded with daily intraperitoneal injection of 400 $\mu g\,kg^{-1}$ *p*-CPA (high dose) but only insignificantly so with a low dose (200 $\mu g\,kg^{-1}$) of *p*-CPA (Table 1, Figs 1, 3). This finding demonstrates that in *Hemidactylus* the retardation effect of *p*-CPA is dose-dependent, with the high dose producing a marked effect. The mechanism of action of *p*-CPA in higher vertebrates has been demonstrated previously. *p*-CPA is a neutral amino acid and can compete with tyrosine for uptake into catecholamine neurones (Wurtman, 1975). It has been shown that *p*-CPA selectively decreases the concentration of 5-HT in the brain without altering the concentration of noradrenaline or dopamine. This selective action is probably effected by inhibition of the enzyme tryptophan hydroxylase (Koe & Weisman, 1966; Walker, 1982).

The perception of light provides important information for the organism about its environment. For this purpose, most animals possess well-developed photoreceptors and neuronal networks in the retina of the lateral eyes. Interestingly, even in species with highly organized ocular photoreceptors, additional photoreceptive structures – extraocular photoreceptors – are utilized in the transmission of photic information about the day–night schedule and seasonal photoperiodic changes. Considerable evidence supports the view that the pineal organ is the principal site of extraocular photoreception in lower vertebrates (see Meissl & Dodt, 1981). The pineal system (pineal organ and parietal eye) has been shown to be light-sensitive on the basis of neurophysiological and cytological evidence (Wurtman *et al.* 1968). Recent studies from our laboratory have demonstrated that continuous light stimulates tail regeneration in the lizard, *H. flaviviridis*, whereas continuous darkness depresses it (P. I. Ndukuba & A. V. Ramachandran, in preparation) and, further, that the lateral eyes, or retinae, do not participate in this photoperiodic response as blinded lizards regenerated their lost (automized) tails as effectively as did their sighted counterparts exposed to the same experimental photoperiodic conditions (Ndukuba & Ramachandran, 1988). It has been shown that the pineal organ is the principal site of extraretinal photoreception in *Hemidactylus*, since pinealectomy, as well as light deprivation to the pineal, abolished the stimulatory influence of continuous illumination and significantly retarded the regeneration process (Ramachandran & Ndukuba, 1988), and also tail regeneration was stimulated by exogenous PRL in lizards kept in continuous darkness (P. I. Ndukuba & A. V. Ramachandran, in preparation). The present report shows that the initiation of regeneration, the daily growth rate, the total length of new growth (regenerate) produced at the end of regeneration, and the

total percentage replacement of the lost (autotomized) tails in lizards exposed to continuous light were all significantly retarded by a daily intraperitoneal injection of 400 μg kg^{-1} *p*-CPA. The results obtained here were similar to those obtained earlier with pinealectomized lizards exposed to continuous illumination (see Figs 1, 3; Ramachandran & Ndukuba, 1988).

PRL has been established as a growth promoter in developing organisms (Crim, 1975) and in regenerating systems (Maier & Singer, 1981; P. I. Ndukuba & A. V. Ramachandran, in preparation) and has been shown to stimulate protein synthesis in developing tadpoles (Yamaguchi & Yasumasu, 1977). Depletion of hypothalamic catecholamines by compounds that inhibit their synthesis resulted in a rise in serum PRL level (Donoso *et al.* 1971). In contrast, pharmacological procedures that enhance the amine levels in brain, the injection of monoamine oxidase inhibitors or L-dopamine, inhibit PRL release (Lu & Meites, 1971). In addition to the vast literature implicating dopamine in the control of PRL secretion, some studies suggest that 5-HT is a neurotransmitter involved in the stimulation of PRL release. Kamberi *et al.* (1971) induced PRL release by injecting 5-HT into the third ventricle, and Lawson & Gala (1976) stimulated PRL release by systemic administration of 5-HT. The 5-HT precursor, 5-hydroxytryptophan (5-HTP) has been shown to induce PRL release in rats (Chen & Meites, 1975). The above reports are consistent with a stimulatory role for 5-HT in the control of PRL secretion. In the present investigation, the marked retardation in tail regeneration in lizards treated with *p*-CPA indicates that 5-HT neurones may be mediating the stimulatory effect of continuous illumination by way of PRL secretion during tail regeneration in lacertilians (P. I. Ndukuba & A. V. Ramachandran, in preparation).

Our recent observations have shown that half the tail is replaced, irrespective of the light factor, since lizards exposed to continuous darkness regenerated 50% of their lost tails (P. I. Ndukuba & A. V. Ramachandran, in preparation). This study, together with that of Ramachandran & Ndukuba (1988), demonstrated that continuous light can increase both the rate and the extent of tail regeneration and that pinealectomy can totally abolish these light-induced effects. Apparently, the intact pineal is the photoreceptor which mediates the favourable influence of light on tail regeneration in *H. flaviviridis*. The present study further reveals that lizards with physically intact pineals, but deprived of their ability to synthesize 5-HT by the injection of *p*-CPA, failed to show the positive influences of continuous light on tail regeneration. This sequence of observations leads to the conclusion that the pineal is not only the photoreceptor but also the essential synchronizer which transduces and translates the photic information into favourable regenerative growth in lacertilians. Hence, it may be tentatively surmised that the purported serotonergic mechanism of PRL release (Clemens *et al* 1977) may be the operative mechanism in lizards, triggered by continuous light, and that such a release of PRL can be blocked at the level of the enzyme tryptophan hydroxylase by its inhibitor, *p*-CPA, leading to the depletion of 5-HT from the brain. However, since *p*-CPA inhibits only the first step in the synthesis of 5-HT, it is possible to

bypass its blocking action, and thereby re-establish the concentration of 5-HT, by injecting the direct precursor of 5-HT following the injection of *p*-CPA. A study of this is now in progress in our laboratory, employing the direct precursor of 5-HT, 5-HTP, which readily crosses the blood–brain barrier. The observation that *p*-CPA did not completely inhibit tail regeneration in *Hemidactylus* (only 50% retardation was obtained) strengthens our earlier inference that 50% tail replacement is an innate ability which is independent of photoperiodism and associated neuroendrocrine mechanisms and, apparently, occurs under basal levels of PRL secretion (Ramachandran & Ndukuba, 1988).

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