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**MORPHOLOGICAL AND HISTOCHEMICAL CORRELATION OF
RECOVERY AFTER SPINAL TRANSECTION IN RAT**

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Introduction

PREVIOUS reports from our laboratory have described a sequential disappearance of the aminergic terminals from the rat spinal cord below the level of the lesion (Rodrigues *et al.*, 1977). Following spinal transection these animals clinically passed through the stage of spinal shock, developing spasticity and finally settled with the spinal-stepping stage. This raised the question as to whether the spinal-stepping represented regeneration of the nerve terminals and, if so, whether these terminals were aminergic or cholinergic.

In this report we present evidence of regeneration of the aminergic axons after spinal cord transection. We have also attempted to correlate this phenomenon with the spinal-stepping.

Materials and methods

Thirty female Sprague-Dawley rats weighing 250 mg were used in this study. In all these animals the spinal cord lesion was surgically produced at the T4 level according to the previously described methods (Rodrigues *et al.*, 1977). The animals were examined for the development of spasticity and spinal-stepping. The skin and bladder care was tended to. All animals that developed decubiti or showed cannibalism were discarded from the study.

Sacrifice: The animals were sacrificed by cardiac puncture at 7, 30, and 90-day intervals. The spinal cord was quickly frozen in isopentane and liquid nitrogen. Specimens from above, at, and below the lesion site were studied for:

- (a) Catecholamine fluorescence (Falck *et al.*, 1962).
- (b) Cholinacetyl transferase reaction (Kassa & Morris, 1972).
- (c) Acetylcholinesterase reaction (Koelle & Friedenwald, 1949).
- (d) H&E Stain.
- (e) Bodian Stain.

The control animals only had laminectomy. These animals were sacrificed and the spinal cord was processed as above.

Results

Clinical: 7 days after transection the animals demonstrated mild spasticity as well as urine and fecal retention. Beginning spinal-stepping returned in two weeks. After 30 days the animals could bear weight on their hind quarter and ambulated within the cage, though clumsily. At 90 days the ambulation became more dexterous though still weak. There was no sensation below the lesion and the sphincter control was automatic.

Control: The characteristic yellow and/or green fluorescence of the biogenic amines was visible throughout the length of the spinal cord. This was seen in the form of small terminals ($0.5\mu-2\mu$). These terminals were most abundant at the tip of

FIG. 1

Monoamine fluorescence in rat spinal cord and anterior horn region from lower thoracic cord. Note the bright monoamine terminals in the neuropil and over the cell bodies. The nerve cells show dark nucleus and lipofuscin. $\times 220$.

FIG. 2

Monoamine fluorescence in the intermediolateral cell column of the thoracic cord. Note the collection of bright terminals. $\times 370$.

the posterior horn, the anteromedial and the lateral regions of the anterior horn (Fig. 1). In the thoracic cord the intermediolateral cell column also showed collection of these terminals (Fig. 2). The terminals were either seen on the surface of the nerve cell bodies or in the neuropil (Fig. 2).

Seven days after transection: The H&E stain showed patchy haemorrhagic areas a few segments above and below the lesion. *Histofluorescence:* There was a marked enhancement of the amine terminals above the level of the lesion (Fig. 3). At the level of the lesion, however, there was an abundance of non-specific yellow fluorescence (Fig. 4). This was distributed in patches all over the parenchyma corresponding to the areas of the haemorrhages. In the lumbar and sacral regions no fluorescent terminals were seen (Fig. 5).

Thirty days after transection: The H&E stain showed marked gliosis and cavitation at the level of the lesion. Above the lesion the fluorescent profile was similar to

FIG. 3

Shows enhancement of the fluorescence above the spinal transection. $\times 350$.

FIG. 4

At the level of the transection note cavitation and aggregates of dense fluorescence. $\times 250$.

that of the control. At the level of the lesion, however, there was a distinct absence of clumps of yellow fluorescence. Instead, a distinct basket-weave pattern of nerve terminals was observed. This pattern extended for 3 to 4 cm below the level of the lesion (Fig. 6). Below that there were no aminergic terminals.

FIG. 5

Shows absence of fluorescent terminals in the lumbar cord. Seven days following spinal transection. $\times 200$.

FIG. 6

Note the dense fluorescent terminals with basket-weave pattern and faint fluorescence in the neuropil.

FIG. 7

Cholinacetyl transferase reaction in the thoracic cord. Note the dark-reacting cell bodies in the anterior horn, intermediolateral cell region and posterior horn. The cavities represent areas of degeneration following transection. $\times 120$.

FIG. 8

Cholinacetyl transferase. Again, note heavy reaction in the cell bodies (lumbar region). $\times 350$.

Ninety days after transection: At and below the level of the lesion, the basket-weave pattern became more pronounced and faint terminals were visible throughout the length of the cord.

Cholinesterase and Cholinacetyl Transferase

Control: The activity of both these enzymes was limited to the cell bodies and the processes. It was distributed in the white matter as well as the posterior horn, intermediolateral cell column and the anterior grey region (Fig. 7).

Seven days after transection: At this time the pattern was similar to the control at and below the level of the lesion. At the level of the lesion the activity was hard to interpret due to haemorrhagic areas.

Thirty and 90 days after transection: The pattern of distribution of these enzymes was similar to the control but at the level of the lesion there was a marked decrease in the enzyme activity. The basket-weave pattern seen with aminergic terminals was difficult to discern with this reaction. Instead, patches of heavy concentration of the reaction product were seen (Fig. 8).

Discussion

The regeneration of nerve tracts in the spinal cord has been observed by many investigators and extensively reviewed (Cajal, 1928; Barnard & Carpenter, 1950; Clemente, 1964). However, the lack of correlation between anatomic and functional restoration was ascribed to the extraneuronal factors like scar tissue, etc. Most of these findings, however, were made using the classical silver staining techniques. The availability of histochemical techniques now permit us to look at the neuropharmacologic nature of these regenerating nerve terminals. Significant collateral sprouting of the adrenergic nerves in the adult rat spinal cord had been previously observed by Olsen (1969). The regeneration of central aminergic axons has also been described by Katzman *et al.* (1971) and Bjorklund *et al.* (1971).

Spinal cord transection resulted in flaccid weakness followed by spasticity and spinal-stepping. The development of spasticity was accompanied by the loss of aminergic terminals. The dexterity of spinal-stepping improved with the passage of time (90 days). At this time new regenerating aminergic terminals were seen. Faint fluorescence was also seen 5 to 6 cm below the lesion level. We feel that these observations suggest regeneration of the aminergic terminals and are, perhaps, related to this improved function. This conclusion is further supported by the observation of Grillner (1975) who discussed the role of spinal noradrenergic mechanisms in locomotion and reflex activity.

In conclusion, we have demonstrated that regenerating nerve terminals are physiologically-active as shown by the presence of the neuro-transmitter and further hypothesise that the appearance of these new terminals may be related to the improved function.

SUMMARY

Previous studies from our laboratory have shown disappearance of aminergic terminals with relative preservation of cholinergic activity below the level of the lesion (Rodriques & Sahgal, 1978). Since then we have extended our study to look at the behaviour of aminergic fibres and neurons at various time intervals following the lesion and attempted to correlate with the functional recovery.

Three to 4 weeks after spinal transection (thoracic), the rats started to show spinal-stepping which became stronger and dexterous with the passage of time. Up to 6 months after thoracic transection, maximum recovery was observed. Studies of catecholamine fluorescence revealed proliferation of new aminergic terminals which were observed four or five segments below the transection. These observations demonstrate regeneration of aminergic fibres in rat. The significance of this data in the light of neurophysiologic studies will be discussed.

RÉSUMÉ

Section transversale de la moelle épinière du rat (T4 niveau) a succédé, dans 90 jours, à flaccidité, l'état spastique, et finalement, pas-spinaux. Cette fonction était associée avec la perte des terminaux aminergiques dedans 7 jours et régénération des axones aminergiques à commence de 2 semaines. Dans cet essai, nous avons démontré régénération des systèmes aminergiques dans la moelle épinière du rat.

ZUSAMMENFASSUNG

Experimentelle Reuckenmarks durchtrennung in der Ratte in der Hoehe von TH4 hatte zuerst schlaaffe Laedmung, spaeter spastische Leohmung endlich einen gang mit rueckenmarksautomatismen zur Folge. Dieses Bild war mit Verlust von aminergischen Nervenendigungen innerhalb von sieben Tagen Verbunden, ferner zeigte sich Regeneration von aminergischen Axonen zwei Wochen nach der Laesion. Diese Studie zeigte die Regeneration aminergischer Systeme in dem Rattenreuckenmark.

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