

THE PROJECTION OF SPINOCEREBELLAR NEURONS FROM THE SACROCOCCYGEAL REGION OF THE SPINAL CORD IN THE CAT. AN EXPERIMENTAL STUDY USING ANTEROGRADE TRANSPORT OF WGA-HRP AND DEGENERATION

Q. XU ¹ and G. GRANT

Department of Anatomy, Karolinska Institutet, S-104 01 Stockholm 60, Sweden

INTRODUCTION

For studying the course and terminal projections of a fiber tract, anterograde methods are, in general, more suitable than retrograde methods. They are more reliable for the demonstration of collateral projections, which, if they are weak, may not be possible to demonstrate by a retrograde method. Furthermore, they are necessary for the selective visualization of the terminal patterns and the morphological character of the terminals. In recent years a large number of studies have been made in which wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) has been used for anterograde tracing. In an early study we applied this tracer for investigating spinocerebellar projections in the cat (35). From the results of that study it was concluded that at least the main part of the labeled profiles in the cerebellar cortex were mossy fiber terminals of spinal origin. The possibility could not be ruled out, however, that part of the profiles were terminals of collaterals of neurons which had been labeled retrogradely, following uptake of tracer from the area of the spinal cord where WGA-HRP had been injected. In a later study from our laboratory, in which the cerebellar projections from the central cervical nucleus were investigated by anterograde WGA-HRP in the cat (42), labeled mossy fiber terminals were found in the same parts of the cerebellum as in a previous study, in which these projections had been investigated by anterograde transport of tritiated leucine (41). This suggested that spinocerebellar projections could be studied reliably by the anterograde WGA-HRP tracing method. The number of labeled terminals was much larger after WGA-HRP injections. This indicated that the anterograde tracing method with WGA-HRP was more sensitive than the autoradiographic tracing method.

In the present study we wanted to investigate the spinocerebellar projections from the sacrococcygeal region of the spinal cord by anterograde approach. This offered the possibility to compare the results of local injections of WGA-HRP with anterograde degeneration, visualized by suppressive silver staining, following

¹ On leave from Beijing Second Medical College, now called Capital Institute of Medicine, Beijing, People's Republic of China.

low spinal cord lesions. The results give further support to the view that the projections of spinocerebellar tracts can be reliably studied by the anterograde WGA-HRP method (19, 23-26, 29, 30, 43-46).

The spinocerebellar projections from the lower part of the spinal cord have been investigated by anterograde WGA-HRP transport by Matsushita recently (19). In that study a very detailed mapping and a quantitative analysis of labeled terminals in different sublobules was made. This allowed conclusions as to the distribution of the terminals both mediolaterally and apico-basally within different sublobules. Such a detailed analysis was not made in the present study. The general findings from this study, however, are with both the methods that we used, the same as in the study by Matsushita. In addition, the present study has given information as to the course taken by the sacrococcygeal spinocerebellar fibers in the spinal cord and their routes of entry into the cerebellum.

MATERIAL AND METHODS

WGA-HRP experiments.

Five adult cats (3.3-4.7 kg) were used. The animals were anesthetized by subcutaneous injections of Rompun (10 mg/kg body weight), followed by Nembutal (30 mg/kg intraperitoneally). After laminectomy of two neighbouring lower lumbar vertebrae, the dura and arachnoid membranes were split open. A cordotomy aimed at interrupting the ventral and lateral funiculi completely on one side was made on the left side at a level between S1 and Ca1 (Table 1). This was followed by injections of 4% (w/v) WGA-HRP (Sigma; dissolved in sterile saline), just caudal and ipsilateral to the cordotomy. All injections were made with a glass micropipette and by a pressure delivery device (2). The injected volume varied between 0.3 and 1.3 μ l.

After three days' survival, the animals were deeply anesthetized with Nembutal and perfused through the ascending aorta with a warm (37 °C) Tyrode's solution, followed

Table 1. — *Details concerning level of cordotomy and survival time for the different animals in the two groups as well as amount and segmental level of injected WGA-HRP in the first of the two groups.*

Cat	Level of cordotomy	WGA-HRP volume (μ l)	injection segmental level	Survival time (days)
<i>WGA-HRP experiments</i>				
C204	S2/S3	1.3	S3	3
C205	S1/S2	0.9	S3	3
C208	S3/Ca1	0.8	Ca1	3
C209	S3	1.0	Ca1	3
C214	S3	0.3	Ca2	3
<i>Degeneration experiments</i>				
C211	L6-L7			2
C212	L7			5
C215	S2-S3			4

by a cold (4 °C) fixative containing 1.5% glutaraldehyde, 1% paraformaldehyde and 5% sucrose in 0.1M phosphate buffer (pH 7.3-7.4). This was followed by a brief perfusion with cold (4° C) 0.1M phosphate buffer containing 10% sucrose.

The cerebellum, brainstem and segments of the spinal cord including the lesion and the injected part were immediately removed and stored overnight at 4° C in 30% sucrose in 0.1M phosphate buffer. The cerebellum and brainstem, from the medulla to the lower midbrain, were cut on a freezing microtome in 80 µm thick serial sections, either in the sagittal (cerebellum, 3 cases; brainstem, one case) or in the frontal (cerebellum, 2 cases; brainstem 4 cases) plane. The sections were collected in groups of three or four. From each group two sections were incubated with 3,3', 5,5', tetramethylbenzidine (TMB) according to Mesulam (27). One of these sections was stained with neutral red. From the spinal cord lesion and the injection site, frozen sections were cut in series at 80-100 µm in the transverse plane. These sections were collected in groups of five. From each such group, one section was processed according to Mesulam (27) and another stained with van Gieson's method or with cresyl violet. In addition, some transverse sections were prepared from segments C2, C5 and T8 and processed with TMB.

In all cases labeled fibers and terminals in the cerebellar cortex were plotted on photographic copies of the sections (10X enlargement), with the aid of an XY-recorder device (3). The findings regarding labeled terminals were summarized on diagrams of the unfolded cerebellar cortex (cf. Fig. 3). Attempts were made to locate the terminals in relation to the longitudinal zones described by Voogd (37, 38; his Figs. 2 and 27, respectively).

Degeneration experiments.

Three adult cats (2.8-3.0 kg) were used. Cordotomies aimed at interrupting the ventral and lateral funiculi completely on one side were made, on the left side, in the lower lumbar or in the sacral spinal cord (Table 1). The animals were anesthetized in the same way as those used for the WGA-HRP experiments. After two to five days' postoperative survival, the animals were killed under deep Nembutal anesthesia by perfusion with a 4% phosphate-buffered paraformaldehyde solution (pH 7.3-7.4) containing 5% sucrose. The cerebellum, brainstem (medulla to lower midbrain) and a block of spinal cord comprising the lesion were dissected out immediately. The spinal cord block was embedded in paraffin and cut serially in transverse sections at 15 µm. One out of ten of these sections were mounted and stained with thionin, another with van Gieson's stain. The cerebellum and brainstem were cryoprotected by immersion in a phosphate buffered 4% paraformaldehyde solution (pH 7.3-7.4) containing 30% sucrose. Twenty micrometer thick sagittal (two cases) or frontal (one case) frozen sections were cut in series from the cerebellum and similar transverse sections from the brainstem. The sections were collected in groups of ten. One from each group was processed according to the Fink-Heimer method, procedure II (10). Another section from each group was treated by bleaching and counterstaining, following the Fink-Heimer impregnation (17). A third section from each group was processed by the cupric silver method of de Olmos and Ingram (7).

Degenerating terminals and axons were plotted by the XY-recorder device and entered on summarizing diagrams of the cerebellum, as for labeled fibers and terminals from the WGA-HRP experiments (cf. Fig. 9).

RESULTS

WGA-HRP Experiments.

Injection sites: The extent of the injections is shown schematically in Figure 1. WGA-HRP activity was always found bilaterally and spread over several segments below the level of the cordotomy. The spinocerebellar cell groups which have earlier been identified at these segmental levels (10, 20) were comprised by the injection, either by the central heavily labeled zone or by the peripheral, lightly labeled zone.

Labeled terminals in the cerebellum: In the cerebellar cortex all labeling was restricted to the granular layer. No labeled neuronal structures were found in the molecular layer. Tortuous labeled fibers could be seen, frequently with fine branches or collaterals, many of which ended as coarse expansions (Fig. 2). The expansions were located in spaces between granule cells, obviously corresponding to cerebellar glomeruli, identifiable in the sections stained with neutral red (Fig. 2). They had the same characteristics as the terminals described in our previous study (35) which were regarded as mossy fiber rosettes. In some areas, such as lobule II, certain parts of the granular layer were packed densely with such expansions.

In two of the cases (C204, C205) there was some labelling suggestive of terminals

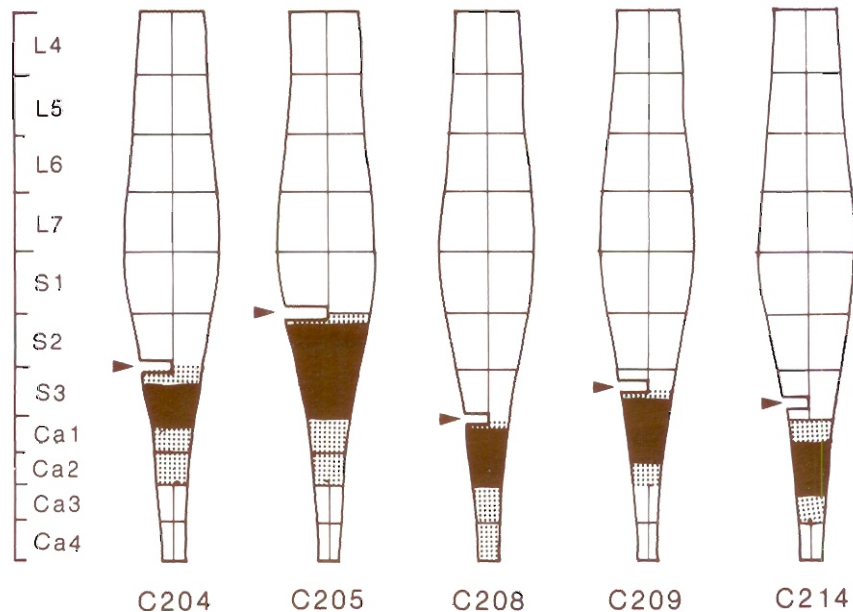


Fig. 1. - Diagrams showing extent of reaction product from injected WGA-HRP in the five cats used for anterograde tracing.

Black indicates heavy WGA-HRP staining and stippled areas light WGA-HRP staining. Arrow-heads indicate levels of cordotomies.

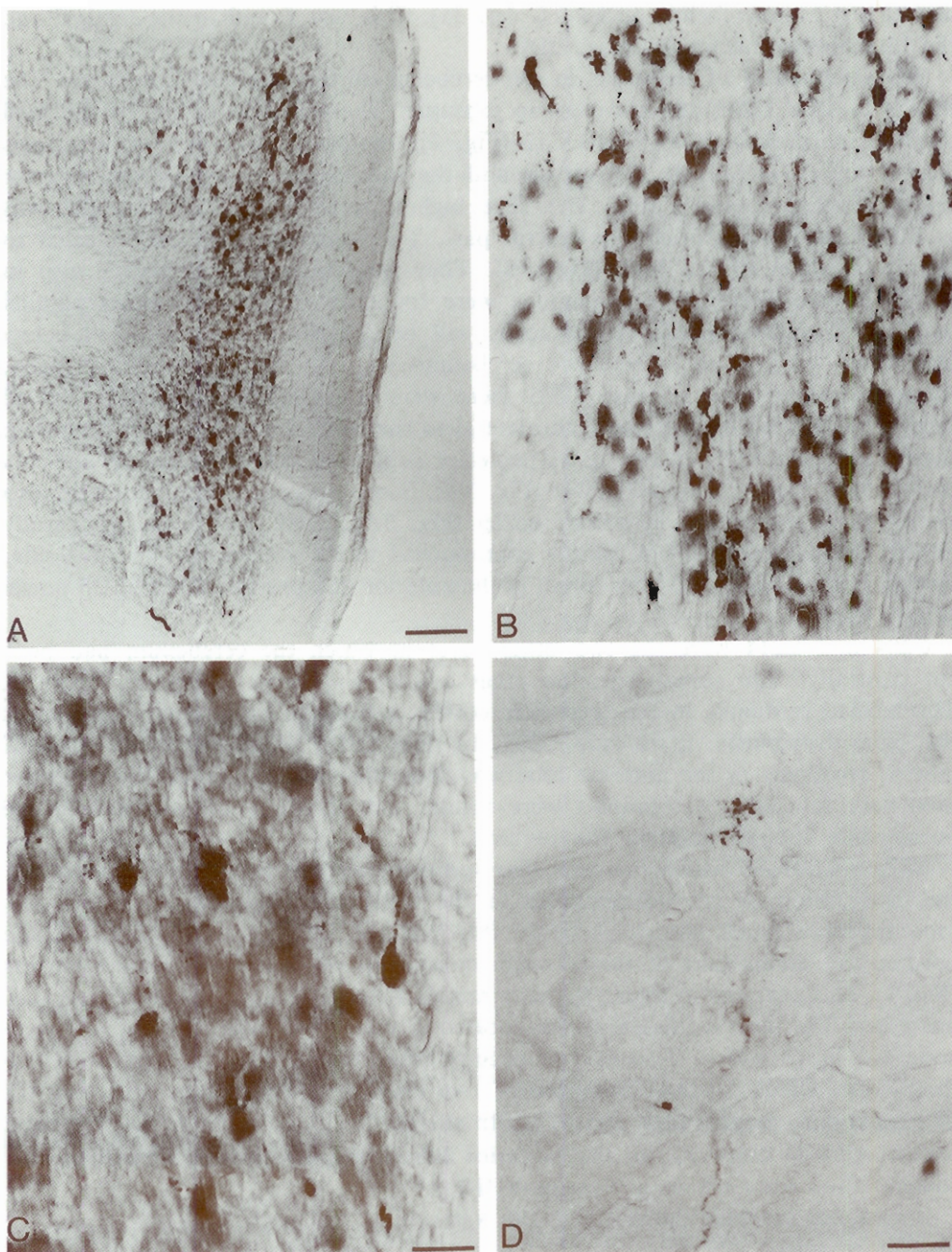


Fig. 2. - Photomicrographs from parts of sagittal sections of the cerebellum of case C204 showing WGA-HRP labeled mossy fiber terminals.

A-C show terminals in the granular layer of lobule II. A and C stained with neutral red. D shows terminals in the fastigial nucleus. A, X80; B and D, X200; C, X320.

also in the intracerebellar nuclei (cf. ref. 35). This was found in the fastigial and the interpositus nuclei (cf. Fig. 2D).

The distribution of terminals in the cerebellar cortex was very consistent. The results of case C205 can serve as an example. The injection in the spinal cord resulted in labeling from S2 to Ca2 (Fig. 1). In the cerebellum most terminals were found bilaterally in the vermis and the intermediate parts of the anterior lobe, including lobule I (Fig. 3A). The highest concentration of terminals was in sublobule IIA, especially its rostral part. The terminals were concentrated to the apical parts of the folia (Fig. 3B). They were unevenly distributed also in the transverse direction. Aggregations were found in longitudinal zones parallel to the mid sagittal plane. These zones will be described more in detail below in another case, C209, where the cerebellum was cut in the transverse plane. In the posterior lobe of case C205 there were some labeled terminals. All of them were located on the side contralateral to the cordotomy. Most of them were found in lobule VIII, chiefly in its posterior sublobule, VIIIB, a few in the pars copularis of the paramedian lobule (Fig. 3A). Some presumed terminals were also identified in the central cerebellar nuclei (Fig. 3A). They were found bilaterally in the fastigial nucleus, but mainly contralateral to the cordotomy. On this side, terminal-like labeling was also found in the anterior and posterior interposed nuclei (Fig. 3A).

C204 and C214 were the two other cases in which the cerebellum had been cut sagittally. The spinal injection areas are shown schematically in Figure 1. The labeled terminals in the cerebellar cortex in both of these cases had almost the same distribution as in case C205. In case C214, however, the quantity of labeled terminals in the anterior lobe was somewhat lower, and in the posterior lobe very few labeled terminals were found. All of them were located in the paramedian lobule, none in the pyramis.

In case C209, in which the cerebellar sections were cut transversely (frontally), the distribution of labeled terminals in the transverse direction in the anterior lobe appeared much more clearly. In this case the labeling at the injection site covered the caudal part of the S3 segment and the whole of the Ca1-2 segments (Fig. 1). As illustrated in figure 4, the terminals appeared concentrated in longitudinal zones. The most medial zone was located in the mid sagittal plane. The second zone was much wider. It seemed to occupy zones B and C1 of Voogd (38). The third zone, which possibly consisted of two (Fig. 4) seemed to occupy zone C3 of Voogd and, maybe, also part of D. In sublobule IIA only the first and second zones could be observed. In the posterior lobe, only few labeled terminals were found. They were all in sublobule VIIIB.

In case 208 in which the injection site was similar, although slightly more caudal to that in case C209 (Fig. 1), the labeled terminals in the anterior lobe were aggregated in longitudinal zones as in case C209.

Fiber course: Many labeled fibers were clearly seen along the peripheral white matter of the spinal cord and brainstem, as well as in the cerebellar peduncles (Fig. 5).

Most of the labeled fibers in the spinal cord (segments T8, C5 and C2) were

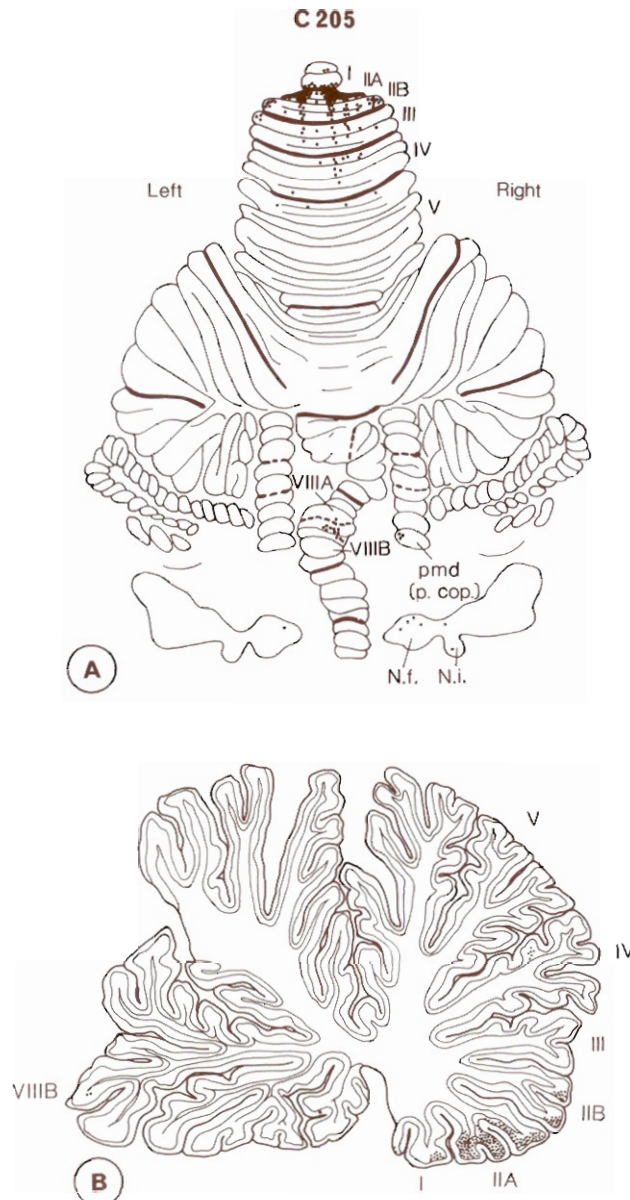


Fig. 3. - A. Distribution of WGA-HRP labeled terminals (dots) within the unfolded cerebellum (slightly modified from Grant, ref. 11) of case C205.

B. Diagram of sagittal section close to the mid plane of the cerebellum showing the distribution of labeled terminals in the cerebellar cortex of the same case as in A.

Spacing of dots indicates relative number of labeled terminals.

I, IIA, IIB, III...X, cerebellar lobules and sublobules, according to Larsell (ref. 18); N.f., nucleus fastigii; N.i., nucleus interpositus; pmd, paramedian lobule; P. cop., pars copularis.

C 209

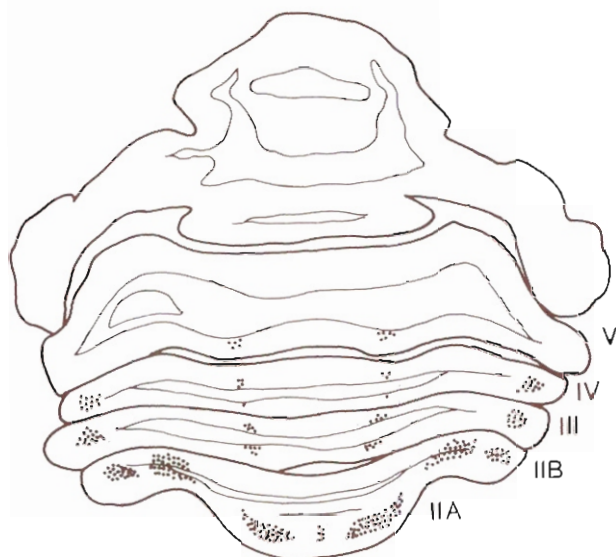


Fig. 4. - Diagram of frontal section through the anterior lobe of the cerebellum of case C209 showing WGA-HRP labeled terminals in longitudinal zones.

located in the lateral funiculus, a few in the ventral funiculus. They were, with these locations, observed exclusively on the side contralateral to the cordotomy. In addition, there were a few labeled fibers in the superficial part of the dorsal funiculi on both sides, although most of them were observed on the side contralateral to the cordotomy.

In the lower part of the medulla oblongata, the labeled axons were distributed within the peripheral white matter, superficial to the spinal trigeminal tract and further ventrally, down to the level of the lateral reticular nucleus. They were located on the same side as the labeled fibers in the lateral funiculus of the spinal cord. In addition, some labeled terminals were found bilaterally in the brainstem, for instance in the dorsal column nuclei.

Many of the labeled fibers turned dorsally to enter the restiform body. In the frontal sections of the cerebellum labeled fibers could be seen with an orientation as if curving medially at some distance dorsal to the central cerebellar nuclei, to reach the rostral lobules of the anterior lobe. Some labeled fibers were seen crossing to the contralateral side (Fig. 5). In sagittal sections fibers were seen curving dorsally at some distance rostral to the lateral cerebellar nucleus (Fig. 6A). Furthermore, labeled fibers were observed in the ventral part of the medulla curving dorsally just rostral to the main sensory nucleus of trigeminal nerve (Fig. 6B). In frontal sections such fibers were seen passing through the superficial part of the brachium conjunctivum, entering the cerebellum just medial to the restiform body (Fig. 5).

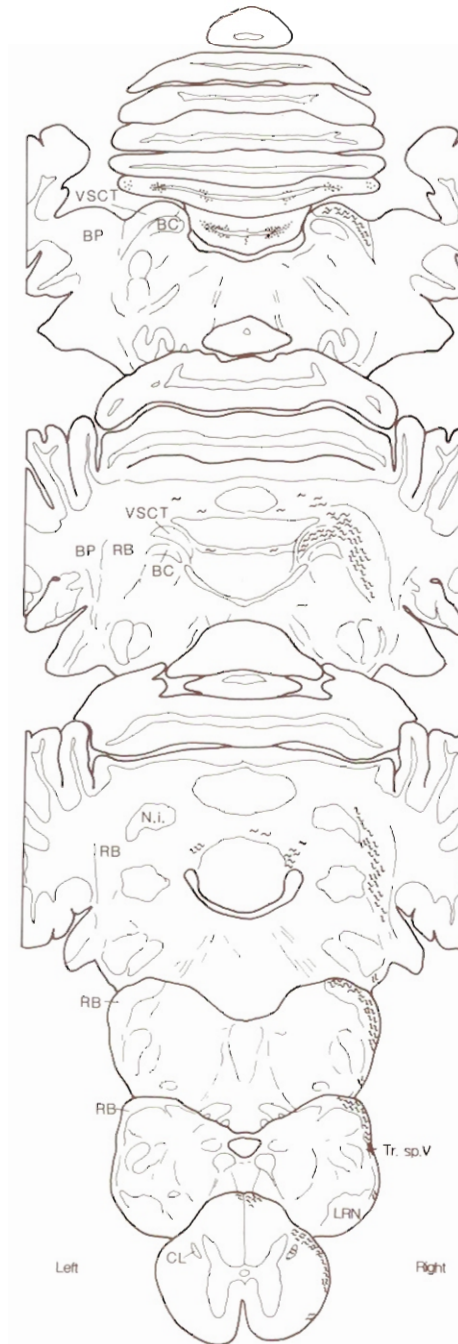


Fig. 5. - Diagrams of transverse sections from the spinal cord, brainstem, and the cerebellum of the case C209 showing distribution of labeled axons (wavy lines) and terminals (dots).

BC, brachium conjunctivum; BP, brachium pontis; CL, lateral cervical nucleus; LRN, lateral reticular nucleus; N.I., nucleus interpositus; RB, restiform body; Tr. sp. V, spinal trigeminal tract; VSCT, ventral spinocerebellar tract.

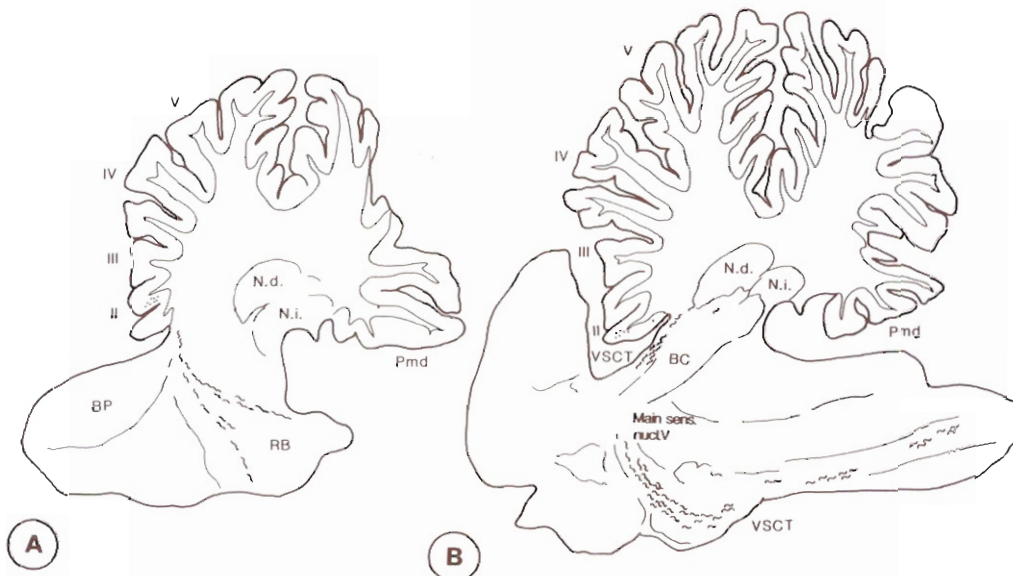


Fig. 6. - Diagrams of sagittal sections of the brainstem and cerebellum of case C214, showing labeled fibers (wavy lines) passing through the restiform body (A) and the brachium conjunctivum (B).

N.d., nucleus dentatus. For other abbreviations see legends for Figs. 3 and 5.

Degeneration experiments.

The extent of the lesions of the spinal cord in the three cats of this group are shown in figure 7. The unilateral transection of the lateral and ventral funiculi was complete in all three cases. In case C215 the contralateral ventral funiculus was also involved.

In the case with two days' survival time (C211; Table 1), groups of small argyrophilic globules were found in the granular layer of the anterior lobe (Fig. 8B, C). These groups had a clear resemblance with degenerating terminal boutons of mossy fibers, as such have been described previously from silver impregnated material (4). The number of such groups was rather small, however, and definitely

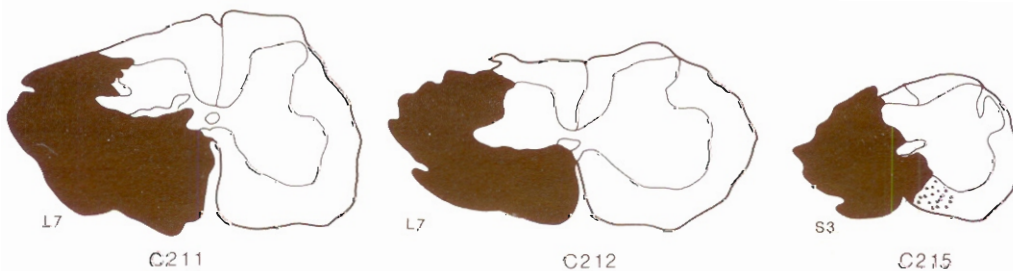


Fig. 7. - Diagrams of transverse sections of the spinal cord, showing the extent of the lesions in the three cats used for the degeneration experiments.

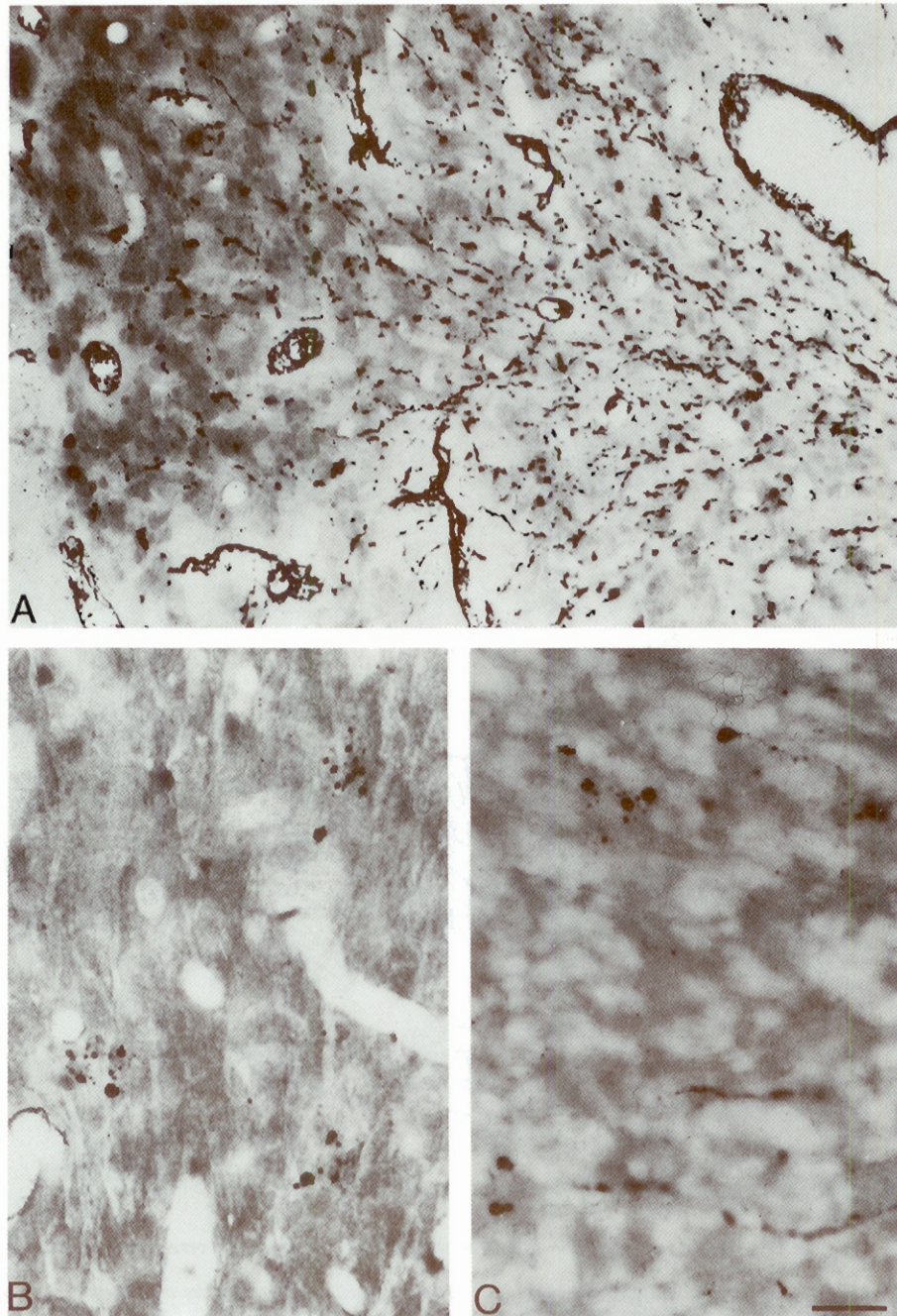


Fig. 8. - *Photomicrographs of silver impregnated sections from the cerebellar cortex.*

A shows degenerating fibers, B degenerating terminals with the Fink-Heimer method, procedure II. C shows degeneration in a cupric silver stained section. A, 5 days' survival. B and C, 2 days' survival. X275.

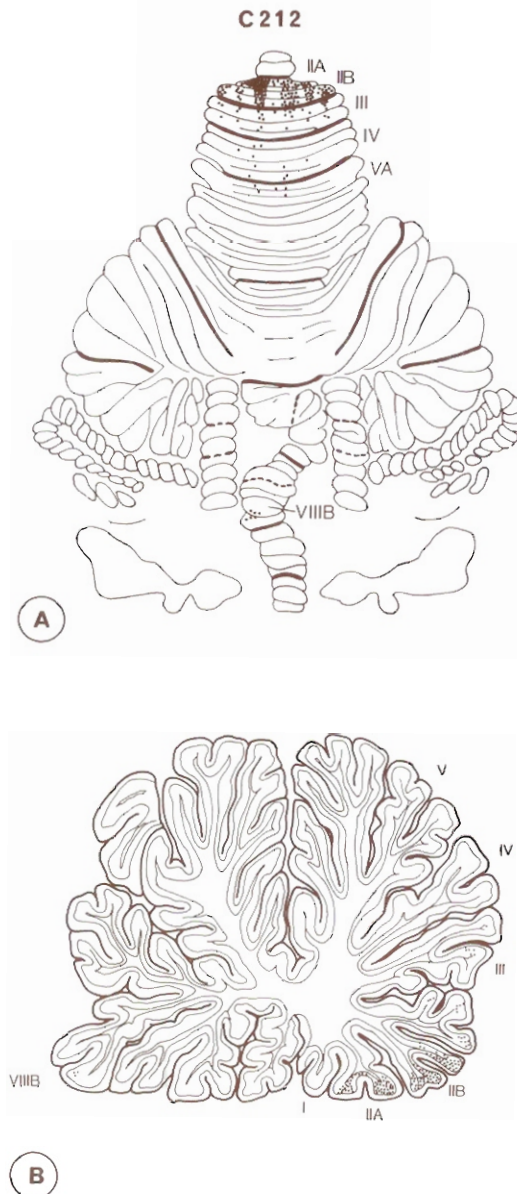


Fig. 9. – A. Distribution of degeneration fibers (dots) within the unfolded cerebellum (modified from Grant, ref. 11) of case C212.

B. Diagram of sagittal section close to the mid plane of the cerebellum, showing the distribution of degenerating fibers (dots) in the cerebellar cortex of the same case as in A.

Spacing of dots indicates relative numbers of degenerating mossy fibers in the granular layer.

smaller than the number of labeled terminals in the WGA-HRP experiments. In the cases with four and five days survival, argyrophilic disintegrating fibers could be seen in the white matter and in the granular layer (Fig. 8A). These fibers had the same general distribution in the granular layer as the labeled fibers in the WGA-HRP experiments, as will be described below.

In case C212, where the cordotomy was at the L7 level, the degenerating fibers were concentrated in lobule II, especially its sublobule IIA on the side of the cordotomy (Fig. 9A). In addition, there were some degenerating fibers in lobules III and IV and in sublobule VA. Although these fibers were found bilaterally they were most numerous on the side where the spinal lesion had been made. Some degenerating fibers were found on this side in lobule VIII B, as well. In the sagittal sections, a great part of the degenerating fibers and terminals were seen in the superficial, apical parts of the folia of lobule II (Fig. 9B). The degenerating fibers in the anterior lobe were aggregated in longitudinal zones. Such zones were easily seen in lobule II of case C215, in which the cerebellum was cut in frontal sections (Fig. 10).

Degenerating fibers were also present in the brachium conjunctivum and the restiform body. They were particularly prominent in Fink-Heimer stained sections which had been bleached and counterstained. These degenerating fibers were concentrated on the side of the spinal lesion, but some were also seen contralaterally.

C 215

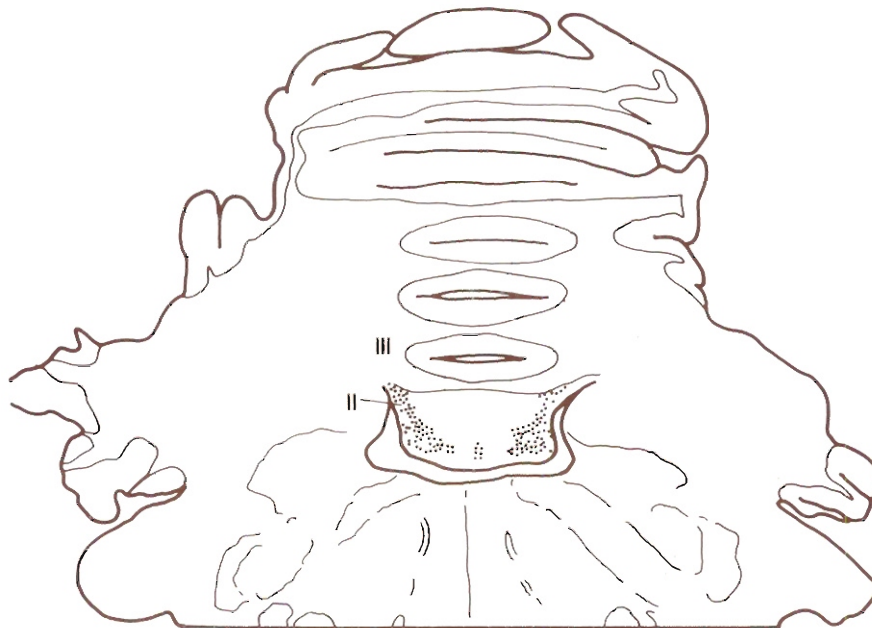


Fig. 10. — Diagram of frontal section through the anterior lobe of the cerebellum of case C215, showing degenerating fibers (dots) aggregated in longitudinal zones.

DISCUSSION

Labeled terminals from the sacro-coccygeal spinocerebellar neurons.

The WGA-HRP labeled terminals in the cerebellum, judged by their appearance, are evidently characteristic of mossy fiber terminals (35). The question is, however, from where these terminals derive. WGA-HRP could have been taken up from the injected area in the spinal cord not only by spinocerebellar cells of origin but also by axon terminals of descending systems, whose cell bodies could have axon collaterals projecting to the cerebellar cortex. The cerebellar cortex could thereby have been supplied indirectly by labeled fibers and terminals. Such a possibility was discussed in one of our previous papers, dealing with spinocerebellar projections studied by anterograde transport of WGA-HRP (35). The present findings clearly demonstrate that the same areas of cerebellar cortex that receive labeled fibers and terminals, as found in the WGA-HRP experiments, are also supplied by degenerating fibers and terminals, as found in the degeneration experiments. This is true not only with respect to the areas in terms of lobules but also with regard to the apico-basal location of the terminal ramifications and terminals within the lobules, as well as with regard to the narrow longitudinal, sagittal zones which are supplied by afferents. Furthermore, degenerating fibers are found both in the superior and the inferior cerebellar peduncles, in which also labeled fibers are found, on the expected side, in the WGA-HRP experiments. These facts support the view that at least the main part of the labeled fibers and terminals are derived from spinocerebellar fibers projecting directly from the spinal cord to the cerebellum.

Distribution of terminals in "hindlimb areas".

The labeled mossy fiber terminals in the present study were found in Larsell's (18) lobules I-IV and the rostral part of lobule V of the anterior lobe, as well as in parts of pyramis, lobule VIIIB, and the paramedian lobule, pars copularis, of the posterior lobe. These parts of the cerebellum have been considered as hindlimb regions (1, 5, 6, 8, 11, 14, 28, 31, 36). They are known to be termination areas of the dorsal and ventral spinocerebellar tracts (11, 31, 37). Later studies, in the cat, have shown that lobule I is related particularly to the neck region (see refs: 15, 16, 39-41). In an early study by one of us (11), in which silver impregnation technique was used, rather heavy degeneration was found located strictly to the anterior part of lobule II, after a hemisection at the level of the third sacral segment in the cat. In the present study, the fact that densely labeled or degenerating terminals were concentrated in the rostral folia of lobule II confirms this observation. In the recent study by Matsushita (19), in which cerebellar cortical projections from the lowest lumbar and sacro-caudal segments of the cat's spinal cord were mapped in very great detail following WGA-HRP injections,

50-80% of the total number of labeled cerebellar cortical terminals were found in lobule II.

As to the posterior lobe of the cerebellum, there are few ventral spinocerebellar fibers projecting to this area (11, 32, 33). By using retrograde transport of HRP, Matsushita and Ikeda (22) found that one group of cells, the medial part of lamina VII from L6 to the caudal segments in the sacro-coccygeal cord, projects to sublobule VIIIB but that no cell group in this caudal part of the spinal cord projects to the paramedian lobule. In the present study, however, some labeled mossy fiber terminals were observed in sublobule VIIIB and a few also in pars copularis of the paramedian lobule, following injections of WGA-HRP into the sacro-coccygeal cord. These results are in agreement with those of the recent anterograde study with WGA-HRP by Matsushita (19). Furthermore, they are compatible with findings from one of our recent studies (13), in which labeled cells were found in sacro-coccygeal segments, including not only the medial part of lamina VII but also laminae VIII-IX (the ventromedial nucleus, VM, according to Grant et al., (12)), after injections of WGA-HRP into pyramis and the paramedian lobule. Experimental evidence was given that many of these neurons send axons to the cerebellum via the inferior cerebellar peduncle (13). This suggests that part of the projection from the sacro-coccygeal cord to the posterior lobe is via the dorsal spinocerebellar tract.

Distribution of terminals in longitudinal zones.

It has been well documented that climbing fiber projections to the cerebellar cortex are longitudinally organized (32, 37, 38). With regard to the distribution of the spinocerebellar tracts, Voogd (38) described that six zones could be distinguished in the anterior lobe. He pointed out, however, that the zones, or maxima, in lobules II and III of the anterior lobe are broad and tend to fuse, which makes a separation of them difficult (38). In present study, there seem to be three, or maybe four zones distinguishable in lobules II and III. The first zone, located at the mid sagittal plane, coincides with the first zone of Voogd (38). The second zone of the present study which is broad, might correspond to the fusion of Voogd's second, third and fourth maxima of spinocerebellar terminals. As to the third zone in our material, which might in fact be two, it might be the equivalent of the fifth and sixth zones in Voogd's 1969 paper (38). In the study by Matsushita (19), in which accurate counting of terminals was carried out, only two zones were described.

Predominant termination in apical parts of folia.

It has been shown in a physiological study on the cuneocerebellar tract that superficial, apical, and deep, basal parts of the cerebellar cortex receive different

mossy fiber inputs (9). This finding has been confirmed in an anatomical study on the external and main cuneate nuclei in the same animal species (34). The results of the physiological study indicated that proprioceptive afferents influenced deep and exteroceptive afferents superficial parts.

In present study the terminals of the spinocerebellar tract neurons from the sacro-coccygeal segments were concentrated mainly in the apical parts of the folia, especially in lobule II. This was found in the WGA-HRP experiments as well as in the degeneration experiments. These findings confirm the results reported by Matsushita (19).

Fiber course through both the restiform body and the brachium conjunctivum.

It has been reported that following injections of WGA-HRP into the cervical or upper lumbar segments of the spinal cord, fine as well as coarse labeled fibers were seen running through both the restiform body and the brachium conjunctivum and continuing through the central cerebellar white matter (35). Our present observations based on WGA-HRP injections in sacro-coccygeal segments confirm these findings as far as the course of the fibers concerns. The labeled fibers passing through the restiform body and the brachium conjunctivum, as well as the central cerebellar white matter could be clearly seen both in frontal and sagittal sections. The fibers entering the cerebellum were observed to take the same course as the ventral and dorsal spinocerebellar tracts, as these have been described in detail by Voogd (37), using silver impregnation technique, in the cat. There should be no doubt, therefore, that axons of spinocerebellar tract neurons in the sacro-coccygeal segments are represented in both the ventral and the dorsal spinocerebellar tracts.

Termination in the cerebellar nuclei.

From studies where degeneration methods were used, and both light and electron microscopy, Matsushita and Ikeda (21) claimed a termination of spinocerebellar neurons in the nucleus medialis and the nucleus interpositus in the cat. Findings from the study by Robertson *et al.* (35), in which injections of WGA-HRP were made in cervical or lumbar segments in the cat, as well as the observations made in the present study, seem to be in direct confirmation of this. In our present WGA-HRP experiments, the labeled terminal-like structures were mainly concentrated on the side contralateral to the cordotomy. On basis of dorsolateral and ventrolateral spinal cord lesions, Matsushita and Ikeda (21) concluded that the degenerating terminals of the ventral spinocerebellar tract were more abundant contralaterally and that the reverse was true for those of the dorsal spinocerebellar tract. The results of the present study could therefore indicate that the sacro-

coccygeal segments of the cat spinal cord send their fibers to the central cerebellar nuclei via the dorsal spinocerebellar tract.

S U M M A R Y

The projection from the sacro-coccygeal region of the spinal cord to the cerebellum was studied by two different techniques in the cat. In five cats wheat germ agglutinin-horseradish peroxidase conjugate (WGA-HRP) was injected caudal to a preceding unilateral cordotomy at the sacral level, aimed at interrupting the spinocerebellar tracts on one side completely, and the distribution of WGA-HRP labeled mossy fibers and mossy fiber terminals was studied in the cerebellum. In three additional cats, degenerating fibers were examined in Fink-Heimer stained sections following unilateral transection of the lateral and ventral funiculi at L7 or S3 level.

In the WGA-HRP experiments the labeled mossy fiber terminals were located bilaterally in lobules I-V. Most of them were found in the anterior part of lobule II. In addition, labeled terminals were observed in sublobule VIIIB and in pars copularis of the paramedian lobule, contralateral to the cordotomy. The terminals in the anterior lobe were concentrated in longitudinal zones parallel to the mid sagittal plane. In lobule II, the terminals were most abundant in the superficial, apical parts of the folia. Some presumed terminals were also seen in the cerebellar nuclei. Labeled fibers were found contralateral, but not ipsilateral to the cordotomy in the superior and inferior cerebellar peduncles, as well as in the spinal cord rostral to the cordotomy.

The results of the degeneration experiments were the same as those of the WGA-HRP experiments with regard to the detailed projections in the cerebellar cortex. This is strong support against the possibility that WGA-HRP labeled cerebellar mossy fiber terminals, following WGA-HRP injections in the spinal cord, would represent terminals of collaterals of retrogradely labeled neurons. It also lends strong support in favour of WGA-HRP as a reliable anterograde tracer for studying cerebellar cortical projections of spinocerebellar neurons in the cat.

Acknowledgements. — Expert technical assistance by Mrs Brita Robertson is gratefully acknowledged. The project was supported by the Swedish Medical Research Council, project no 553.

R E F E R E N C E S

1. ADRIAN, E.D. Afferent areas in the cerebellum connected with the limbs. *Brain*, **66**: 289-315, 1943.
2. ALHEID, G.F., EDWARDS, S.B., KITAI, S.T., PARK, M.R. and SWITZER, R.C. 111 Methods for delivering tracers. Pp. 91-116, In: HEIMER L., and ROBARDS M.J., (Eds.), *Neuroanatomical Tract-Tracing Methods*. New York and London, Plenum Press, 1981.
3. BOIVIE, J., GRANT, G. and ULFENDAHL, H. The X-Y recorder used for mapping under the microscope. *Acta physiol. scand.*, **74**: 1A-2A, 1968.

4. 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**: 67-87, 1962.
5. CHAMBERS W.W. and SPRAGUE, J.M. Funcional localization in the cerebellum. I. Organization in longitudinal corticonuclear zones and their contribution to the control of posture, both extrapyramidal and pyramidal. *J. comp. Neurol.*, **103**: 105-130, 1955.
6. COMBS, C.M. Electro-anatomical study of cerebellar localization. Stimulation of various afferents. *J. Neurophysiol.*, **17**: 123-143, 1954.
7. DE OLMOS, J.S. and INGRAM, W.R. An improved cupric-silver method for impregnation and terminal degeneration. *Brain Res.*, **33**: 523-529, 1972.
8. DOW, R.S. and ANDERSSON, R. Cerebellar action potentials in response to stimulation of proprioceptors and extrareceptors in the rat. *J. Neurophysiol.*, **5**: 363-371, 1942.
9. EKEROT, C.F. and LARSON, B. Differential termination of the exteroceptive and proprioceptive components of the cuneocerebellar tract. *Brain Res.*, **36**: 420-424, 1972.
10. FINK, P. and HEIMER, L. Two methods for selective silver impregnation of degenerating axons and their synaptic endings in the central nervous system. *Brain Res.*, **4**: 369-374, 1967.
11. GRANT, G. Spinal course and somatotopically localized termination of the spinocerebellar tracts. An experimental study in the cat. *Acta physiol. scand.*, **56**, suppl. 193: 1-45, 1962.
12. GRANT, G., WIKSTEN, B., BERKLEY, K.J. and ALDSKOGIUS, H. The location of cerebellar-projecting neurons within the lumbosacral spinal cord in the cat. An anatomical study with HRP and retrograde chromatolysis. *J. comp. Neurol.*, **204**: 336-348, 1982.
13. GRANT G. and XU, Q. Routes of entry into the cerebellum of spinocerebellar axons from the lower part of the spinal cord. An experimental anatomical study in the cat. *Exp. Brain Res.*, **72**: 543-561, 1988.
14. GRUNDFEST, H. and CAMPBELL, B. Origin, conduction, and termination of impulses in the dorsal spino-cerebellar tracts. *J. Neurophysiol.*, **5**: 275-294, 1942.
15. HIRAI, N., HONGO, T. and YAMAGUCHI, T. Spinocerebellar tract neurones with descending axon collaterals. *Brain Res.*, **142**: 147-151, 1978.
16. HIRAI, N., HONGO, T. and SASAKI, S. A physiological study of identification, axonal course and cerebellar projection of spinocerebellar tract cells in the central cervical nucleus of the cat. *Exp. Brain Res.*, **55**: 272-285, 1984.
17. JOS IKKEMA-PAASSEN, P.O.G. and GRAMSBERGEN A. Bleaching and counterstaining of Fink-Heimer stained section. *Stain Technol.*, **55**: 111-113, 1980.
18. LARSELL, O. The cerebellum of the cat and the monkey. *J. comp. Neurol.*, **99**: 135-200, 1953.
19. MATSUSHITA, M. Spinocerebellar projections from the lowest lumbar and sacro-caudal segments in the cat, as studied by anterograde transport of wheat germ agglutinin-horseradish peroxidase. *J. comp. Neurol.*, **274**: 239-154, 1988.
20. MATSUSHITA, M., HOSOYA, Y. and IKEDA, M. Anatomical organization of the spinocerebellar system in the cat, as studied by retrograde transport of horseradish peroxidase. *J. comp. Neurol.*, **184**: 81-106, 1979.
21. MATSUSHITA, M. and IKEDA, M. Spinal projections to the cerebellar nuclei in the cat. *Exp. Brain Res.*, **10**: 501-511, 1970.
22. MATSUSHITA, M. and IKEDA, M. Spinocerebellar projections to the vermis of the posterior lobe and the paramedian lobule in the cat, as studied by retrograde transport of horseradish peroxidase. *J. comp. Neurol.*, **192**: 143-162, 1980.
23. MATSUSHITA, M. and IKEDA, M. Spinocerebellar projections from the cervical enlargement in the cat, as studied by anterograde transport of wheat germ agglutinin-horseradish peroxidase. *J. comp. Neurol.*, **263**: 223-240, 1987.
24. MATSUSHITA, M. and TANAMI, T. Spinocerebellar projections from the central cervical

- nucleus in the cat, as studied by anterograde transport of wheat germ agglutinin-horseradish peroxidase. *J. comp. Neurol.*, **266**: 376-397, 1987.
25. MATSUSHITA, M., TANAMI, T. and YAGINUMA, H. Differential distribution of spinocerebellar fiber terminals within the lobules of the cerebellar anterior lobe in the cat: an anterograde WGA-HRP study. *Brain Res.*, **305**: 157-161, 1984.
 26. MATSUSHITA, M. and YAGINUMA, H. Spinocerebellar projections from spinal border cells in the cat as studied by anterograde transport of wheat germ agglutinin-horseradish peroxidase. *J. comp. Neurol.*, **288**: 19-38, 1989.
 27. MESULAM, M.M. Tetramethyl benzidine for horseradish peroxidase neurohistochemistry: a non-carcinogenic blue reaction product with superior sensitivity for visualizing neural afferents and efferents. *J. Histochem. Cytochem.*, **26**: 106-117, 1978.
 28. MORIN, F. and LINDNER, D. Pathways for conduction of tactile impulses to the paramedian lobule of the cerebellum of the cat. *Amer. J. Physiol.*, **175**: 247-250, 1953.
 29. OKADO, N., YOSHIMOTO, M. and FURBER, S.E. Pathway formation and the terminal distribution pattern of the spinocerebellar projection in the chick embryo. *Anat. Embryol.*, **176**: 165-174, 1987.
 30. OKADO, N., ITO, R. and HOMMA, S. The terminal distribution pattern of spinocerebellar fibers. An anterograde labelling study in the posthatching chick. *Anat. Embryol.*, **176**: 175-182, 1987.
 31. OSCARSSON, O. Functional organization of the spino- and cuneocerebellar tracts. *Physiol. Rev.*, **45**: 495-522, 1965.
 32. OSCARSSON, O. Functional organization of spincerebellar paths. Pp. 338-380. In: IGGO, A. (Ed.), *Handbook of Sensory Physiology*, Vol. II, *Somatosensory System*. Berlin, Heidelberg, New York, Springer Verlag, 1973.
 33. OSCARSSON, O. and UDDENBERG, N. Identification of a spinocerebellar tract activated from forelimb afferents in the cat. *Acta physiol. scand.*, **62**: 125-136, 1964.
 34. RINVIK, E. and WALBERG, F. Studies on the cerebellar projections from the main and external cuneate nuclei in the cat by means of retrograde axonal transport of horseradish peroxidase. *Brain Res.*, **95**: 371-381, 1975.
 35. ROBERTSON, B., GRANT, G. and BJÖRKELAND, M. Demonstration of spinocerebellar projections in cat using anterograde transport of WGA-HRP with some observations on spinomesencephalic and spinothalamic projections. *Exp. Brain Res.*, **52**: 99-104, 1983.
 36. SNIDER, R.S. and STOWELL, A. Receiving areas of the tactile, auditory and visual systems in the cerebellum. *J. Neurophysiol.*, **7**: 331-357, 1944.
 37. VOOGD, J. *The Cerebellum of the Cat*. Assen, van Gorcum and Comp. N.V., 215 pp., 1964.
 38. VOOGD, J. The importance of fiber connections in the comparative anatomy of the mammalian cerebellum. Pp. 493-514. In: LLINAS, R. (Ed.), *Neurobiology of Cerebellar Evolution and Development*. Chicago, Amer. Med. Assoc., 1969.
 39. WIKSTEN, B. The central cervical nucleus — a source of spinocerebellar fibers, demonstrated by retrograde transport of horseradish peroxidase. *Neurosci. Lett.*, **1**: 81-84, 1975.
 40. WIKSTEN, B. The central cervical nucleus in the cat. II. The cerebellar connections studied with retrograde transport of horseradish peroxidase. *Exp. Brain Res.*, **36**: 155-173, 1979.
 41. WIKSTEN, B. The central cervical nucleus in the cat. III. The cerebellar connections studied with anterograde transport of ³H Leucin. *Exp. Brain Res.*, **36**: 175-189, 1979.
 42. WIKSTEN, B. Further studies on the fiber connections of the central cervical nucleus in the cat. *Exp. Brain Res.*, **67**: 284-290, 1987.
 43. YAGINUMA, H. and MATSUSHITA, M. The projection fields of spinal border cells in the cerebellar anterior lobe in the cat: an anterograde WGA-HRP study. *Brain Res.*, **384**: 175-179, 1986.
 44. YAGINUMA, H. and MATSUSHITA, M. Spinocerebellar projection fields in the horizontal

- plane of lobules of the cerebellar anterior lobe in the cat: an anterograde wheat germ agglutinin-horseradish peroxidase study. *Brain Res.*, **365**: 345-349, 1986.
45. YAGINUMA, H. and MATSUSHITA, M. Spinocerebellar projections from the thoracic cord in the cat as studied by anterograde transport of wheat germ agglutinin-horseradish peroxidase. *J. comp. Neurol.*, **258**: 1-27, 1987.
 46. YAGINUMA, H. and MATSUSHITA, M. Spinocerebellar projections from the upper lumbar segments in the cat as studied by anterograde transport of wheat germ agglutinin-horseradish peroxidase. *J. comp. Neurol.*, **281**: 298-319, 1989.