CHAPTER VIII

PARACHLOROPHENYLALANINE, A SPECIFIC DEPLETER OF BRAIN SEROTONIN, RETARDS TAIL REGENERATION IN THE GEKKONID LIZARD, HEMIDACTYLUS FLAVIVIRIDIS EXPOSED TO CONTINUOUS LIGHT.

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 (Jannes 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 inhibiting 5-HT biosynthesis (see Costa et al., 1962a,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 and 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, SQ 10,631 and cyproheptadine, 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 and Singer, 1981). A recent observation has shown that exogenous PRL improves tail regeneration in lizards exposed to continuous darkness (Ndukuba and Ramachandran, 1988a). 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 :

A total of 30 lizards was used for the investigation, and they were divided into three groups of 10 lizards each and exposed to continuous light (24L : 0D) of 2500 lx intensity as described on pages 12 and 13.

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.

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. 0.6g of reagent grade sodium chloride (NaCl) was dissolved in 100 ml of redistilled water and stored in a refrigerator for daily use.

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, depending on the length of each tail from the third segment to the tip of the tail. The length of new growth (regenerate), in mm, was measured daily with a meter rule and recorded at fixed time 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-test was 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. Values which were different at the $P < 0.05$ level was considered to be statistically significant

RESULTS

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

The regeneration blastema appeared in saline and 200 µg-

TABLE 1. APPROXIMATE NUMBER OF DAYS TAKEN TO REACH THE VARIOUS ARBITRARY STAGES OF TAIL REGENERATION IN *p*CPA TREATED AND CONTROL LIZARDS, H. FLAVIVIRIDIS EXPOSED TO CONTINUOUS LIGHT DURING THE SUMMER MONTH.

EXPERIMENTAL ANIMALS	WOUND HEALING	BLASTEMA	EARLY DIFFEREN-TIATION	MID DIFFEREN-TIATION	LATE DIFFEREN-TIATION	GROWTH
			DAYS POST - CAUDAL AUTOTOMY			
CONTROLS	1	3 - 5	5 - 7	8	14	20
200 µg PCPA	1	3 - 5	5 - 7	8	14	20
400 µg PCPA	5	8 - 10	12 - 14	16	20	24

*p*CPA - PARACHLOROPHENYLALANINE

TABLE 2. LENGTH OF TAIL REGENERATED AND TOTAL PERCENTAGE REPLACEMENT IN PCPA AND SALINE TREATED H. FLAVIVIRIDIS EXPOSED TO CONTINUOUS LIGHT DURING THE SUMMER SEASON.

EXPERIMENTAL ANIMALS	DAY 10	DAY 20	DAY 30	TOTAL % TAIL REPLACEMENT
NL + SAL	10.3 ± 0.17	18.0 ± 0.22	27.7 ± 0.52*	54.5%
NL + 200 µg Kg^{-1}	8.5 ± 0.17	16.6 ± 0.42	26.3 ± 0.43	52.4%
NL + 400 µg Kg^{-1}	3.6 ± 0.08	10.3 ± 0.24	13.2 ± 0.28	28.0%

NL + SAL - NORMAL LIZARDS TREATED WITH SALINE
NL + 200 µg Kg^{-1} - NORMAL LIZARDS TREATED WITH 200 µg Kg^{-1} p-CPA
NL + 400 µg Kg^{-1} - NORMAL LIZARDS INJECTED WITH 400 µg Kg^{-1} p-CPA
PCPA - PARACHLOROPHENYLALANINE
* - TOTAL LENGTH OF REGENERATE IN mm.

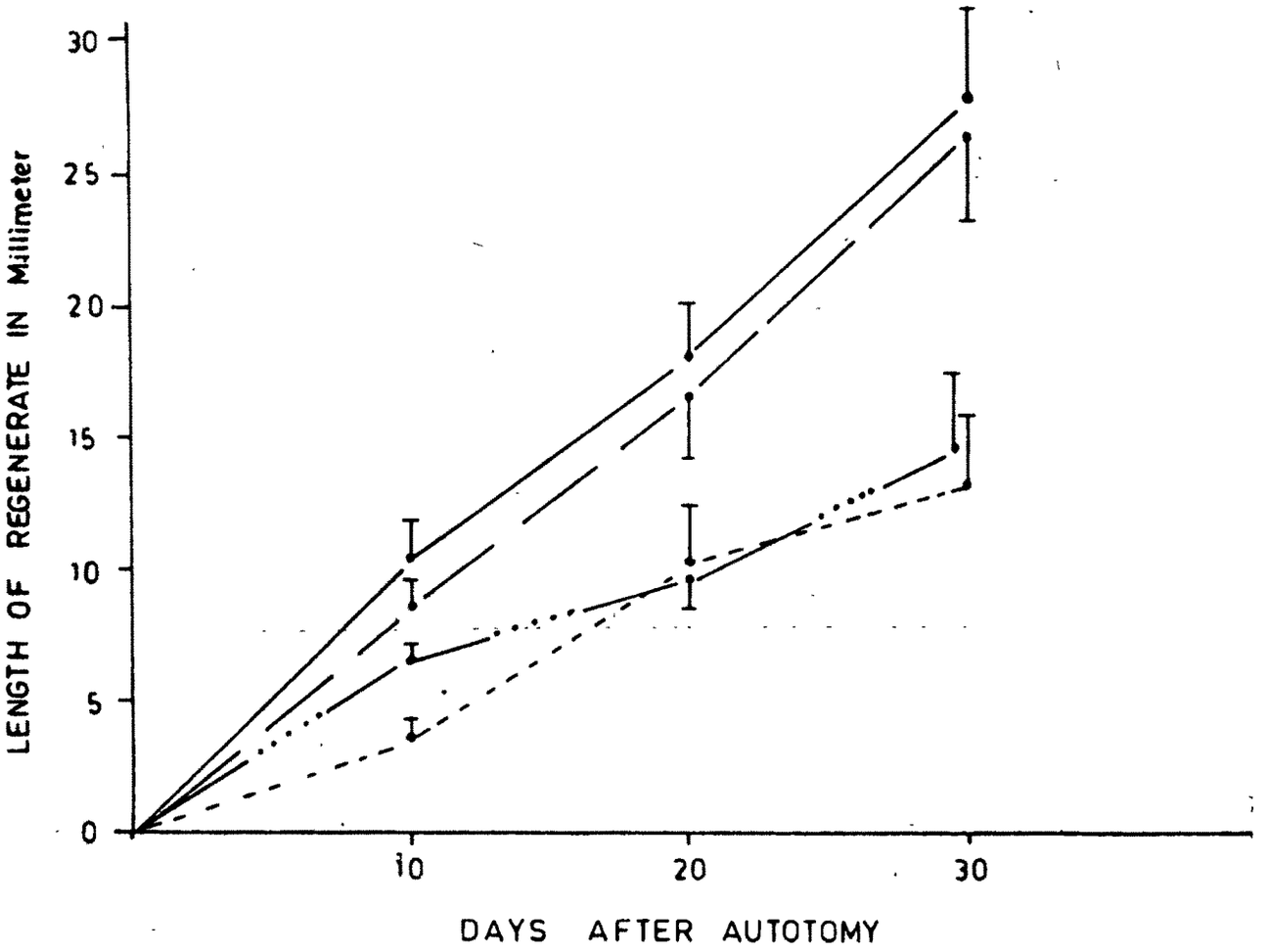

FIG.-1 LENGTH OF TAIL REGENERATED AT THE END OF 30 DAYS IN CONTROL (•——•) AND p-CPA TREATED (•— —•,- 200 µg, •---•400 µg) LIZARDS EXPOSED TO CONTINUOUS LIGHT. VERTICAL LINES (± S.D. n= 10) •——·····——•, PINEALECTOMISED AND EXPOSED TO CONTINUOUS LIGHT. (data from Ramachandran and Ndukuba, 1983a)

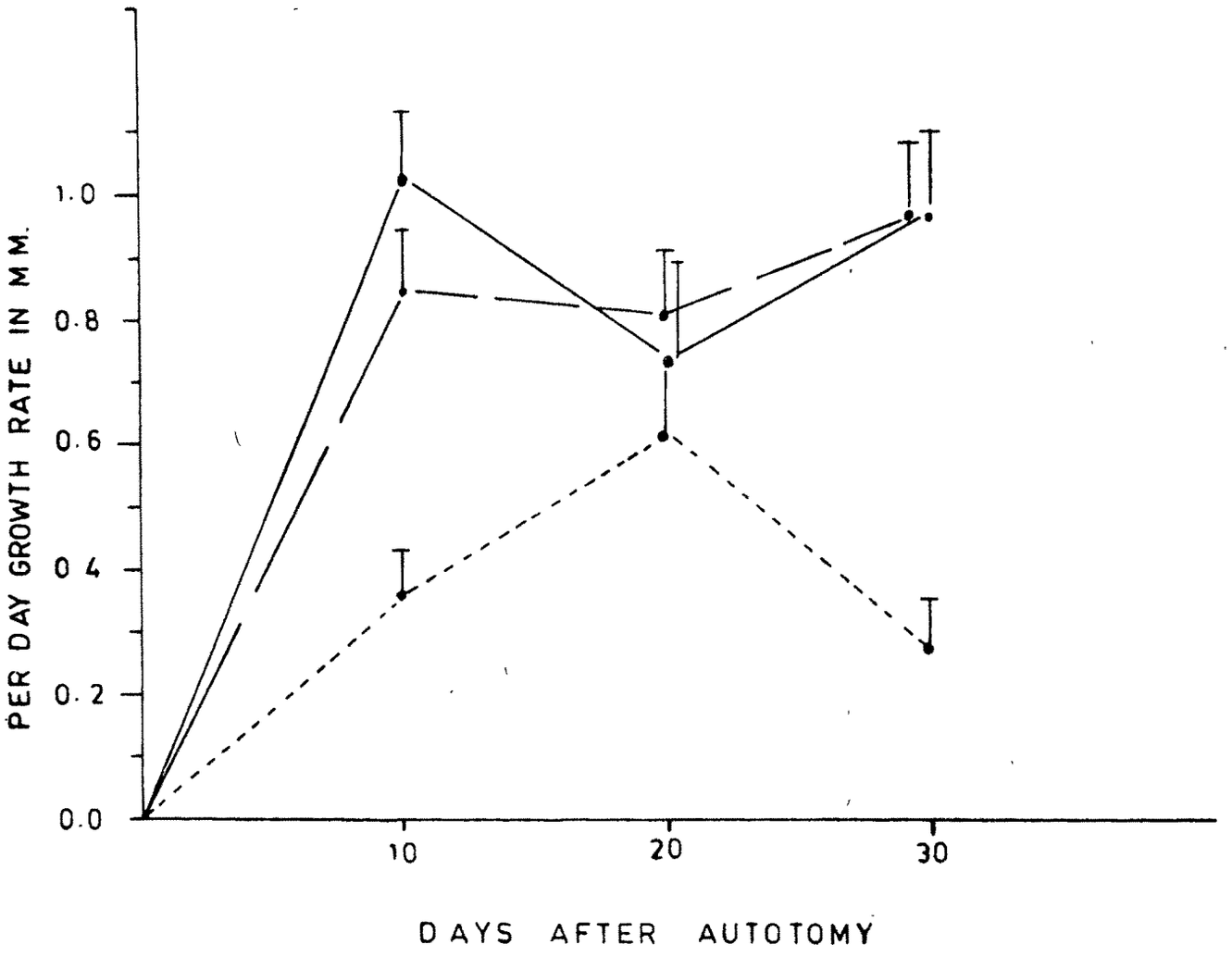

FIG.- 2 PER DAY RATE OF GROWTH IN BLOCKS OF 10 DAYS IN CONTROL (⟵⟶) AND P - CPA TRATED (⟵ ⟶, 200 µg ;⟵---⟶,400 µg) LIZARDS EXPOSED TO CONTINUOUS LIGHT. MEAN ± S.D. (n = 10)

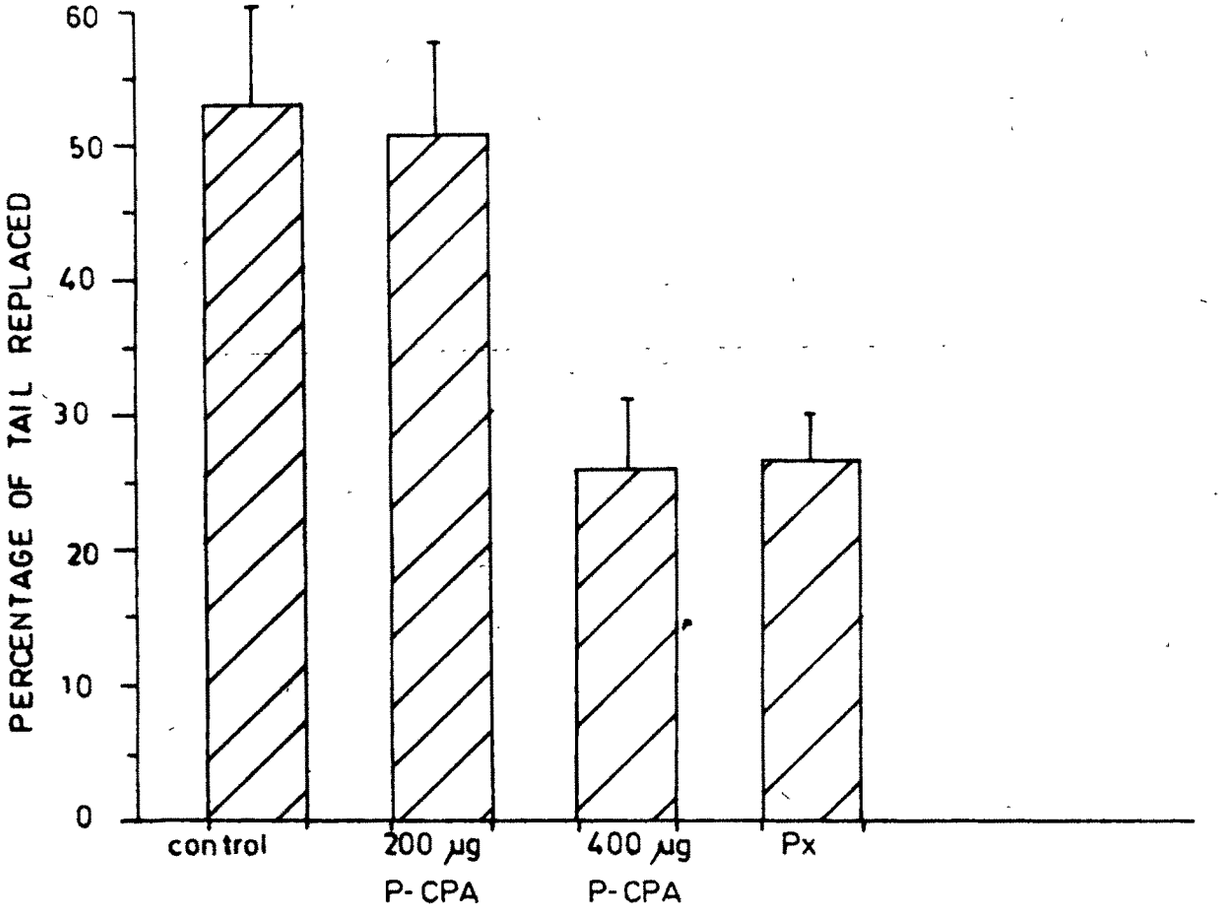

FIG-3 PERCENTAGE OF TAIL REPLACED AT THE END OF 30 DAYS IN CONTROL AND p-CPA TREATED LIZARDS EXPOSED TO CONTINUOUS LIGHT.

Px - pinealectomised and exposed to continuous light (taken from Ramachandran and Ndukuba, 1989a)

treated animals by day 5 and in 400 µg p-CPA injected groups of lizards 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 µg Kg^{-1} p-CPA and 400 ug Kg^{-1} p-CPA were 27.7mm, 26.3mm and 13.2mm, 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 peaking at 15-20 days in animals treated with 400 µg Kg^{-1} p-CPA. However, the saline-injected lizards produced a biphasic growth rate curve, one within the first 10 days and the second between 20 and 30 days, whereas the lizards treated with 200 µg Kg^{-1} p-CPA showed an insignificant biphasic growth rate curve.

Comparisons (total length of tail regenerated and total percentage replacement) between the three groups of animals (Student's t-test) revealed no statistically significant difference between the saline and 200 µg Kg^{-1} p-CPA groups. However, comparisons between the control and 400 µg Kg^{-1} p-CPA groups and between 200 µg Kg^{-1} p-CPA and 400 µg 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 not significantly so with a low dose (200 $\mu g.Kg^{-1}$) p-CPA (Table1, Figures 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 competes 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 either noradrenaline or dopamine. This selective action is probably effected by inhibition of the enzyme tryptophan hydroxylase (Koe and Weisman, 1966; Walker, 1983).

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 retinae 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 and Dodt, 1981). The pineal system (pineal organ

and parietal eye) is light-sensitive on the basis of neurophysiological and cytological evidence (Wurtman et al., 1968). Recent results from our laboratory demonstrated that continuous light stimulates tail regeneration in the lizard, H. flaviviridis, whereas continuous darkness depresses it (Ndukuba and Ramachandran, 1989c) and further, that the lateral eyes, or retinae, do not participate in this photoperiodic response as blinded lizards regenerated their lost (autotomized) tails as effectively as did their sighted counterparts exposed to the same experimental photoperiodic conditions (Ndukuba and Ramachandran, 1988j). 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 and Ndukuba, 1988e) and also tail regeneration was stimulated by exogenous PRL in lizards kept in continuous darkness (Ndukuba and Ramachandran, 1989a). 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 $\mu g.Kg^{-1}$ p-CPA. The results obtained here were similar to those obtained earlier with pinealectomized lizards exposed to

continuous illumination (see Figs 1,3; Ramachandran and Ndukuba, 1982, Chapter 3).

PRL has been established as a growth promoter in developing organisms (Crim, 1975) and in regenerating systems (Maier and Singer, 1981; [illegible] Ndukuba and [illegible] Ramachandran, [illegible] Chapter 7) and has been shown to stimulate protein synthesis in developing tadpoles (Yamaguchi and 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, either by the injection of monoamine oxidase inhibitors or L-DOPA, inhibit PRL release (Lu and 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 and 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 and 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.

Recent observations have shown that half of the tail is replaced, irrespective of the light factor, since lizards exposed to continuous darkness regenerated 50% of their lost tails (Ndukuba and Ramachandran, 1989c; Chapter 1). This study, together with that of Ramachandran and Ndukuba (1989a; Chapter 3) demonstrated that continuous light can increase both the rate and extent of tail regeneration and that pinealectomy can totally abolish these light - induced effects. Apparently, an 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 depict 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. This study is now in progress in our laboratory, employing the direct precursor of 5-HT, 5-HTP, which readily crosses the blood-brain barrier. The fact 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 and Ndukuba, 1985, Chapter 3).