

AN EXPERIMENTAL STUDY OF THE COURSE AND TERMINATION OF THE SPINO-CEREBELLAR SYSTEMS IN A LIZARD (*LACERTA VIRIDIS*)

VIRGIL L. JACOBS*

Netherlands Central Institute for Brain Research, Amsterdam (The Netherlands) and Department of Anatomy, Wayne State University, Detroit, Mich. (U.S.A.)

(Accepted April 27th, 1968)

INTRODUCTION

Early studies by De Lange²⁸, Huber and Crosby²², Larsell^{29,31}, Kappers *et al.*²⁷ and Weston⁴² on the reptilian spino-cerebellar system utilized normal brain specimens stained by various silver methods. Most of these authors concluded that this system, as its mammalian equivalent, can be divided into a dorsal and a ventral component. Larsell^{29,31}, Weston⁴² and others^{10,12,20,22,27} described fibers of the dorsal or nucleo-cerebellar tract as sweeping dorsolaterally over the descending tract and nucleus of the trigeminal to enter the cerebellar peduncle. Larsell considered the dorsal spino-cerebellar fibers to be of bulbar³¹ and cervical cord origin²⁹. On the basis of several species of reptiles prepared by the methylene blue, Weigert and rapid Golgi techniques, Banchi¹ identified a Clarke's nucleus which gives rise to dorsal spino-cerebellar fibers and which were considered to have a comparable mammalian counterpart.

More recently, Goldby and Robinson¹³ used the Nauta and Holmes methods on the *Lacerta viridis* (all lesions were caudal to the 9th spinal segment) and found no evidence of degenerated spinal cord fibers passing laterally over the descending nucleus and tract of the trigeminal. The spino-cerebellar fibers which they followed to the cerebellum are comparable to the mammalian ventral spino-cerebellar fibers. Ebbesson⁸ employed the Nauta method on the Tegu lizard and found the dorsal spino-cerebellar tract to consist of only a few fibers which were later described⁹ as a component of the dorsal funicular system. The course and relationships of the ventral spino-cerebellar system is generally agreed upon by most authors^{7,27,33}.

The differences in the literature regarding the location of the dorsal spino-cerebellar tract, its relationship to the trigeminal complex, its importance phylogenetically and even its presence or absence in the reptilian brain has prompted this study. Additional findings, other than spino-cerebellar relations, have resulted and lend some support to the hypothesis of Larsell and others¹⁹ that the anterior lobe of the

* Present address: Department of Anatomy, Wayne State University, Detroit, Michigan.

cerebellum of lizards and its spinal connections demonstrate a fundamental organization that is basic to the mammalian pattern.

MATERIALS AND METHODS

The lizards (*Lacerta viridis*) selected for this investigation were healthy adult specimens measuring from 9 to 11 cm in length (tip of nose to the base of the tail) and weighing from 22 to 30 g. Pre- and postoperatively the animals were kept in screen covered glass containers at a thermostatically controlled temperature of 27–32°C and maintained on water and mealworms.

Twenty-five animals were operated on under ether anesthesia and aseptic precautions were taken. Spinal cord exposures were carried out under a binocular dissecting microscope. Usually one spinous process and its left lamina were removed with a dental drill following the exposure of two to three adjacent spinous processes. Then the pigmented dura was incised and hemichordotomies and partial cord lesions were attempted with an iridectomy knife or a dissecting needle. Skin closure was by fine nylon thread and globenicol surgical spray, after the wound had been lightly sprinkled with a globenicol powder and loosely packed with spongostan.

The mortality rate of operated animals was high (almost 50%), and at least in some cases, was due to dehydration, excessive anesthesia and/or interruption of CNS respiratory pathways. Although the animals were previously acclimated to the elevated temperature of 27–32°C, such a warm environment cannot be eliminated as possibly contributing to this mortality rate. Only ten of the surviving animals gave satisfactory staining results and are used in this report.

Following a selected postoperative survival time, the animals were given an overdose of MS 222 (Sandoz) anesthetic intraperitoneally and perfused by gravity via the heart with a 0.65% saline solution followed by a 10% non-neutral formalin solution. The skull and vertebral column were removed *in toto*, placed in formalin and then under a dissecting microscope the brain and spinal cord were partially exposed for further fixation. After 3–8 days of fixation the brains and spinal cords were dissected out *in toto*, the meninges removed and the site of the lesion identified and isolated. Usually 1 segment rostral and 1 segment caudal to the lesion together with the 1st cervical segment were removed for a cross-sectional study. The remainder of the spinal cord was cut alternately into 1- and 2-segment lengths. The brains and spinal cord segments were cut at 20–30 μ on a freezing microtome. The brains were sectioned in the transverse or in the sagittal plane (Table I) while the 1-segment spinal cord pieces were sectioned transversely and the 2-segment spinal cord pieces were sectioned horizontally.

To retrieve a complete serial arrangement of transverse brain sections on slides, the following method was utilized. As the sections were cut they were placed into 8 separate staining containers in a clockwise fashion whereby each container held every 9th section. After staining by the Nauta–Gygax³⁴ method the sections in each container were arranged in a rostrocaudal order. Then the most caudal section from each container was removed, transferred to a gelatin–alcohol mixture and

TABLE I

Tr = transverse; Sag = sagittal.

<i>Lizard No.</i>	<i>Segment of cord lesion</i>	<i>Survival time (days)</i>	<i>Brain cut</i>
L8	27	28	Sag
L3	18	28	Sag
L15	13	11	Tr
L4	8	28	Sag
L5	7	28	Sag
L10	5	20	Tr
L6	4	28	Sag
L16	1	14	Tr
L18	1	12	Sag
L21	1	11	Tr

placed serially on a slide. The remaining sections were similarly processed in groups of 8. A similar procedure was followed for sagittal sections. This method of arranging the sections to maintain a complete serial order proved valuable in following degenerated fiber systems.

All spinal cord segments with lesions were observed macroscopically for possible hemorrhagic sites and later processed for the Nauta, hematoxylin or toluidine blue techniques. The extent of the lesion was confirmed microscopically and plotted with a projection apparatus. Projection drawings of the brain stem and cerebellum were also made to portray the pattern of axonal degeneration.

The normal lizard brains used in this study were cut in various planes and stained either by the Bodian silver method or toluidine blue.

Nature of degeneration

In lizards the Nauta technique reveals axonal remnants as intensely stained argentophilic material that closely resembles the descriptions of mammalian axon degeneration. At longer survival periods (20–28 days) the elements of degeneration (segments, fragments, granules, beads and debris) are highly irregular in shape with large interfragmental separations in the fiber path. Occasionally fine axonal threads interconnect the smaller irregular elements of degeneration. A beaded arrangement along the path of smaller axons is a common feature. In shorter survival animals (11–14 days) the degenerated elements are separated by smaller interfragmental spaces presenting the appearance of a more compact mass of argentophilic material. Many fragments are interconnected by thin threads of impregnated axons. In the spinal cord gray matter and cerebellar cortex many cells are surrounded by argentophilic clusters of granules, ringed or oval shaped beads and irregular debris. Some pericellular material is considered as degenerated terminals when a very thin axonal filament (preterminal) is present. In longer survivals such pericellular degeneration is commonly observed as irregular swollen or glob-like material.

Occasionally normal fibers are stained but they can be distinguished from true

axon degeneration by their smooth and uniform surfaces. Axonal swellings with smooth surfaces and interconnected by a slender axon are not considered as degeneration. The reticular network of the Lacertilian pia-arachnoid stained occasionally but this lattice network can be easily distinguished from true axonal degeneration.

OBSERVATIONS

Degeneration in the spinal cord

Macroscopically, the spinal cord of *Lacerta viridis* shows brachial and lumbosacral enlargements which extend from segments 5 to 10 and 25 to 31, respectively. According to Romer³⁷ there are 27 presacral, 3 sacral and an inconstant number of caudal (tail) vertebrae in lizards.

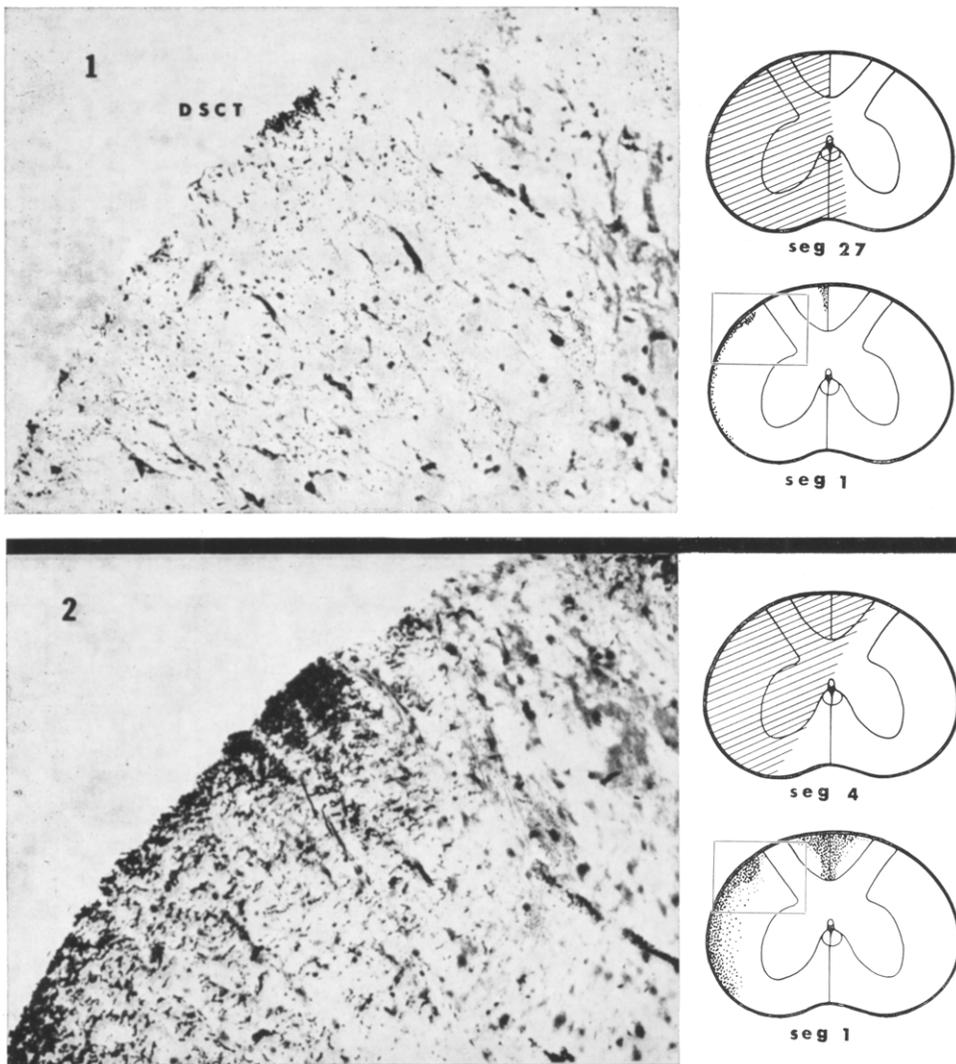
The term 'dorsal spino-cerebellar tract' (DSCT) refers to those spino-cerebellar fibers situated dorsally in the periphery of the lateral funiculus of the spinal cord, dorsal to the ventral spino-cerebellar tract. The DSCT sweeps around the rostral edge of the root of the trigeminal nerve. The term 'ventral spino-cerebellar tract' (VSCT) refers to those nerve fibers having an intermediate position in the periphery of the lateral funiculus of the spinal cord, ventral to the DSCT. The VSCT also curves around the trigeminal nerve and is rostral to the DSCT in the cerebellar peduncle.

The lateral funiculus was destroyed unilaterally in some cases (Figs. 1, 2, 3) and partially in others (Fig. 4). In all instances there are varying degrees of involvement of the dorsal funiculus. Rostral to the lesion there are always a few degenerated fibers in the contralateral lateral funiculus resulting, no doubt, from destruction of the spinal cord gray matter. However, only the ipsilateral ascending degeneration in the spinal cord and brain stem will be described.

Postbrachial lesions

The first spinal cord segments of those animals with lesions below the brachial enlargement (Fig. 1) show ascending degeneration in the lateral funiculus at the periphery of the spinal cord. The distinct group of degenerated fibers, the dorsal spino-cerebellar tract (DSCT), located dorsally in the area ventral to the dorsal horn is compact and can be followed to the cerebellum in the serially stained Nauta sections. DSCT fibers are more numerous in animals with higher spinal cord lesions, the fibers of more rostral origin are added primarily to the medial and ventral sides of the DSCT fibers of a more caudal origin (compare Figs. 1 and 6). At the periphery of the cord between the dorsal horn and the dorsal edge of the DSCT is a small area of lateral funiculus devoid of degeneration (Fig. 1). It contains fibers of the lateral division of the dorsal roots^{13,25,27} and perhaps some descending fibers of brain stem origin. Thus, the DSCT does not completely border upon the dorsal horn gray matter in animals with postbrachial spinal cord lesions.

The more ventral group of degenerated fibers, the ventral spino-cerebellar tract (VSCT), is less distinct and lies immediately ventral to the DSCT. Its ventral

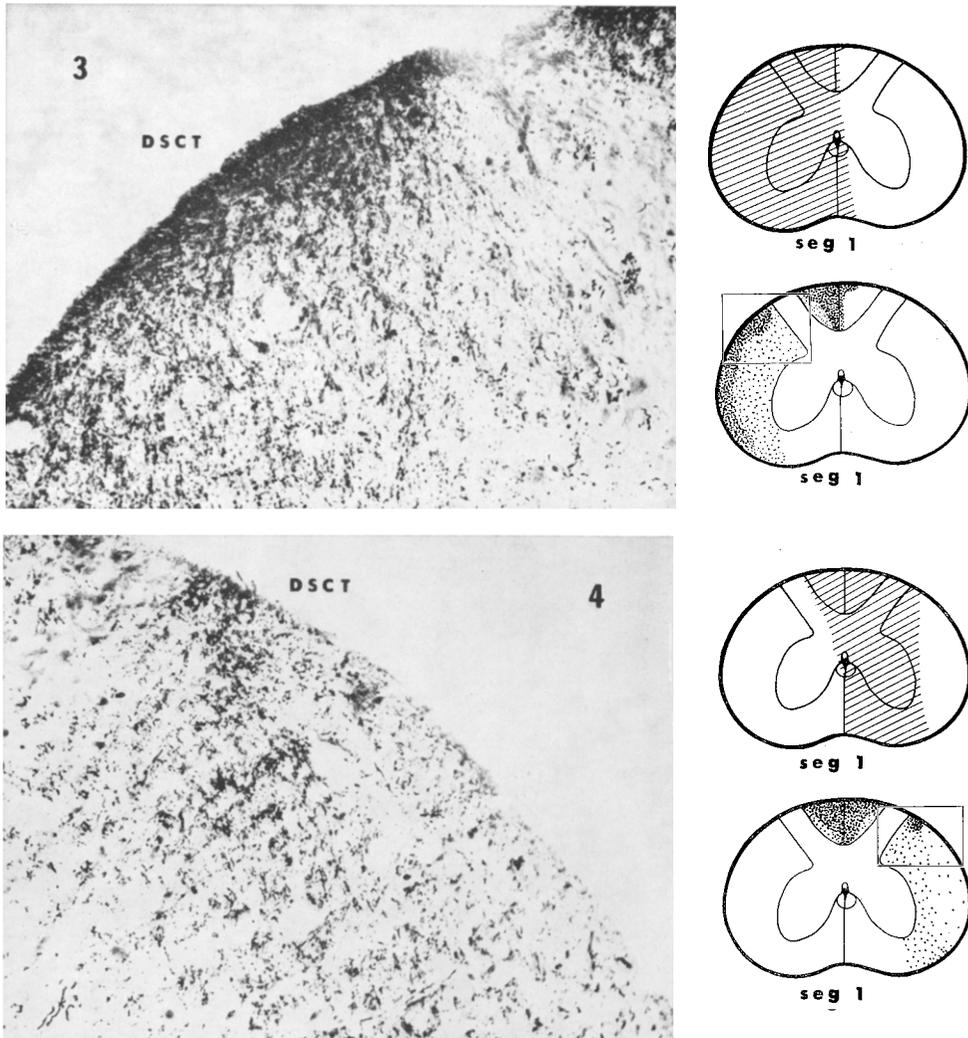


See opposite page for legend.

extent could not be adequately determined in these animals for it is mixed with spino-reticular, spino-vestibular and spino-mesencephalic fibers.

Prebrachial lesions

Lesions within and above the brachial enlargement (Figs. 2, 3) produce a dense peripheral rim of degeneration in the lateral funiculus at the first spinal segment, the amount of which is approximately doubled as compared to that caused by post-brachial lesions. The increased degeneration in the DSCT, derived from the higher spinal segments, is added primarily to the medial and ventral sides of the spino-



Figs. 1-4 are photomicrographs (left) of Nauta stained spinal cord sections and projection drawings of cross-sections of the spinal cord (right). The hatchings in the upper right spinal cord drawings represent the extent of the lesion with the level of segmental involvement indicated below. The spinal cord drawings in the lower right represent the pattern of degeneration found at the level of the 1st spinal cord segment. The photomicrographs were taken from cross-sections of the 1st spinal segment and correspond to the area blocked off in the spinal cord drawings. Approx. 175 \times . See text for further explanation.

cerebellar fibers coming from more caudal levels. Unexplicably a few fibers from the cervical and brachial regions of the cord pass to the area between the dorsal horn and the DSCT.

Somatotopic pattern

Collectively, these experiments give some indication of a somatotopic organi-

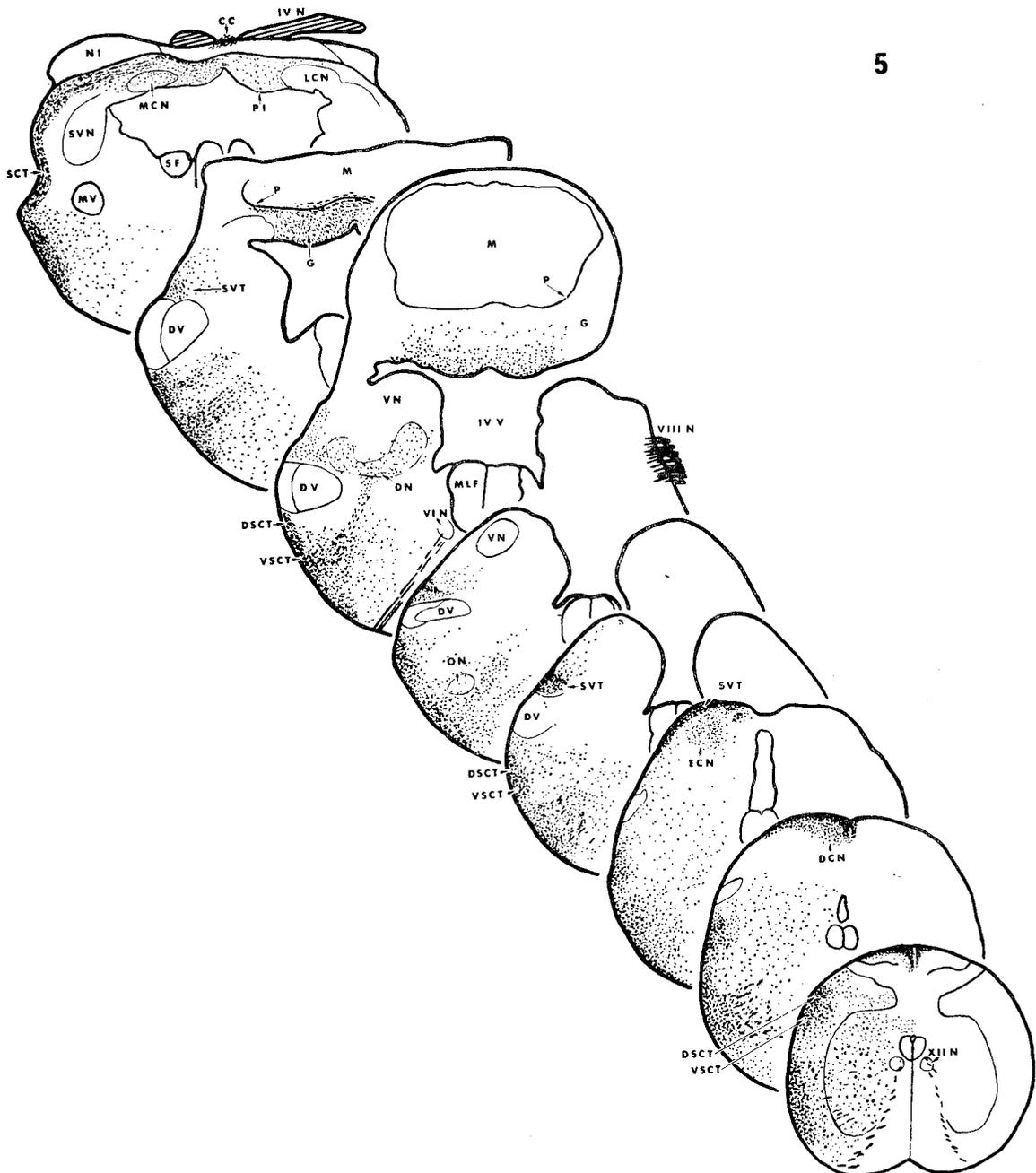


Fig. 5. A series of projection drawings from Nauta stained sections through various brain stem levels of a lizard (L6) showing the distribution and relative density of degeneration following a lesion of the 4th spinal cord segment (Fig. 3). The relationship of the DSCT, VSCT and SVT to the DV are presented. For list of figure abbreviations see page 173.

zation in the DSCT so that: the tail, hindlimb and lower thoracic regions are dorsal; the upper thoracic region and the forelimb are more medial and ventral; the cervical region is most medial (Fig. 7). In both pre- and postbrachial lesioned animals there are many scattered degenerated fibers, adjacent to the medial side of the spino-cerebellar tracts, that give off trunk or collateral fibers to the spinal cord gray matter. The medial half of the lateral funiculus contains a few ascending fibers that eventually terminate at higher spinal levels.

Degeneration in the brain stem

In both sagittal and transverse serial sections, the rostral continuation of the degeneration seen in the lateral funiculus is easily followed through the medulla where the DSCT and VSCT maintain essentially the same relative relationships as described in the spinal cord. On entering the medulla the spino-cerebellar fibers are altered from their relatively straight course by: the pronounced ventral flexure of the medulla; the dorsoventral elongation of the lateral funiculus of the medulla caudally; and the continuation of the descending tract and nucleus of the trigeminal with Lissauer's tract and the dorsal horn. At the level of the dorsal column nuclei the DSCT and VSCT are directed ventrally and rostrally at approximately a 40° angle with reference to the longitudinal axis of the brain stem (Fig. 5). The spino-cerebellar fibers lie at the peripheral edge of the medulla, directly ventral to the descending trigeminal complex. This course and relationship of the spino-cerebellar fibers is maintained up to the peripheral root of the trigeminal nerve. In transverse sections the DSCT appears as a compact group of degenerated fibers with the contiguous VSCT located more ventrally. In all animals, but especially those containing more rostral lesions numerous degenerated fibers, medial and ventral to the spino-cerebellar tracts, send trunk or collateral fibers to the reticular nuclei while other degenerated fibers course medially and then dorsally in relation to the descending nucleus of V to terminate in the vestibular area (Fig. 5). A careful examination of the region containing the descending tract and nucleus of V was done in all brains under high power magnification and not one brain showed evidence of spino-cerebellar fibers passing laterally over the descending trigeminal complex and contributing to an inferior cerebellar peduncle. The eminence or tuberculum produced by the descending root of V clearly demarcates the dorsal border of the DSCT (Fig. 5). Lizards with a first cervical segment lesion show an occasional fiber within the lateral substance of the caudal-most part of the descending tract of V but these appear to pass medially into the medulla. Possibly some efferent fibers from the descending nucleus of V are involved in a 1st cervical segment lesion and this may explain the presence of an occasional fine degenerated fiber within the caudal part of the trigeminal complex of *Lacerta*.

At the level of the trigeminal nerve root, degenerated spino-cerebellar fibers curve around the rostral edge of this nerve and ascend dorsally and medially in the cerebellar peduncle to enter the anterior commissure of the cerebellum (Fig. 6). The DSCT, located just ventral to the descending trigeminal complex, makes a sharp

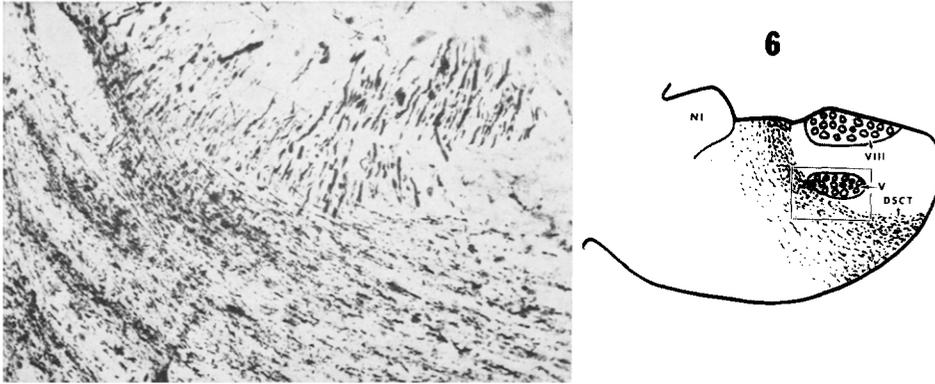


Fig. 6. A projection drawing (right) of a sagittal section of animal L18 showing the relative density of degenerated fibers and the relationship of the DSCT to the trigeminal nerve root (V). The blocked area in the drawing was photographed from a Nauta section and is depicted in the photomicrograph (left). Approx. 210 \times . See text for further explanation.

bend around the trigeminal nerve root and lies just caudal to the VSCT in the cerebellar peduncle. An occasional degenerated fiber passes through a few of the rostral-most rootlets of the trigeminal nerve and later joins the main group of spino-cerebellar fibers in the cerebellar peduncle. No degenerated DSCT fibers could be found passing caudodorsally to the main trigeminal nerve root. The VSCT makes a broader rostradorsal sweep around both the DSCT and the trigeminal nerve root and attains a position more rostral in the cerebellar peduncle.

Anterior cerebellar commissure

Laterally the anterior cerebellar commissure, a somewhat oval-shaped structure in parasagittal sections, is situated caudal to the nucleus isthmi and rostral to the vestibular nuclei. Medially the commissure is quite distinct and lies directly caudal to the IVth nerve decussation. The fibers in the afferent component of the cerebellar commissure decrease in number lateromedially and at the midline there are only a few fibers wedged between the decussating IVth nerve rostrally and the cerebellar efferent system caudally. This latter fiber bundle (probably originating from Purkinje cells and cells of the cerebellar nuclei) replaces the medial cerebellar nucleus medially and makes up most of the midline portion of the anterior cerebellar commissure. The medial portion of the cerebellar efferent system is free of degeneration in all the specimens studied. Thus, the cerebellar commissure in *Lacerta* is composed mainly of cerebellar afferents laterally and cerebellar efferents medially.

In the cerebellar commissure the DSCT and VSCT are indistinguishable from each other, nevertheless, here the degenerated spino-cerebellar fibers show some somatotopy. A comparison of sagittal sections taken at about the same level (approximately 1 mm from the midline) from each animal shows that degenerated spino-cerebellar fibers from the spinal cord below the 18th segment are confined mainly to the most dorsal area of the afferent divisions of the anterior cerebellar

commissure. Lesions at the 8th and 4th cord segments produce degeneration mainly in the caudodorsal one-half of the commissure with some additional degeneration from a 4th segment lesion added ventrally near the caudal border. With involvement mainly of the DSCT at the 1st cord segment degeneration is restricted primarily to the middle third of the caudal one-half of the commissure. In all these animals a few degenerated fibers are diffusely scattered throughout the remainder of the afferent portion of the cerebellar commissure with some fibers passing rostrally near the lateral edge of the ipsilateral IVth nerve decussation and entering the central gray of the tectum. These findings suggest that the caudal spinal cord segments project fibers to the more dorsal regions of the anterior cerebellar commissure while rostral spinal segments project slightly to the more ventral and caudal regions of the commissure.

Degeneration patterns in the cerebellum

Larsell's²⁹⁻³² terminology for the reptilian cerebellum is employed in this account. In general the cerebellum of *Lacerta viridis* is a vertically oriented structure, slightly everted, with a concave rostral surface, and a convex caudal surface. It consists of two major divisions: the corpus cerebelli and the pars auricularis. The former is subdivided into a pars interpositus (medially) and a pars lateralis (laterally). The pars interpositus is represented in transverse sections (Fig. 5) as a convex swelling (caudally and ventrally) on either side of the cerebellar surface.

A constant feature of crocodylians^{23,27-29} is the presence of two transversely directed furrows or sulci that divide the corpus cerebelli into three lobes — lobus anterior, lobus medius and lobus posterior. So far these sulci have not been described for other groups of reptiles. I am, however, of the opinion that they also occur in lacertilians, at least in *Lacerta viridis*. In this species the anterior sulcus (primary fissure), separating lobus anterior from lobus medius, is more distinct than the posterior sulcus (secondary fissure), separating lobus medius from lobus posterior. The indentations formed by the sulci on the rostral cerebellar surface show concomitant changes in the histological pattern of the cerebellar cortex: (a) the granular layer extrudes into the sub-Purkinje zone and the Purkinje cell layer and (b) the Purkinje cells scatter into the molecular layer toward the sulcus and appear pseudostratified.

A general observation made on the lizards shows that degenerated spino-cerebellar fibers leave the anterior cerebellar commissure to enter the anterior lobe of the corpus cerebelli where the majority of them terminate. The quantity of degeneration found ipsilaterally and contralaterally is dependent upon the extent and level of cord involvement. Spino-cerebellar degeneration in the lobus medius is always present in slight and variable amounts but the pars auricularis is free of any axonal degeneration.

The spino-cerebellar fibers enter the cerebellum near the level of the lateral border of the pars lateralis. Sagittally cut brains show degenerated axons entering the granular cell layer (Fig. 9). Some degeneration continues dorsomedially in the sub-

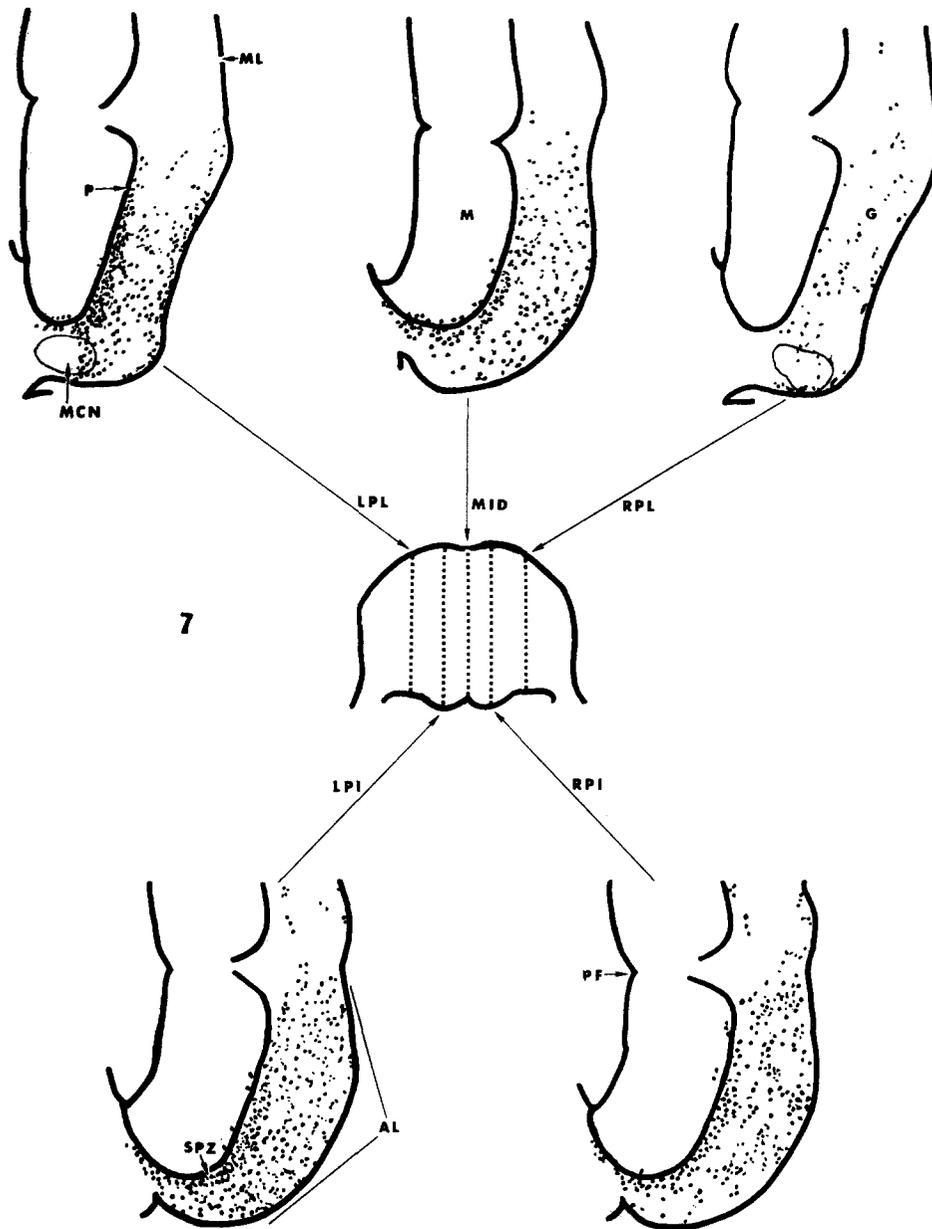


Fig. 7. A sagittal series of projection drawings with reference to a diagrammatic frontal view taken from Nauta stained sections of the anterior lobe of the cerebellum. The lesion occurred at the 4th segment in animal L6 (see Fig. 2). Note the relative density and distribution of degeneration in the granular layer (caudal) of the right and left pars lateralis as compared to the right and left pars interpositus. A few fibers of degeneration are present in the middle lobe. Compare with Fig. 8. See text and list of figure abbreviations for further explanation.

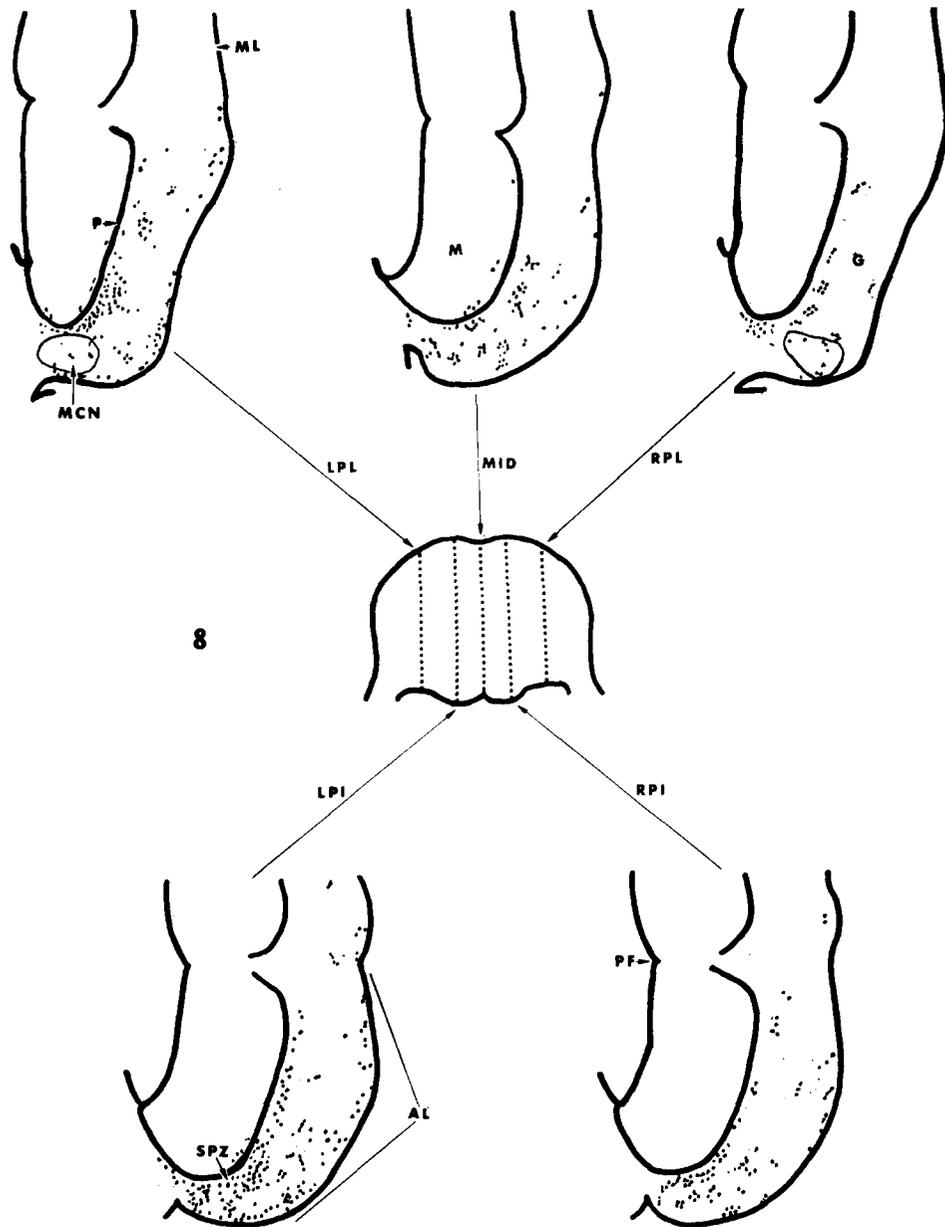


Fig. 8. A schema as presented in Fig. 7. The degeneration pattern in the pars lateralis and pars interpositus of the cerebellum result from a lesion at the 18th spinal cord segment in lizard L3. Note the relatively greater density of degeneration in the proximal third of the cerebellum as compared to the more distal areas. Compare with Fig. 7. See text and list of figure abbreviations for further explanation.

Purkinje zone and courses in a lateromedial (transverse) direction (Fig. 10) while other fibers entering the deeper part of granule cell layer pass more caudally and run dorsally along the caudal border of the cerebellum. Degenerated fibers also pass caudoventrally through the medial part of the medial cerebellar nucleus to reach the most ventral part of the granule cell layer.

Within the sub-Purkinje zone — a course network of nerve fibers beneath the Purkinje cell layer — there are many long degenerated spino-cerebellar axons which weave their way transversely (lateromedially) across the anterior lobe of the cerebellum. This is most evident in transverse sections (Figs. 10, 12), where the degenerated axons run parallel to the plane of section and can be followed for a considerable distance. A few degenerated fibers enter the Purkinje cell layer and some pass along the bases of several Purkinje cell dendrites. The sub-Purkinje zone appears to be the major route for decussating spino-cerebellar fibers. The afferent portion of the anterior cerebellar commissure in the midline contains very few degenerated fibers. The small number of fibers in this commissure at the midline does not account for very much of the degeneration found contralaterally in the cerebellum and presumably this afferent commissure contains only a few, if any, crossing spino-cerebellar fibers.

Lesions caudal to the brachial enlargement of the spinal cord (animals L8, L3, L15) result in spino-cerebellar degeneration that is primarily distributed to the proximal part of the anterior lobe (Fig. 8). A lesion of the cord at the 27th segment produces degeneration that is evenly but lightly scattered throughout the ipsilateral, proximal one-fourth of the anterior lobe while cord lesions at the 18th and 13th segments result in degeneration to the proximal one-third of the anterior lobe. The distal parts of the anterior lobe contain only a few fibers scattered throughout the

Figs. 9–14 are photomicrographs of sections of the anterior lobe of the cerebellum stained by the Nauta method. Approx. $260 \times$. See list of figure abbreviations.

Fig. 9. A saggittal view of the left pars lateralis near the entrance of spino-cerebellar fibers into the cerebellum (animal L6). A lingula of the granular cell layer between the Purkinje cell layer and the medial cerebellar nucleus (MCN) contains dense degeneration. Degenerated fibers are closely associated with a few Purkinje cell bodies. A few fibers of degeneration are present in the MCN.

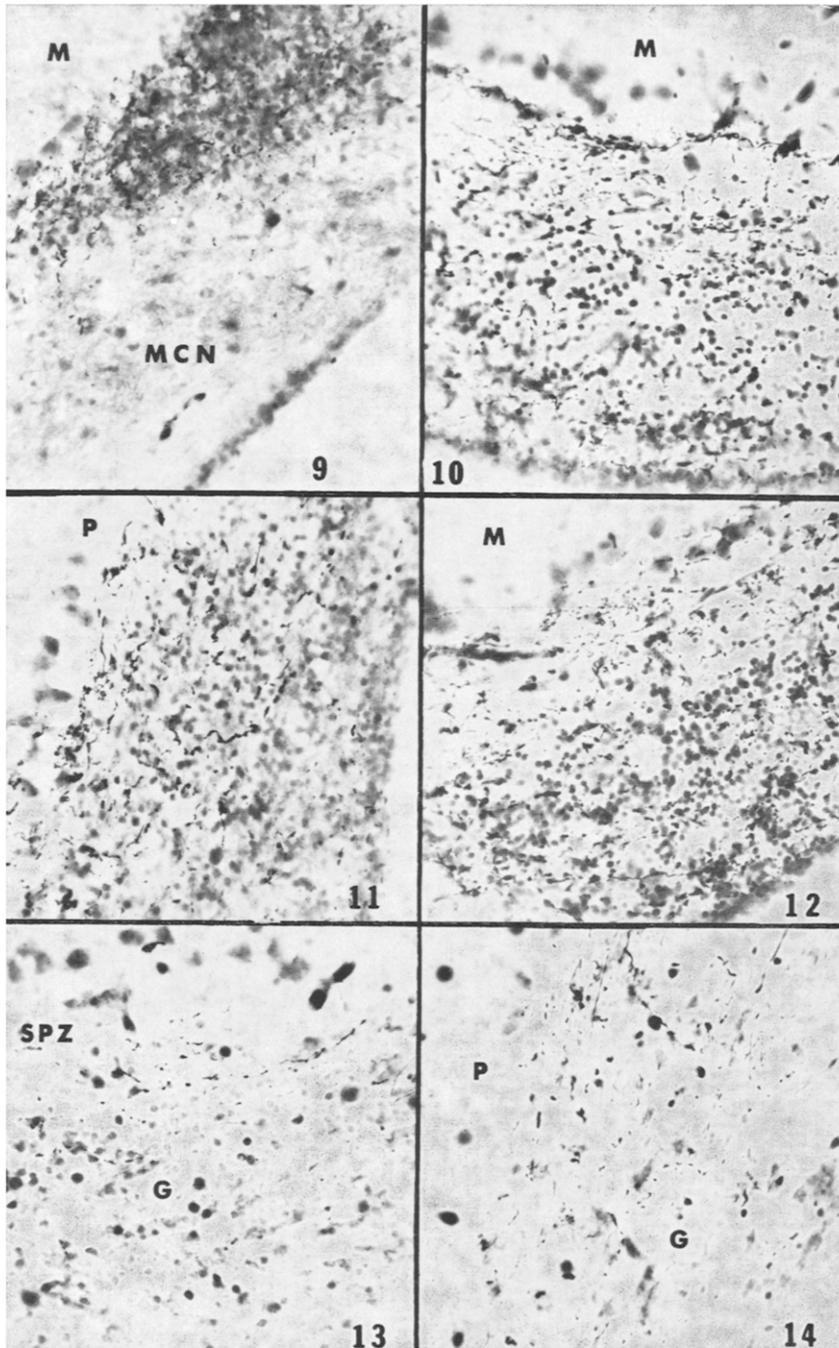
Fig. 10. A transverse section through the left pars interpositus at about its midportion (animal L16). Degeneration is uniformly scattered throughout the granular layer. The sub-Purkinje zone contains degenerated fibers directed transversely.

Fig. 11. A sagittal section taken from the left pars lateralis (more medially than Fig. 9) of lizard L6. Less degeneration is present in the deeper part (caudal) of the granular layer than the more superficial part (near the Purkinje cell layer). Several Purkinje cells are related to degenerated axonal elements. The apparent space within the sub-Purkinje zone (also Figs. 10 and 13) represent some tissue shrinkage and numerous unstained normal fibers coursing in this area.

Fig. 12. A transverse section taken from an area just to the left of the midline (lizard L16). Numerous Purkinje cells are laden with degenerated axons coursing transversely. The granular layer contains an even distribution of degenerated axons.

Fig. 13. A transverse section through the midportion of the right pars interpositus of lizard L21. Degenerated axons are located almost exclusively in the sub-Purkinje zone and superficial granular layer. Compare with Fig. 10.

Fig. 14. A sagittal section taken from the left pars lateralis of lizard L18. Degeneration is relatively more dense in the superficial granular zone than in the deeper granular layers.



granular layer. The middle portion of the contralateral pars interpositus displays a few degenerated fibers which are approximately one-half of that found ipsilaterally. The caudal part of the lobus medius contains a few fibers bilaterally.

The lesion at the 8th spinal segment results in moderately heavy degeneration in the ipsilateral ventral one-half of the anterior lobe. Fewer fibers are scattered in the dorsal half of the ipsilateral pars lateralis than in the pars interpositus. Spino-cerebellar fibers decussate in the sub-Purkinje zone (mainly its ventral two-thirds) to attain the contralateral side. The most ventromedial part of the contralateral pars interpositus shows only a few degenerated fibers while the remainder of the pars interpositus has approximately one-half the amount found ipsilaterally. The few degenerated fibers present bilaterally in the lobus medius are limited mainly to the pars interpositus. These fibers ascend from the anterior lobe (ventrodorsally) along the caudal wall of the granular layer.

Lizards L10, L6 and L16 with lesions at segments 5, 4 and 1, respectively, show generally similar patterns of degeneration in the anterior lobe. The animals with more rostral lesions, however, show increased amounts of degeneration (Fig. 7). The ipsilateral pars interpositus displays dense degeneration, especially ventrally (Figs. 10, 12), and its contralateral counterpart contains approximately one-half the amount with the most ventromedial area showing only a few fibers. The distal one-fourth of the pars interpositus shows approximately equal degeneration bilaterally. The ipsilateral pars lateralis (Figs. 9, 11) has heavy degeneration — most dense ventrally, subjacent to the sub-Purkinje zone. The contralateral (right) pars lateralis shows a slight amount of degeneration. Decussation of spino-cerebellar fibers is evident in the entire anterior lobe.

Somatotopy in the anterior lobe

The results from all the animals with hemisections of the spinal cord suggest a ventrodorsal somatotopic arrangement of spino-cerebellar fibers in the ipsilateral anterior lobe. On the basis of greatest density of degeneration in the anterior lobe; the tail, hind limb and lower trunk are represented in the ventral one-third; the upper trunk and upper limb in the middle one-third; and the neck in the dorsal one-third.

Several lizards with subtotal lateral funicular involvement provide additional evidence for localizing the DSCT and VSCT in the spinal cord and their apparent differences in termination. The lesion in 1 animal at the 18th segment spares the ventral portion of the lateral funiculus but the degeneration pattern and distribution in the cerebellum is nearly the same as that in animals with hemisections of the entire lateral funiculus. These results indicate that most, if not all, of the spino-cerebellar fibers below the brachial enlargement are located in the dorsal half of the lateral funiculus.

When the dorsal half of the lateral funiculus is not involved in the lesion the spino-cerebellar systems remain intact. This occurred in an animal having a lesion at the 7th spinal segment that involves only the dorsal funiculus and gray matter ipsilaterally.

Regarding the distribution of the DSCT in the cerebellum, 2 lizards (L21, L18) have lesions at the first spinal segment that selectively involve the dorsal funiculus, the spinal gray matter and only the dorsal portion of the lateral funiculus. In both animals the majority of VSCT fibers remain intact. The lesion in lizard L21 (Fig. 4) involves approximately the dorsal half of the DSCT whereas the entire DSCT is transected in animal L18. The distribution of degeneration in the anterior cerebellar lobe is similar in both animals. In animal L21 (Fig. 13) most of the degenerated fibers are in the sub-Purkinje zone and the superficial portion of the granular layer. Contralaterally (left) there are only a few fibers of degeneration scattered in the granular layer. The deeper portion of the granular layer bilaterally remains relatively free of degeneration. The degeneration pattern in a sagittally cut brain (animal L18; Fig. 14) confirms these results. In this animal there are a few more fibers in the granular layer but most of them, as in the previous animal, are in the sub-Purkinje zone and superficial granule cell layer of the anterior lobe. In both animals there are a few degenerated fibers in the lobus medius.

The results from these 2 animals (L18, L21) suggest that the DSCT projects mainly to the sub-Purkinje zone and the adjacent superficial granule cell layer of the entire anterior lobe. It seems highly probable that the VSCT is distributed to the deeper portion of the granular layer since a complete lateral funicular involvement at the first spinal segment (Fig. 3) produces heavy degeneration throughout the entire granular layer.

Terminations in the cerebellum

The degenerated mossy fiber terminations in the granular layer appear as highly irregular and sometimes bifurcated structures (Figs. 15, 16). Animals which survived for 11–20 days demonstrate beaded segments of an axon that are closely related to relatively large, coiled or twisted masses or simply swollen and irregular bag-like profiles. These are obvious in the clear areas surrounded by granule cells but other degenerated fragments, suggestive of terminations, appear very near the cell somas.

Other spino-cerebellar terminations appear close to the Purkinje cell soma. Both sagittal and transverse sections of the cerebellum show such degeneration (Figs. 9, 12, 17, 18, 19) as swollen granules or tear-like droplets. A first segment cord lesion produces such axonal degeneration that is in the vicinity of approximately 4–6 out of 25 Purkinje cells of the anterior lobe. These suggestive axosomatic terminations appear to be derived mainly from the lateromedially coursing fibers which are a continuation of the DSCT.

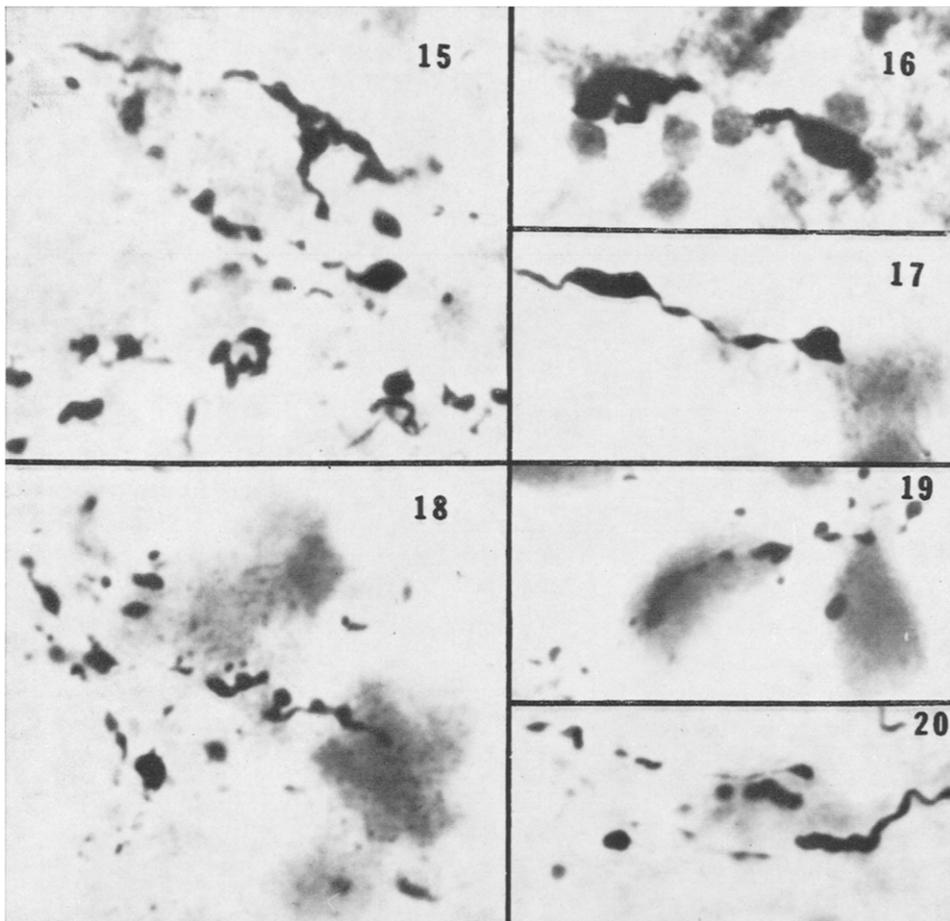
In the superficial granule cell layer relatively large cells are found which, because of their size and location, may well be Golgi cells. Occasionally such cells are surrounded by what appear to be degenerated terminals (Fig. 20) as suggested by the swollen ends with attached preterminals. DSCT fibers appear to be the major source of this degeneration.

Some spino-cerebellar terminations are closely related to cells of the medial

cerebellar nucleus (Fig. 9). While some fibers are undoubtedly in passage, the presence of granular debris in the neuropil and the argyrophilic fragments related to the cell soma suggest preterminal formations in this nucleus.

DISCUSSION

Anatomical descriptions of the reptilian spino-cerebellar systems based on normal preparations of the brain have been extensively reviewed by Larsell^{31,33}, Kappers *et al.*²⁷, Weston⁴² and Nieuwenhuys³⁵. The identification of spino-cerebellar



Figs. 15 and 16 show degenerated axonal elements (mossy fiber terminations) within the granular layer following 20 days degeneration. Approx. $1500\times$ and $1900\times$, respectively. Nauta stain. Figs. 17, 18 and 19 represent terminal and preterminal degeneration related to the Purkinje cell soma. 28, 20 and 14 days degeneration, respectively. Approx. $1500\times$. Nauta stain. Fig. 20. The outline of a large cell located in the superficial granular layer, which based on its location and size is thought to be a Golgi cell soma. Terminal and preterminal degeneration is related to the cell soma. 11 days degeneration. Approx. $1500\times$. Nauta stain.

tracts in the reptilian brain stem is based primarily on their course and relationship to the trigeminal nerve root⁴². The dorsal spino-cerebellar tract (DSCT) is described³³ as passing dorsolaterally over the descending tract of V, caudal to the Vth nerve root, while the ventral spino-cerebellar tract (VSCT) courses ventral and rostral to the Vth nerve root before passing dorsally into the cerebellum.

The DSCT described by Ebbesson⁹ is so called because of its position in the medulla (dorsal to the descending tract of V) and its termination in the cerebellum. Joseph and Whitlock²⁶, working on the toad, found evidence which suggests that dorsal root fibers project directly into the cerebellum by way of the dorsal funicular system. Preliminary studies on a single lizard²⁵ show that a few degenerated fibers from the left 4th dorsal root follow the dorsal funicular region and continue rostrally to terminate ipsilaterally in the granular layer of the cerebellum. Holbrook and Wilcox²¹ followed degeneration to the cerebellum after sectioning the posterior roots at various levels in a goat. Thus it seems that the dorsal funiculus carries a few spino-cerebellar fibers of dorsal root origin. Spinal cord lesions involving the dorsal column would interrupt dorsal root projections to the cerebellum. In the present material the few dorsal column fibers of the lizard which continue on rostral to the vestibular nuclear region toward the cerebellar commissure cannot be properly identified as spino-cerebellar fibers²⁴. Based on the present results in *Lacerta*, the DSCT in the lateral funiculus is considered homologous to the mammalian tract by the same name. Preliminary work on snakes shows that the DSCT is not present in the lateral funiculus caudal to the 5th spinal cord segment²⁵. This suggests that the DSCT may be a 'limb dependent' tract.

Differences regarding the description of the DSCT and VSCT in reptiles may represent variations in species as well as a lack of agreement among authors in defining such tracts or fiber groups. In mammals the DSCT consists of those spino-cerebellar fibers of the lateral funiculus that are situated dorsal to the denticulate ligaments of the spinal cord while the VSCT lies ventral to the ligaments. The DSCT enters the inferior cerebellar peduncle while the majority of VSCT fibers of hind limb origin join the superior cerebellar peduncle after coursing around the Vth nerve root. Anatomical⁴¹ and physiological investigations³⁶ have shown that some VSCT fibers of forelimb origin in the cat are present in the inferior cerebellar peduncle. Thus, utilizing the topographical relationship in the spinal cord rather than the relationship to the trigeminal complex, *Lacerta viridis* demonstrates both dorsal and ventral spino-cerebellar tracts in the lateral funiculus and the route taken by these spino-cerebellar fibers to the cerebellum is a common one through the brain stem. It was not determined whether denticulate ligaments exist in the *Lacerta*.

There was no attempt to homologize all the spino-cerebellar tracts described by Grant^{14,15} and Oscarsson³⁶ for the cat although it appears that some similarities may be present in the lizard. The area immediately ventral to the dorsal horn lacks degeneration from caudal lesions but when the lesions occur above the brachial enlargement a few fibers of cervical origin enter the area dorsomedial to the DSCT.

Since no spino-cerebellar fibers pass over the descending tract of V, this possibly represents a primitive arrangement in the lizard. The mammalian somatic sensory

area of the medulla which gives rise to pontine gray in addition to other nuclei may influence the separation of spino-cerebellar fibers into DSCT and VSCT groups. In lower mammals the first developing pontine fibers lie near the level of the Vth nerve root while in higher mammals the Vth nerve root is shifted caudalward as a result of increased development of the pons caudally²⁷. Perhaps the spino-cerebellar groups are also separated in the course of pons development. The absence of a massive neo-pontine gray and a brachium pontis in the *Lacerta* may account for the DSCT and VSCT systems remaining inseparable and thus following a common course around the trigeminal nerve root to enter a common cerebellar peduncle.

The spino-cerebellar fibers in the anterior commissure of the cerebellum appear to have some general topographical arrangement in the *Lacerta* which is not too unlike the description offered for mammals. In general the mammalian anterior cerebellar commissure contains a rostral afferent part and a caudal efferent part — the latter representing the interfastigial commissure⁴¹. It is doubtful if any of the few midline afferent fibers in the cerebellar commissure decussate to subsequently enter the cerebellum contralaterally. Ebbesson⁹, however, has described a moderate decussation of spino-cerebellar fibers in the commissure of the Tegu lizard.

The pattern of spinal afferents to the anterior lobe of the Lacertilian cerebellum shows some similarities to the anatomical¹⁴ and physiological³⁶ results described for the mammalian anterior lobe. In the Tegu lizard, Ebbesson⁹ noted that the ipsilateral ventral portion of the cerebellum receives most of the spino-cerebellar fibers with a moderate amount in the contralateral side. Goldby and Robinson¹³ noted bilateral degeneration in the lacertilian cerebellum. In the present investigation of *Lacerta* the contralateral anterior lobe receives approximately half the amount found ipsilaterally.

Mossy fiber terminations in the cerebellum have been described by Larsell³³ and Kappers *et al.*²⁷ for various reptiles. The results from degeneration studies show the mossy terminations in the granular layer to be the predominant mode of spino-cerebellar termination^{9,13}. Such terminations in the short survival lizards appear as clusters of axonal segments with apparent short bifurcated segments (Fig. 15) which closely follow the descriptions of Brodal and Grant⁵, Brodal and Drabløs⁶ and Fox *et al.*¹¹ for mammals. Evidence for a DSCT and VSCT stratification in mammals is inconclusive although Szentágothai³⁹ noted spino-cerebellar terminations in the deeper aspects of the granular layer.

There is an additional mode of termination of spino-cerebellar fibers which to the author's knowledge has not been previously described and concerns the axosomatic terminations on Purkinje cells. Some of the Purkinje cells (roughly 20% in the anterior lobe) show related argyrophilic swellings which are suggestive of degenerated terminals or axonal endings. Using light and electron microscopy, Boycott *et al.*⁴ have studied the structure of axonal endings in various nuclei of *Lacerta viridis*. In the cerebellum they observed neurofilamentous structures in the mossy fiber terminals and in the axosomatic terminals on Purkinje cells but with no mention of the origin of the latter terminals. Since the deposition of silver in degenerated axons appears to be highly dependent on the presence of neurofilaments or some axoplasmic

constituent^{3,4,16,17} the large argyrophilic swellings of degeneration related to the Purkinje cells may very well represent axosomatic terminations derived from spino-cerebellar fibers. Further studies employing electron microscopy on normal and experimental lizards are needed to verify the presence of synapses in these argyrophilic swellings. In those animals sustaining only a DSCT involvement such axosomatic relations are present. This mode of termination may represent an anatomical basis for local cerebellar arcortical activity². To the author's knowledge such axosomatic terminations of mossy fibers with Purkinje cells have not been described in mammals.

Based on neurofilamentous and synaptic vesicular arrangements Szentágothai^{18,39} has described two morphologically different types of synaptic relationships of the Golgi cell soma in the cat. In the present study, the silver impregnation of degenerated endings on or near very large cells (Golgi cells?) in the superficial granular layer may represent the heavy filamentous type and thus be of spinal origin.

In agreement with Ebbesson⁹ the medial cerebellar nucleus contains fragments of degeneration on the ipsilateral side. Many of the swollen argyrophilic elements are related to the cell soma and appear to represent terminations. Smaller degeneration debris within the neuropil of the nucleus may represent axodendritic terminations.

LIST OF ABBREVIATIONS USED IN THE FIGURES

AL	= anterior lobe of cerebellum	MV	= motor nucleus of V
CC	= cerebellar commissure	NI	= nucleus isthmi
DCN	= dorsal column nuclei	ON	= olivary nucleus
DN	= Deiters' nucleus (vestibularis ventro-lateralis)	P	= Purkinje cell layer of cerebellum
DSCT	= dorsal spino-cerebellar tract	PF	= primary fissure of cerebellum
DV	= descending nucleus and tract of V	RPI	= right pars interpositus
ECN	= external (lateral) cuneate nucleus	RPL	= right pars lateralis
G	= granule cell layer of cerebellum	SCT	= spino-cerebellar tract
IV N	= trochlear nerve	SF	= solitary fasciculus
IV V	= fourth ventricle	SPZ	= sub-Purkinje zone
LCN	= lateral cerebellar nucleus	SVN	= superior vestibular nucleus
LPI	= left pars interpositus	SVT	= spino-vestibular tract
LPL	= left pars lateralis	V	= trigeminal nerve
M	= molecular layer of cerebellum	VI N	= abducens nucleus
MCN	= medial cerebellar nucleus	VIII N	= stato-acoustic nerve
MID	= midline	VN	= vestibular nuclei
ML	= middle lobe of cerebellum	VSCT	= ventral spino-cerebellar tract
MLF	= medial longitudinal fasciculus	XII N	= hypoglossal nucleus

SUMMARY

The Nauta silver method for staining degenerated axons was applied to 10 lizards (*Lacerta viridis*) having spinal cord lesions at various levels (1st–27th segment). The ascending spino-cerebellar tracts of each animal were observed at the 1st spinal segment and in the serial sections of the brain stem and cerebellum. The following results were obtained.

(1) Dorsal and ventral spino-cerebellar tracts are identified in the spinal cord on the basis of their position in the lateral funiculus. Ventral to the dorsal horn the DSCT fibers of forelimb origin are medial and ventral to lumbar fibers. Some cervical fibers are dorsal and medial to the main DSCT group. The VSCT lies immediately ventral to the DSCT.

(2) All DSCT and VSCT fibers remain ventral to the descending nucleus and tract of V through their course in the brain stem and pass around the rostral edge of the trigeminal nerve root to ascend dorsomedially in the common cerebellar peduncle.

(3) Within the lateral part of the afferent division of the cerebellar commissure the spino-cerebellar fibers of a more caudal origin are located dorsally while the spino-cerebellar fibers of a more rostral origin are located ventrally.

(4) Spino-cerebellar fibers leave the cerebellar commissure ipsilaterally to enter the granular layer of the cerebellum. Very few if any spino-cerebellar fibers decussate in the afferent part of the anterior cerebellar commissure.

(5) Almost all spino-cerebellar fibers terminate in the anterior lobe of the corpus cerebelli. Only a few fibers enter the middle lobe.

(6) Within the granular layer of the anterior lobe the spino-cerebellar fibers in the sub-Purkinje zone take a transverse or lateromedial course. Spino-cerebellar fibers in the deeper granular layer run somewhat vertically. The sub-Purkinje zone and superficial granular layer are the major sites of decussation.

(7) The spino-cerebellar fibers of a more caudal origin terminate largely in the proximal part of the anterior lobe while the fibers of a more rostral origin terminate in the more distal aspect of the anterior lobe. This somatotopic arrangement of spino-cerebellar terminations is similar to the spino-cerebellar organization found in the anterior lobe of the mammalian cerebellum.

(8) There is some evidence to indicate that the DSCT occupies the sub-Purkinje zone and the superficial granular layer ipsilaterally while the VSCT fibers are in the deeper granular layer bilaterally. The ipsilateral corpus cerebelli contains approximately twice the amount of degeneration found contralaterally.

(9) Spino-cerebellar fibers terminate mainly as mossy endings in the granular layer. Some spino-cerebellar fibers (DSCT in origin?) have axosomatic terminations on approximately 20% of the Purkinje cells. Evidence for degenerated axon terminals on large cells (Golgi cells?) and cells of the medial cerebellar nucleus is presented.

(10) Some similarities with the mammalian spino-cerebellar systems, the cerebellar commissure and terminations in the anterior lobe of the cerebellum are discussed.

ACKNOWLEDGEMENTS

The author is grateful to Professor J. Ariëns Kappers for providing the laboratory materials and the assistance which made this project possible. Special thanks are due Dr. Rudolf Nieuwenhuys for his advice and suggestions and Dr. Elizabeth C. Crosby for her help in evaluating and interpreting some of the findings.

Appreciation is extended to Dr. Clement A. Fox and Dr. Duncan T. Kennedy for their helpful criticisms during the preparation of the manuscript.

REFERENCES

- 1 BANCHI, A., Sulle vie di connessione del cervelletto, *Arch. ital. Embriol.*, 2 (1903) 291-307.
- 2 BELL, C. C., AND DOW, R. S., Cerebellar circuitry, *Neurosci. Res. Progr. Bull.*, 5 (1967) 162-163.
- 3 BERESFORD, W. A., An evaluation of neuroanatomical methods and their relation to neurophysiology, *Brain Research*, 1 (1966) 221-249.
- 4 BOYCOTT, B. B., GRAY, E. G., AND GUILLERY, R. W., Synaptic structure and its alteration with environmental temperature: a study by light and electron microscopy of the central nervous system of lizards, *Prod. roy. Soc. B*, 154 (1961) 151-172.
- 5 BRODAL, A., AND GRANT, G., Morphology and temporal course of degeneration in cerebellar mossy fibers following transection of spinocerebellar tracts in the cat, *Exp. Neurol.*, 5 (1962) 67-87.
- 6 BRODAL, A., AND DRABLØS, P. A., Two types of mossy fiber terminals in the cerebellum and their regional distribution, *J. comp. Neurol.*, 121 (1963) 173-187.
- 7 CHRISTENSEN, K., The morphology of the brain of *Sphenodon*, *Univ. Iowa Stud. nat. Hist.*, 12 (1917) 3.
- 8 EBBESSON, S. O. E., Ascending fiber projections from the spinal cord in the Tegu lizard (*Tupinambis nigropunctatus*), *Anat. Rec.*, 154 (1966) 341-342.
- 9 EBBESSON, S. O. E., Ascending axon degeneration following hemisection of the spinal cord in the Tegu lizard (*Tupinambis nigropunctatus*), *Brain Research*, 5 (1967) 178-206.
- 10 EDINGER, L., *Vorlesungen über den Bau der nervösen Central-Organen des Menschen und der Thiere*, Teil 7, Leipzig, 1908.
- 11 FOX, C. A., HILLMAN, D. E., SIEGSMUND, K. A., AND DUTTA, C. R., The primate cerebellar cortex: a Golgi and electron microscopic study. In C. A. FOX AND R. S. SNIDER (Eds.), *The Cerebellum, Progress in Brain Research*, Vol. 25, Elsevier, Amsterdam, 1967, pp. 174-225.
- 12 FREDERIKSE, A., *The Lizard's Brain. An Investigation on the Histological Structure of the Brain of Lacerta vivipara*, Ph. D. Dissertation, Amsterdam, 1931.
- 13 GOLDBY, F., AND ROBINSON, L. R., The central connexions of dorsal spinal nerve roots and the ascending tracts in the spinal cord of *Lacerta viridis*, *J. Anat. (Lond.)*, 96 (1962) 153-170.
- 14 GRANT, G., Spinal course and somatotopically localized termination of the spinocerebellar tracts. An experimental study in the cat, *Acta physiol. scand.*, 56, Suppl. 193 (1962) 1-45.
- 15 GRANT, G., Projection of the external cuneate nucleus onto the cerebellum in the cat: An experimental study using silver methods, *Exp. Neurol.*, 5 (1962) 179-195.
- 16 GUILLERY, R. W., Some electron microscopical observations of degenerative changes in central nervous synapses. In M. SINGER AND J. P. SCHADÉ (Eds.), *Degeneration Patterns in the Nervous System, Progress in Brain Research*, Vol. 14, Elsevier, Amsterdam, 1965, pp. 57-76.
- 17 GUILLERY, R. W., AND RALSTON, H. J., Nerve fibers and terminals: Electron microscopy after Nauta staining, *Science*, 143 (1964) 1331-1332.
- 18 HAMORI, J., AND SZENTÁGOTHAJ, J., Participation of Golgi neuron processes in the cerebellar glomeruli: An electron microscope study, *Exp. Brain Res.*, 2 (1966) 35-48.
- 19 HAUSMAN, L., The comparative morphology of the cerebellar vermis, the cerebellar nuclei and the vestibular mass, *Res. Publ. Ass. nerv. ment. Dis.*, 6 (1929) 193-237.
- 20 HINDENACH, J. C. R., The cerebellum of *Sphenodon punctatum*, *J. Anat. (Lond.)*, 65 (1931) 283.
- 21 HOLBROOK, J. R., AND WILCOX, H., Observations on the spinocerebellar tracts following dorsal root transection in the goat, *Anat. Rec.*, 148 (1964) 292.
- 22 HUBER, G. C., AND CROSBY, E. C., On thalamic and tectal nuclei and fiber paths in the brain of the American alligator, *J. comp. Neurol.*, 40 (1926) 97-228.
- 23 INGVAR, S., Zur Phylo- und Ontogenese des Kleinhirns, *Folia neuro-biol.*, 1 (1918) 14-277.
- 24 JACOBS, V. L., A spino-vestibular component of the dorsal funiculus in a lizard (*Lacerta viridis*), *Anat. Rec.*, 157 (1967) 264-265.
- 25 JACOBS, V. L., Unpublished observations.
- 26 JOSEPH, B. S., AND WHITLOCK, D. G., Central connections of caudal dorsal root ganglion in the toad (*Bufo marinus*), *Anat. Rec.*, 154 (1966) 364.
- 27 KAPPERS, C. U. A., HUBER, G. C., AND CROSBY, E. C., *The Comparative Anatomy of the Nervous System of Vertebrates, Including Man*, Hafner, New York, 1936.
- 28 DE LANGE, S. J., Das Hinterhirn, das Nachhirn und das Rückenmark der Reptilien, *Folia neuro-biol.*, 10 (1916) 385-422.
- 29 LARSELL, O., The cerebellum of reptiles: Lizards and snake, *J. comp. Neurol.*, 41 (1926) 59-94.
- 30 LARSELL, O., The comparative morphology of the membranous labyrinth and the lateral line organs

- in their relations to the development of the cerebellum. The cerebellum, an investigation of recent advances, *Res. Publ. Ass. nerv. ment. Dis.*, 6 (1929).
- 31 LARSELL, O., The cerebellum of reptiles: Chelonians and alligator, *J. comp. Neurol.*, 56 (1932) 299-345.
- 32 LARSELL, O., The cerebellum, *Arch. Neurol. Psychiat. (Chic.)*, 38 (1937) 580-607.
- 33 LARSELL, O., *The Comparative Anatomy and Histology of the Cerebellum from Myxinooids through Birds*, Univ. Minnesota Press, Minneapolis, Minn., 1967.
- 34 NAUTA, W. J. H., AND GYGAX, P. A., Silver impregnation of degenerating axons in the central nervous system: A modified technique, *Stain Technol.*, 29 (1954) 91-93.
- 35 NIEUWENHUYNS, R., Comparative anatomy of the spinal cord. In J. C. ECCLES AND J. P. SCHADÉ (Eds.), *Organization of the Spinal Cord, Progress in Brain Research, Vol. 11*, Elsevier, Amsterdam, 1964, pp. 1-57.
- 36 OSCARSSON, O., Functional organization of the spino- and cuneocerebellar tracts, *Physiol. Rev.*, 45 (1965) 495-522.
- 37 ROMER, A. S., *The Vertebrate Body*, Saunders, Philadelphia, 1949.
- 38 SHANKLIN, W. M., The central nervous system of *Chameleon vulgaris*, *Acta zool.*, 11 (1930).
- 39 SZENTÁGOTHAJ, J., Anatomical aspects of junctional transformation. In F. W. GERARD AND J. W. DUIJFF (Eds.), *Information Processing in the Nervous System*, Excerpta med. (Amst.), Congr. Ser., No. 49, 1962, pp. 119-136.
- 40 TERNI, T., Sulla correlazione fra ampiezza del territorio di innervazioni e grandezza delle cellule gangliari. 2. Ricerche sui gangli spinali che innervano la coda rigenerata, nei sauri (*Gongylus ocellatus*), *Arch. ital. Anat. Embriol.*, 17 (1920) 507.
- 41 VOOGD, J., *The Cerebellum of the Cat. Structure and Fibre Connexions*, Ph. D. Dissertation, Leyden, 1964.
- 42 WESTON, J. K., The reptilian vestibular and cerebellar gray with fiber connections, *J. comp. Neurol.*, 65 (1936) 93-199.