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# ANALYSIS OF CNS PROJECTIONS TO SPINAL CORD ROSTRAL TO MIDTHORACIC TRANSECTION IN LIZARD *Anolis carolinensis* USING HRP - LABELLING TECHNIQUE

# ANALISIS SYARAF-SYARAF PUSAT YANG AXONNYA MENUJU MEDULLA SPINALIS ROSTRAL PERTENGAHAN THORAX PADA *Anolis carolinensis*, DENGAN TEKNIK PENANDAAN *HRP*

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## ABSTRACT

Projections of supraspinal and intraspinal neurons to spinal cord rostral to midthoracic transection in lizard *Anolis carolinensis* was studied. This experiment utilized retrograde transport technique with HRP to determine the structure and the number of the neurons projecting axon to spinal cord rostral to midthoracic transection as compared to the number of the projecting axon in midthoracic region.

The result revealed that the majority of the supraspinal neurons projecting axon to spinal cord rostral to midthoracic transection were more heavily labelled than those in animals with transection in midthoracic spinal cord. On the other hand, intraspinal neurons were heavily labelled in both animals. Further, it was found that the number of supraspinal and intraspinal neurons projecting axon to spinal cord rostral to midthoracic transection were greater than those of the descending axons to midthoracic spinal cord.

Keyword : Supraspinal and intraspinal neurons - spinal cord - HRP.

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## ABSTRAK

Axon syaraf-syaraf supraspinal dan intraspinal menuju rostral bagian medulla spinalis yang dipotong di pertengahan thorax telah diteliti pada *Anolis carolinensis*. Dalam penelitian ini digunakan teknik transport retrograd dengan *HRP* untuk menentukan struktur dan jumlah syaraf yang mengirim axon ke medulla spinalis di rostral pertengahan thorax dibandingkan dengan jumlah axon di medulla spinalis pertengahan thorax.

Hasil penelitian menunjukkan bahwa sebagian besar syaraf supraspinal yang axonnya menuju rostral bagian medulla spinalis yang dipotong di pertengahan thorax, terlabel lebih kuat dibandingkan dengan yang menuju medulla spinalis pertengahan thorax. Syaraf-syaraf intraspinal yang mengirim axon baik ke bagian rostral medulla spinalis pertengahan thorax maupun ke medulla spinalis pertengahan thorax terlabel kuat. Selanjutnya didapatkan bahwa syaraf-syaraf supraspinal dan intraspinal yang mengirim axon ke medulla spinalis di bagian rostral pertengahan thorax lebih banyak dibandingkan dengan syaraf serupa yang mengirim axon ke medulla spinalis pertengahan thorax.


Kata kunci: syaraf supraspinal dan intraspinal - medulla spinalis -*HRP*

## INTRODUCTION

The ability of many lizards to cast off (autotomize) their tails is a widely known phenomenon. Autotomy or amputation of the tail of lizards are followed by the tail regeneration. During tail regeneration most lizards also regenerate the tail spinal cord. The regenerated spinal cord is deficient and consisting only of descending fiber tracts from the old cord, the ependymal lining of the central canal and scattered glial cells (Simpson, 1970). Although in goldfish and newts the spinal cord regeneration occurs at midtrunk level, it does not regenerate when transected or ablated at midtrunk level in lizards (Simpson, 1983).

Duffy *et al* (1990) demonstrated that the majority of the CNS nerve fiber that grow into the regenerated spinal cord of *Anolis carolinensis* are





of intra (local) spinal neurons. While supraspinal axons in the regenerated tail spinal cord represent a very small percentage of total number of axon. In contrast to the almost total absence of supraspinal fibers within the regenerated tail spinal cord, there is a significant increase in the supraspinal projection rostral to the regenerated tail (74 percent greater than seen in non - regenerating lizards).

Relatively little attention has been directed toward the number of supraspinal and intraspinal neurons projecting axon to spinal cord rostral to midthoracic transection.

Microscopic examination of living axon shows that membrane vesicles move along the axon, in a relatively rapid, and it is called axonal transport. Axonal transport occurs in two directions, from the cell body to terminal (anterograde transport) and from terminal to the cell body (retrograde transport). It is recognized that almost all macromolecular synthesis occurs in the pericaryon and the functional significance of anterograde transport become obvious. Retrograde transport returning worn out materials from nerve terminals to the cell body (Schwartz, 1979). Radioactively labelled substances or enzymes like horseradish peroxidase (HRP) can be injected near the synaptic terminal. These substances are taken up at the synapse and transported retrogradely in the axon back to the cell body (Reichert, 1992).

The enzyme horseradish peroxidase was discovered for retrograde tracing of neuroanatomical pathways by Kristenson and Olson (1974). Presynaptic terminals take up HRP which is then transported in vesicles back to the somata. HRP also taken up by damaged axon, packed and transported back to the cell bodies. In this case the result is commonly found as granular labelling of somatic cytoplasm (Kristenson & Olson, 1974).

The present study has utilized retrograde transport technique with HRP to determine the structure and the number of supraspinal and intraspinal neurons, project axons into the spinal cord rostral to mid-thoracic transections.

## MATERIALS AND METHODS

### Animals

*Anolis carolinensis* lizards were obtained from Charles Sullivan Farm (Tennesse). Exsperiment with lizards were performed over 2 month period. Animals were maintained on wax worm. Ten animals were divided into experimental and control groups.

### Surgery

All animals were anesthetized with anesthesia grade ethyl ether to surgery. Transection of the spinal cord in the midthoracic level was made following the removal of the tissue over the vertebrae and laminectomy. Sufficient vertebrae were removed to allow the spinal cord to be picked up between the open blades of a microdissecting scissors and then were cut. The gap between the cut ends of the spinal cord was flushed with sterile saline to allow visualization of both ends of the spinal cord and sterile cotton swab was used to remove any remaining pieces of tissue. On completion of the transection the two ends of the spinal cord retracted producing a 1-2 mm gap. Wound were closed with sterile 7- 0 polypropylene suture. Descending projection were examined by placing a pledget of HRP {(50% HRP, 1% DMSO, and 10% lysophosphatidyl choline dissolved in distilled water) (Frank *et al.*, 1980)} in the gap before suturing the wound.

### Experiments

**1. Supraspinal and intraspinal projections to normal midthoracic spinal cord**

For the projection to midthoracic levels, five lizards recieved laminectomies in midthoracic spinal cord. After exposing the spinal cord, a complete transection was made as described above and pledget of HRP was placed in the gap and the wound closed. Animals survived for 7-14 days (one of the animals died) and were then anesthetized and perfused transcardially with 50% glutaraldehyde, 4% paramorfaldehyde in 0.1 M phosphate buffer. The brain and spinal cord were postfixed in this solution





for 4-16 hours and then placed in a 30% sucrose solution overnight. Tissue was embedded in gelatin hardened with glutaraldehyde, section at 50 m on a sliding microtome, and collected in buffer filled tissue trays. HRP was visualized with the glucose-oxidase method of Adam (1977).

**2. Supraspinal and intraspinal projections to spinal cord rostral to midthoracic transection**

These exsperiments were done to asses the number of neurons whose axon project to at least 2 mm rostral to midthoracic transection. Five lizards recieved laminectomies in midthoracic vertebrae. After exposing the spinal cord, a first complete transection was made as described above, and the wound closed. Following a 21 day survival time (however 3 animals died), a second complete transection was carried out 2 μm rostral to the first transection. A pledged of HRP was placed in the gap and the wound closed. Following HRP application, animals were maintained for 7-14 days, and processed for histological examination as described above.

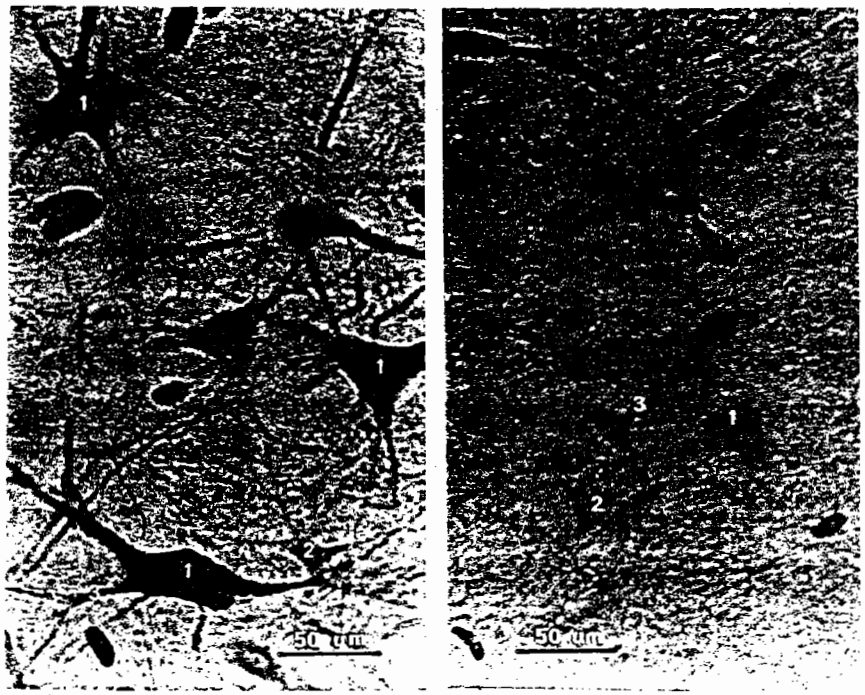
### Anatomical analysis for retrograde studies

Tissue section of the brain and spinal cord were examined at 100x total magnification on an Olympus Vanox AH2-PC photomicroscope. The location of HRP labelled cells was noted and marked on the corresponding traces.

## RESULTS AND DISCUSSION

The HRP labelling of supraspinal and intraspinal neurons were extensive in the experimental animals. Although the intensity of the label varied from cell to cell in the same animal, the majority of the experimental animals displayed a predominance of heavily labelled neurons. In the Figure 1A, heavily labelled neurons as well as several intermediately labelled neurons containing varying quantities of HRP reaction granules were showed. In the control animals, most of the supraspinal neurons were intermediately labelled (Figure 1B), while the

intraspinal neurons in control and experimental animals showed heavily labelled neurons (Figure 2).



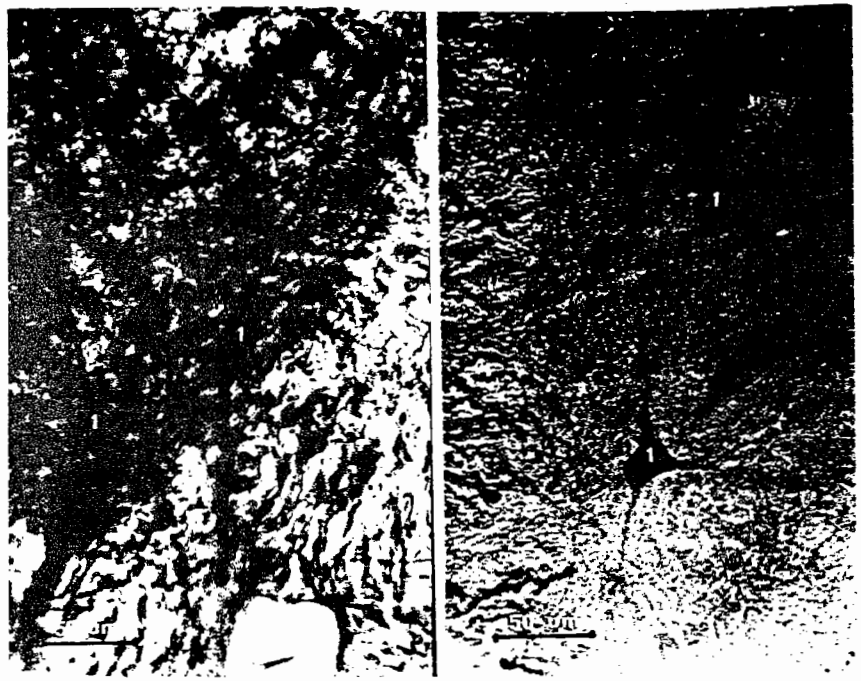

A B

Figure 1. Differential labelling of HRP in supraspinal neurons of experimental animals (A) and control animals (B).

Legend : 1. Heavily labelled neurons (solid black)
2. Intermediately labelled neurons (granular black)
3. Dendrite








A B

Figure 2. Labeling of HRP in intraspinal neurons of experimental animals (A) and control animals (B)

Legend : 1. Heavily labelled neurons
2. Dendrite

Following application of HRP to midthoracic spinal cord, the number of labelled neurons in supraspinal nuclei ranges from 215 to 1.054, and from 83 to 573 in intraspinal nuclei. When HRP was applied immediately rostral to midthoracic transections, between 1.421 to 1.619 neurons in supraspinal nuclei and 249 to 1.218 neurons in intraspinal nuclei were labelled (Table 1). The number of supraspinal and intraspinal

neurons projecting axon to spinal cord rostral to midthoracic transection were greater than those of the descending axons to midthoracic spinal cord. Thus, this result showed that many more axons were present at the rostral to midthoracic spinal cord transection than were able to enter the midthoracic spinal cord. It is necessary to study the processes which inhibit axon elongation, as evidenced by accumulation of axons rostral cord transection.

**Table 1. Number of HRP labelled supraspinal neurons and intraspinal neurons following application of HRP to the midthoracic spinal cord and immediately rostral to midthoracic spinal cord transection.**

| Level of HRP application | | Number of HRP labelled supraspinal neurons | Number of HRP labelled intranspinal neurons |
|---|---|---|---|
| Midthoracic spinal cord | Rostral to midthoracic spinal cord transection | | |
| Lizard No. | | 1,054 | 573 |
| 1 | | 215 | 176 |
| 2 | | 487 | 83 |
| 3 | | 537 | 370 |
| 4 | | | |
| | Lizard No. | 1.421 | 1.218 |
| | 5 | 1.619 | 249 |
| | 6 | | |

A similar accumulation of supraspinal axons, just rostral to a spinal lesion, has been reported for midback transections of *Rana catesbeiana* tadpoles (Forehand & Farel, 1982) while increases in the growth of collateral axon is seen following thoracic hemisection in neonatal and weanling rats (Prendergast & Stelzner, 1976).





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