

NEURAL TISSUE AND COMPLETE REGENERATION OF THE TAIL OF THE GEKKONID LIZARD, *Hemidactylus flaviviridis*

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ABSTRACT

Tails of three groups of the Gekkonid lizard, Hemidactylus flaviviridis, were amputated (group I) or autotomized (groups II and III). The animals were exposed to 12 hours of light and 12 hours of darkness. In group I experiment, previously regenerated tails were amputated (repeated autotomy RA) with a pair of sharp scissors, after anesthetizing the animals with ice cubes, at point equivalent to three autotomy segments. The original planes of autotomy have been replaced by ependymal tubes and there were no blood exudates. In group II, the spinal cord at the local site of autotomy was carefully removed (spinal cord removed, SCR), with dissecting instruments, for a length equal to one autotomy segment. Lizards in group III served as controls (Normal Lizard NL). The results show that the initiation of regeneration, the growth rate, the total length of new growth (regenerate) produced, and the total percentage replacement of the lost (amputated/autotomized) tails 30 days after excision were all significantly less in lizards of group II, ($p < 0.01$) and insignificantly less in group I lizards, when compared with the controls (group III). The results show that for complete regeneration of the lizard tail neural tissue must be present.

Keywords: Neural tissue, Regeneration, Tail, Gekkonid lizard, *Hemidactylus flaviviridis*

INTRODUCTION

With the exception of most tissues, many lower vertebrates are capable of replacing amputated limbs, a process that invariably involves, and is usually dependent upon, the regeneration of the severed spinal cord (Goss, 1964). However, the completeness of cord regeneration varies from one form to another. In the larval lamprey, and in both larval and adult urodele amphibians, the regenerating tail or limb is complete, including the differentiation of new neurons and the production of paired spinal ganglia in each segment (Goss, 1969). In contrast, the spinal cord of larval amphibians and lizards regenerate little more than their ependymal tubes accompanied by elongating nerve fibres; new neurons do not differentiate (Goss, 1969).

Cavanaugh (1951) demonstrated that if some of the cells in a spinal ganglion of a rat are destroyed, as a result of cutting their peripheral fibres, the remaining ones which must grow back into the entire peripheral field undergo enlargement. Considerable evidence suggests that a comparable situation prevails in the regenerating lizard tail (Panness, 1962). This author reported that no new spinal ganglia are differentiated in the process of lacertilian tail regenerating, the regenerate itself becomes innervated by fibres derived from the three pairs of sensory ganglia immediately anterior to the level of amputation or autotomy.

The role of ependyma in the initiation of regeneration and cartilage differentiation has been well documented (Simpson, 1964). The present study attempts to elucidate the effects of repeated amputation and the removal of the spinal cord from the local site of autotomy on tail regeneration in *Hemidactylus flaviviridis*.

MATERIALS AND METHODS

Adult *H. flaviviridis* of both sexes measuring 6 ± 2 cm (\pm S. D. snout - vent length) weighing 10 ± 5 gm (\pm S. D. body mass) were obtained from a local supplier and maintained *ad libitum* on a diet of cockroaches and grasshoppers for a period of 7 days for acclimation to laboratory conditions. 90 lizards were used for the investigation. They were divided into three groups of 10 lizards each and exposed to the normal photoperiod (12 hours of light and 12 hours of darkness).

All the lizards were immobilized with ice cubes at 4 °C before autotomy or amputation was performed. Ten *H. flaviviridis* with their tails fully regenerated previously, thus lacking planes of autotomy, were amputated with a pair of sharp scissors at points equivalent to three autotomy segments from the vent. This forms the group I lizards. In group II, ten *H. flaviviridis*, with normal planes of autotomy were autotomized at the third segment from the vent. The spinal cord at the local site of autotomy was carefully removed with dissecting instruments in order to study the possible influence of the distal spinal cord on tail regeneration in *H. flaviviridis*. In group III, ten *H. flaviviridis* were autotomized at the third segment from the vent. This group of lizards served as the control and was exposed to the same light and ambient temperature conditions. All the lizards were fed *ad libitum* inside their respective cages. The experimental set up was replicated thrice.

The length of new growth (regenerate) was measured in mm, with a graduated meter rule at fixed time intervals of 10, 15, 20, 25, and 30 days post tail amputation or autotomy. This investigation was conducted during the harmattan months (December – March). The recorded average monthly

ambient and cage temperatures are given in Table 1. The data on the length of tail regenerated and the percentage replacement were subjected to an analysis of variance and mean separation using New Duncan's multiple range test at 0.05 and 0.01 probabilities (Duncan, 1955)

Table 1: Average ambient temperature and cage temperatures during the Harmattan season

Months	Ambient Temperature	Cage Temperature
January	30 – 31 °C	31- 32 °C
February	31 - 32 °C	32 –33 °C
March	32 - 33 °C	33 - 34 °C
April	33 - 34 °C	34 –35 °C
Average	32 – 32.5 °C	32.50 – 33.5 °C

RESULTS

Growth Rate and Total Length Regenerated and Total Percentage Replacement: A measurable growth occurred in normal (NL) group (Group III) of animals by day 3 while in repeated amputation (RA) group (Group I) and spinal cord removed (SCR) group (Group II) lizards, measurable growth occurred by day 9 and 10, respectively (Table 2). There was also a delay in the appearance of the regeneration blastema in RA when compared to normal *H. flaviviridis* (Table 3).

Effect of Repeated Amputation (RA): The total lengths of tails regenerated as well as the total percentage replacement of the RA lizards were less when compared with the controls (Figure 1 and 2). RA *H. flaviviridis* produced an average tail length of 19.18 mm out of a total amputated length of 40.0 mm, accounting for a replacement rate of 49.5% (Table 3)

Effect of Removal Spinal Cord (SCR) from the Local Site of Autotomy: The results showed that the initiation of regeneration, the total length of tail regenerated and the total percentage replacement of the lost (autotomized) tails on day 30 post autotomy were all significantly different in SCR group of *H. flaviviridis*, when compared with those of the controls ($P < 0.01$). SCR animals produced an average tail replacement of 9.0 mm out of a total autotomized length of 40.0 mm, accounting for a replacement rate of 22.5% (Table 3).

Normal Lizards (NL): On day 30 after tail autotomy, the normal lizards produced an average tail replacement of 2.2 mm out of the total autotomized length of 41.0 mm, accounting a replacement rate of 51.7% (Table 3).

Comparison of the total length of tail regenerated and the total percentage replacement between the three groups of *H. flaviviridis* (ANOVA and Duncan's multiple range test) revealed no statistically significant difference between the RA and the controls (Figures 1 and 2). However, all other

comparisons between the controls and SCR group of lizards on one hand, and between RA and SCR on the other hand, were significantly different at 5% level (Duncan, 1955).

DISCUSSION

Several lines of evidence suggest that the central nervous system (CNS) and its associated endocrine organs play pivotal roles in vertebrate appendage regeneration (Singer and Salpeter, 1961; Tassava *et al.*, 1987; Goldharmer, 1988). As reviewed by Wallace (1981), the importance of the wound epithelium to amphibian limb regeneration was demonstrated by several successful experiments in which the formation or function of the wound epithelium was inhibited. Pannese (1962) has shown that in the regenerating lizard tail, no new spinal ganglia were differentiated; the regenerate itself became innervated by fibers derived from the three pairs of sensory ganglia immediately anterior to the level of amputation/autotomy.

In a similar series of investigations, Ndukuba and Ramachandran (1988, 1989) and Ramachandran and Ndukuba (1989 a,b) had earlier demonstrated the influence of both intrinsic and extrinsic factors on tail regeneration in *H. flaviviridis*. These authors had shown that there was a positive influence of increasing photoperiodism as well as intensity on lacertilian tail regeneration and a negative influence of decreasing lengths of light from the intermediate photoperiod regimen of 12 hours of light and 12 hours of darkness. Lizards under continuous illumination produced the best regenerative performance while those in continuous (total) darkness produced the worst performance.

When *H. flaviviridis* were blinded by surgical removal of the two lateral eyes, the regenerative process was unaffected as blinded lizards regenerated their lost tails like their sighted counterparts exposed to the same experimental photoperiodic conditions (Ndukuba and Ramachandran, 1988). However, pinealectomy produced a 50% retardation effect in lizards exposed to continuous light, suggesting the involvement of the pineal organ of *H. flaviviridis* in photoperiodic photoreception (Ramachandran and Ndukuba, 1989).

Furthermore, the administration of exogenous prolactin (PRL) enhanced both the length of new tail and the percentage replacement in unoperated lizards exposed to continuous darkness, but did not affect their pinealectomized counterparts, indicating a more intriguing, interdependent interaction among photoperiodism, pineal, and PRL (Ndukuba and Ramachandran 1989). The influence of temperature and seasonal variations on lacertilian tail regeneration showed that the best regenerative performance was obtained during the summer months (temperature: 30 °C) and the worst performance in the winter months (temperature: 17 °C) with the regenerative performance during the monsoon season (temperature: 26 °C) falling in between. (Ramachandran and Ndukuba, 1989).

Table 2: Approximate number of days taken to reach various arbitrary stages of tail regeneration in the house lizard, *Hemidactylus flaviviridis*

Experiment Animals	Wound Healing	Blastema	Early Differentiation	Mid Differentiation	Late Differentiation	Growth ^a
Experiment I (Repeated amputation)RA	1	3	5	6	8	30 ^b
Experiment II (Spinal cord removed) SCR	3	5	8	10	12	30
Experiment III (Normal Lizards (NL))	1	3	5	6	8	30

a = Stages of tail regeneration, *b* = Total number of days after tail autonomy, RA = Repeated amputation, SCR = Spinal cord removed, NL = Normal lizards, Average daily room and cage temperature = 32 ± 1 °C.

Table 3: Length of tail regenerated and percentage replacement in three groups of the Gekkonid lizard, *H. flaviviridis*

Group of Lizards	Length of tail removed	Length of tail regenerated	Percentage replacement *
1. Repeated autotomy (RA)	40 mm	19.8 mm	49.5 %
2. Spinal cord removed (SCR)	40 mm	9.0 mm	22.5 %
3. Normal Lizard (NL)	40 mm	21.2 mm	51.7 %

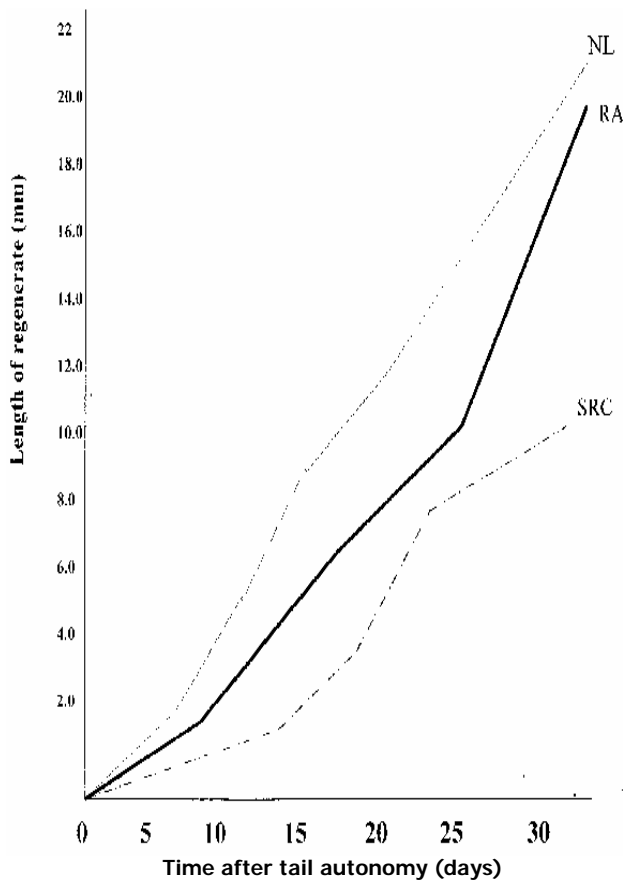


Figure 1: Length of tail regenerated at the end of 30 days in Normal Lizards (NL), repeated amputation (RA) and spinal cord removed (SCR) *H flaviviridis*

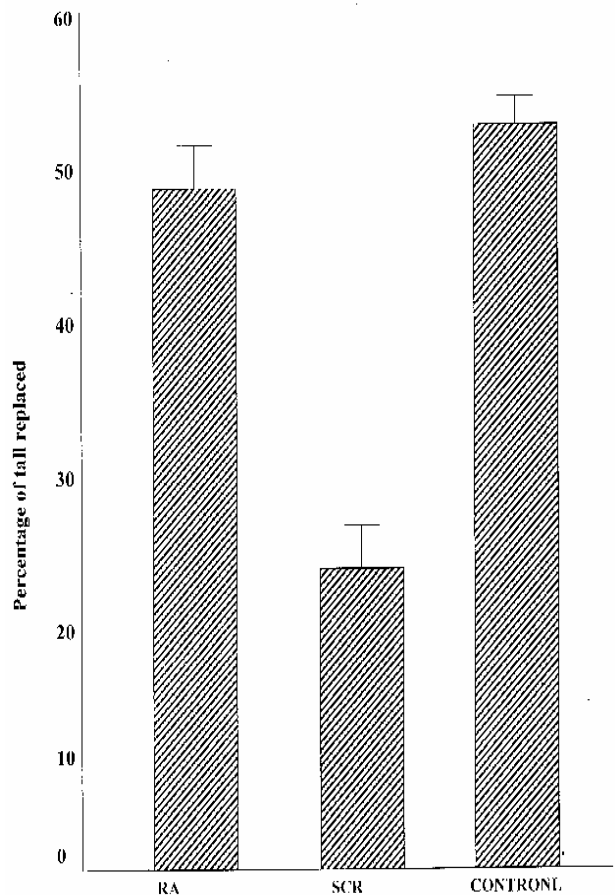


Figure 2: Repeated amputation (RA) , Spinal Cord removed (SCR) and normal lizards (NL) *H. flaviviridis*. Vertical lines are ± S. D. (N = 30)

Results of the present study showed that tail regeneration in Gekkonid lizard, *H. flaviviridis*, was inhibited by the removal of the spinal cord from the local site of autotomy, but insignificantly retarded when previously regenerated tails were amputated for a second regrowth as compared with the controls. Since according to Goss (1969) lacertilian tails do not regenerate new spinal cords; in their place are regenerated ependymal tubes accompanied by elongating nerve fibers, it could be concussively surmised that the excellent regenerative performance by previously regenerated tails may be due to a possible influence by the ependymal tubes, and their accompanying elongated nerve fibers. Simpson (1964) demonstrated the role of ependyma in the initiation of regeneration and cartilage differentiation in the lizard, *Lygosoma laterale*.

The active participation of the distal spinal cord on tail regeneration in *H. flaviviridis* was evident by a 60 % retardation effect on lizards deprived of their spinal cords at the local sites of autotomy. This finding suggests that for complete tail regeneration in *H. flaviviridis* neural tissue must be present. It further strengthens our earlier report (Ndukuba and Ramachandran, 1989; Ramachandran and Ndukuba, 1989) that 50 % tail replacement is an innate ability which is independent of photoperiodism and associated neuroendocrine mechanism and apparently, occurs under basal level of prolactin secretion.

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