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The Cerebellar Corticonuclear and Nucleocortical Projections in the Cat as Studied with Anterograde and Retrograde Transport of Horseradish Peroxidase

II. Lobulus simplex, Crus I and II

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Summary. The cerebellar corticonuclear and nucleocortical connections of lobulus simplex, crus I and II in the cat were studied by means of anterograde and retrograde transport of HRP. Previous experimental studies give evidence that the cortex of the cerebellar hemisphere in a lateromedial direction can be subdivided into five longitudinal zones. These are recognized as zones D_2, D_1, C_3, C_2 and C_1. Our observations indicate that each cortical zone has its own field of termination in the cerebellar nuclei, and that these nuclear fields are similar to those receiving afferents from the corresponding zones within the paramedian lobule (Dietrichs and Walberg, 1979).

The Purkinje axons from each folium terminate from medial to lateral along a continuous band which loops through the cerebellar nuclei from the ventromedial part of nucleus interpositus posterior to the dorsolateral part of the same nucleus, from where it proceeds into the lateral part of nucleus interpositus anterior and the transition area between nucleus interpositus anterior and the dentate nucleus, to end within the latter. In addition to this arrangement there is a rostrocaudal organization within the hemispheral cortex so that the nuclear bands receiving Purkinje axons from the rostral folia (lobulus simplex) are situated slightly ventral to those receiving terminal fibres from the middle folia (crus I), which again are situated ventral to the terminal bands for the caudal folia (crus II).

The nucleocortical projection shows largely the same zonal arrangement as the corticonuclear, but labelled nuclear neurons are in some cases found bilaterally within the fastigial nucleus. This nucleus does not receive Purkinje axons from lobulus simplex, crus I and crus II.

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The findings are discussed with reference to previous investigations on the cerebellar corticonuclear and nucleocortical connections, and some comments are made concerning the use of HRP as an anterograde tracer.

Key words: Cerebellar corticonuclear projection – Cerebellar nucleocortical projection – Anterograde transport of HRP – Retrograde transport of HRP – Cerebellar hemispheres.

Introduction

Several previous anatomical and physiological studies have given evidence that the cerebellar cortex can be divided into a number of narrow longitudinal zones (see Dietrichs and Walberg 1979 for references to the literature), and that as concerns the cerebellar hemispheres, five zones may be distinguished. Each zone appears to extend rostrocaudally along the entire length of lobulus simplex, crus I and II, and in a lateromedial direction these five zones are recognized as D_2, D_1, C_3, C_2 and C_1. (See especially Brodal and Kawamura, in press; Voogd and Bigaré, in press). Furthermore, there is also a zonally arranged cerebellar corticonuclear connection, so that each cortical zone sends its Purkinje axons to the corresponding zone within the cerebellar nuclei (see especially Voogd 1964, 1969; Voogd and Bigaré, in press; Oscarsson 1979). Certain observations on the corticonuclear projection from the paramedian lobule in addition indicate that the fibers from this cortical lobule terminate along a continous mediolateral band which loops through the cerebellar nuclei (CN) (Dietrichs and Walberg 1979).

In their degeneration study, Brodal and Courville (1973) showed a precisely organized somatotopical pattern within the corticonuclear projection from crus II, but these authors were not able to demonstrate a corticonuclear longitudinal zonal arrangement. We therefore found it of interest to reinvestigate the corticonuclear projection from the simple and ansiform lobules with the new method of anterograde transport of horseradish peroxidase (HRP). This method can with advantage be used to demonstrate even a sparse fibre connection within the cerebellum of the cat (Dietrichs and Walberg 1979), especially when the sections are treated according to the very sensitive Mesulam (1978) technique (for a comparison of the currently used techniques, see Mesulam and Rosene 1979). Furthermore, since HRP can be used as a retrograde as well as an anterograde tracer, studies of afferent as well as of efferent cerebellar cortical connections can be made in the same animal.

A cerebellar corticonuclear-nucleocortical reciprocity has recently been demonstrated by several authors (Tolbert et al. 1976; Haines 1978; Gould 1979; Haines and Pearson 1979; Tolbert and Bantli 1979; Dietrichs and Walberg 1979). However, a detailed study of the reciprocity between the simple and ansiform lobule and the cerebellar nuclei has not been performed. In the present study, where HRP has been used as an anterograde and retrograde tracer, new details concerning the organization within the corticonuclear and nucleocortical connections of the simple and ansiform lobules have been obtained.

Table 1. Table summarizing the number, weight and survival time for all animals, and the type, concentration, added agents and injected amount/ejection time for the HRP used

A. Pressure injections

Cat	Weight kg	HRP				Survival time days
		Type	Conc.	Amount	Added agents	
C.Co.L.197	3.8	Sigma VIP	50% w/v	0.25		2
B.St.L.635	3.5	Sigma VIP	50% w/v	0.5		2
B.St.L.666	3.4	Sigma VIP	50% w/v	0.5		2
B.St.L.751	1.8	Sigma VIP	50% w/v	0.1		1
B.St.L.779	2.5	Serva	25% w/v	0.07		2
B.St.L.784	3.3	Serva	25% w/v	0.2	DMSO	1
B.St.L.789L	4.0	Serva	25% w/v	0.1		2
B.St.L.791	2.2	Serva	25% w/v	0.15		2
B.St.L.825	1.5	Serva	25% w/v	0.2		2
B.St.L.827	2.1	Serva	25% w/v	0.1		2
B.St.L.828	2.8	Serva	25% w/v	0.07		2
B.St.L.831	2.0	Serva	25% w/v	0.1		2
B.St.L.832	1.4	Serva	25% w/v	0.15		1
B.St.L.835	1.3	Serva	25% w/v	1.3		1

B. Iontophoretical ejections

Cat	Weight kg	HRP			Ejection		Survival time days
		Type	Conc.	Added agents	Current uamp	Time min.	
B.St.L.830	4.5	Serva	5% w/v		5	10	2
B.St.L.869	2.5	Serva	4% w/v	Ornitine	5	2	3
B.St.L.870	2.1	Serva	4% w/v	Ornitine	5	$3^1/_2$	3
B.St.L.878	3.5	Serva	4% w/v	Ornitine	5	3	2
B.St.L.879	2.8	Serva	4% w/v	Ornitine	5	5	4

Material and Methods

Altogether nineteen cats were used in this study. The operations were performed under Mebumal or Nembuthal anaesthesia, and various amounts of HRP were injected into lobulus simplex, crus I and II. In some cats poly-L-ornitine, 50 µg/ml (Itaya et al. 1978), or dimethyl sulfoxide (DMSO), 2% (Keefer et al. 1976), was added to the HRP. The delivery was performed either iontophoretically or by pressure. Details concerning the injection, the type, amount and concentration of HRP used, and the weight and survival times of the animals, are found in Table 1. The cats were killed under deep Mebumal or Nembuthal anaesthesia by intracardiac perfusion with 0.1 M phosphate buffer at pH 7.4, followed by 0.25% glutaraldehyde and 1% paraformaldehyde in phosphate buffer [1] and finally with 10% sucrose. The cerebellum and the brain stem were immediately isolated and removed, and were placed in a 30% sucrose solution for about 24 hours at 4° C. The cerebellum was cut sagittally or frontally, and the brain stem transversally into serial sections at 50 µm.

[1] Some cats (B.St.L. 635, 666, 751, 779, 784, 789L, 791 and C.Co.L 197) were perfused with a solution containing 0.4% formaldehyde and 1.25% glutaraldehyde in phosphate buffer

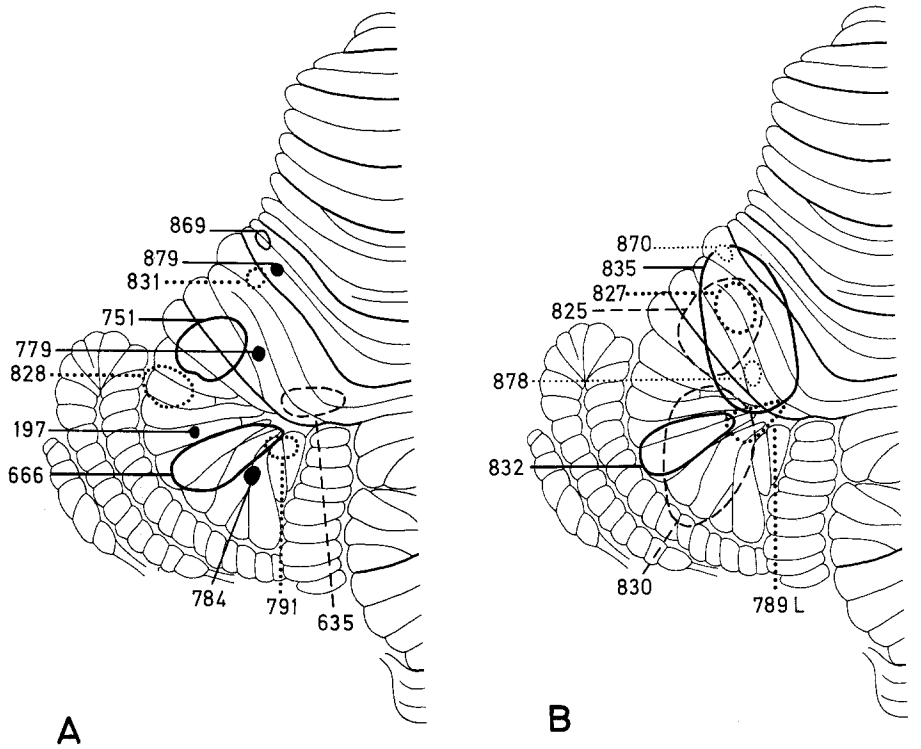

Fig. 1. Diagrams of the cerebellar surface of the cat (from Larsell 1970) showing the extent of the cortical staining in all cases presented in this study. Note that labelled Purkinje axons in some cases descended from a small part of the cortically stained area only (see Table 2). For the sake of convenience cases injected on the right side have been transferred to the left in these diagrams

Two out of five consecutive sections were selected and mounted. One series was left unstained, the other was weakly stained with neutral red, thionin or cresyl violet. Eleven cats were treated with tetramethylbenzidine (TMB) as the chromogen, as described by Mesulam (1978). In these 3.5 ml 0.3% H_2O_2 per 100 ml of medium was used for incubation. The remaining eight cats were processed with diaminobenzidine (DAB) according to the Graham and Karnovsky (1966) method. All these cats (B.St.L. 635, 666, 751, 779, 784, 789L, 791 and C.Co.L.197) have been used in previous studies made in our laboratory (see Pierce et al. 1977; Kotchabhakdi et al. 1978).

Several uninjected brains were included as control cases. They were treated according to the same procedures as used for the experimental material. No endogenous peroxidatic activity was found in the cerebellum of these cats.

The sections from the experimental material and the control cases were studied with bright-field and interference contrast microscopy. The location and extent of cortical staining at the injection site were carefully transferred to a standard diagram of the cerebellar cortex (Larsell 1970; see Fig. 1), and the precise location of anterogradely filled preterminal and terminal[2] Purkinje axons and retrogradely labelled nuclear neurons was mapped in drawings taken at equal intervals through the CN. These were later transferred to standard diagrams to enable a comparison of the different cases.

[2] The term "terminal" is used for the plexus of thin fibers found within localized parts of the cerebellar nuclei. Thicker fibres which branch inside the nuclei are considered "preterminal" (see Fig. 8C and Dietrichs and Walberg 1979)

When mapping anterogradely labelled fibres, special care was taken not to include artificial deposits of blue or brown reaction product in cells lining capillaries.[3] These deposits may mimic anterogradely labelled axons in sections where capillaries are cut longitudinally (see Dietrichs and Walberg 1979). The readers should consult the same paper for a detailed discussion of other methodological difficulties and for a description of the nomenclature used for our delimitation of the CN.

For the sake of convenience, in this study all folia in the cerebellar hemispheres will be considered to have a largely mediolateral orientation. The D_2 zone of each folium thus represents its lateral margin. Likewise, when the cerebellar surface is imagined unfolded, the upper parts of the diagram will be referred to as rostral (see Figs. 1 and 7).

Results

After the injections into lobulus simplex, crus I and II, anterogradely labelled Purkinje axons could in most cases be followed along their entire route from the injection site to their terminations within the CN. In some of the animals the anterogradely labelled fibres originated only from a narrow strip of the stained cortical area, presumably from one or two zones only. In others, the labelled fibres took their origin from the greater part of the stained cortical area. This observation is in keeping with our findings concerning the origin of the nuclear afferents after injections into the paramedian lobule (Dietrichs and Walberg 1979).

For our identification of the cortical zones we have put particular emphasis on the results of the HRP studies on the olivocerebellar projection performed in this laboratory, where the width of the HRP injections in the hemisphere was estimated from cases where the distribution of labelled cells was restricted to what is considered to be corresponding zones within the olivary complex (see especially Kotchabhakdi et al. 1978). Furthermore, in previously not used cases we have mapped the distribution of retrogradely labelled olivary cells, thereby permitting a tentative delimitation of the width of the cortical longitudinal zones to which the injected HRP was delivered. Table 2 gives the extent of the total cortical staining of the cortical zones, and the cortical origin and nuclear termination of the anterogradely labelled Purkinje axons. In addition, the table shows the nuclear zones of origin of the retrogradely labelled nucleocortical neurons in each case. The reader should consult this table concerning details for all experimental animals.

Anterogradely labelled Purkinje axons could be followed along their entire route. However, it should be emphasized that in certain cats some of the stained fibres could be traced only into the first part of the white matter. In other cases axons could be followed through the white matter into the nuclei where the preterminal fibres got lost. No terminal plexus was visible in these cases. Usually, such unfilled fibres took their origin from the peripheral parts of the cortically injected area (see Discussion, Methodological comments, for further details).

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[3] In our previous paper (Dietrichs and Walberg 1979) such artifacts were only observed in TMB activated sections. In the present study even some of our DAB activated cases showed deposits of brown reaction product in capillary walls

Table 2. Table summarizing the findings made in all cases used in this study. Column A shows the total HRP stained cortical area, column B gives the cortical zones from which anterogradely labelled Purkinje axons take their origin, column C lists the nuclear zones receiving terminal and preterminal fibres in each cat, and column D gives the nuclear zones where labelled nucleocortical neurons were located. Numbers and letters in square brackets are preterminal fibres, round brackets give weak connections. – in column D refers to DAB activated cases with no or only faintly labelled nucleocortical neurons not included in this study

Cat	A		B	C	D
	Cortical staining		Cortical origin of descending axons zones	Nuclear terminal zones for Purkinje axons	Nuclear zones of origin of cortical afferents
	Area	Zones			
B.St.L.635	Cr. I	A	A	NM	–
B.St.L.666	Cr. II	$D_2 \rightarrow C_1$	$D_2 \rightarrow D_1$	$D_2(D_1)$	D_2
B.St.L.751	Cr. I+II	D_1	D_1	$[D_2]D_1$	–
B.St.L.779	Cr. I	C_2	C_2	$[D_2, D_1]C_2$	–
B.St.L.784	Cr. II	$D_1 \rightarrow C_2$	$D_1(C_2)$	$D_1(D_2)$	D_1
B.St.L.789L	Cr. II	$D_1 \rightarrow C_2$	$D_1 \rightarrow C_2$	$[D_2]D_1 \rightarrow C_2[C_1]$	$D_2 \rightarrow C_2$
B.St.L.791	Cr. II	$C_3 \rightarrow C_2$	$(C_3)C_2$	$(C_3)C_2$	–
B.St.L.825	Cr. I(II)	$D_2 \rightarrow C_2$	$D_2 \rightarrow D_1$	$D_2 \rightarrow D_1$	$D_2 \rightarrow D_1$ + NM
B.St.L.827	Cr. I	$D_2 \rightarrow D_1$	$D_2 \rightarrow D_1$	$D_2 \rightarrow D_1$	$D_2 \rightarrow D_1$ + NM
B.St.L.828	Cr. II	D_2	D_2	D_2	no cells
B.St.L.830	Cr. II	$D_2 \rightarrow C_1$	$D_1 \rightarrow C_2$	$D_1 \rightarrow C_2$	$D_1 \rightarrow C_2$
B.St.L.831	Cr. I	D_1	D_1	$[D_2]D_1$	D_1
B.St.L.832	Cr. II	$D_2 \rightarrow C_1$	$D_2 \rightarrow C_1$	$D_2 \rightarrow C_1$	$D_2 \rightarrow C_1$
B.St.L.835	Cr. I	$D_2 \rightarrow C_1$	$D_2 \rightarrow C_2$	$D_2 \rightarrow C_2$	$D_2 \rightarrow C_1$ + NM
B.St.L.869	Simplex	D_2	D_2	D_2	no cells
B.St.L.870	Cr. I	D_2	D_2	D_2	no cells
B.St.L.878	Cr. I	$C_2 \rightarrow C_1$	$(C_2)C_1$	$(C_2)C_1$	C_2
B.St.L.879	Simplex	D_1	D_1	D_1	no cells
C.Co.L.197	Cr. II	D_1	D_1	$[D_2]D_1$	–

Five main terminal areas are found in our material: One in the lateral part of the lateral (dentate) nucleus (NL), another in the transition area between NL and nucleus interpositus anterior (the NL-NIA transition area),[4] a third in the medial part of the NL-NIA transition area and the lateral part of NIA, a fourth in the dorsolateral part of nucleus interpositus posterior (NIP), and a fifth in the ventromedial NIP. As will be evident from the results presented below, however, no well defined borders can be drawn between these regions of corticonuclear termination. In many of our cases, the anterogradely visualized termination area extends from one of these regions into the neighbouring one. Our small injections gave rise to fibres within only one or two of these terminal fields, whereas the largest injections had terminations within all five regions.

[4] For details concerning the delimitation of the cerebellar nuclei in the cat, see Courville and Brodal (1966)

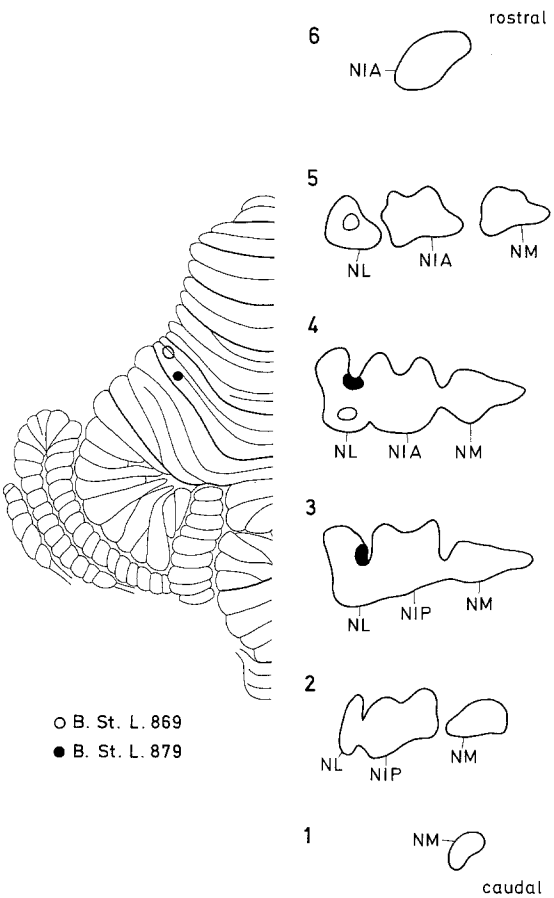

Fig. 2. Diagrams showing the injection sites in cats B.St.L. 869 and 879 and the distribution of anterogradely labelled terminal Purkinje axons in the cerebellar nuclei, as seen in two series of six equally spaced frontal sections. *NIA* nucleus interpositus anterior; *NIP* nucleus interpositus posterior; *NL* nucleus lateralis (dentatus); *NL-NIA*, transition area of nucleus lateralis (dentatus) and nucleus interpositus anterior; *NM* nucleus fastigii

Retrogradely labelled neurons were found in most of our cases, and were usually located among the anterogradely filled, corticonuclear terminal fibres. In addition, retrogradely labelled cells occurred bilaterally within nucleus fastigii (NM) in some cats.

In the presentation given below, the experimental material has been divided in three groups, those with injections in (1) lobulus simplex, (2) in crus I, and (3) in crus II.

Injections in Lobulus simplex

The cortical staining was restricted to lobulus simplex in two cats. B.St.L. 869 (Figs. 1A and 2) had a small injection which covered the lateral part,

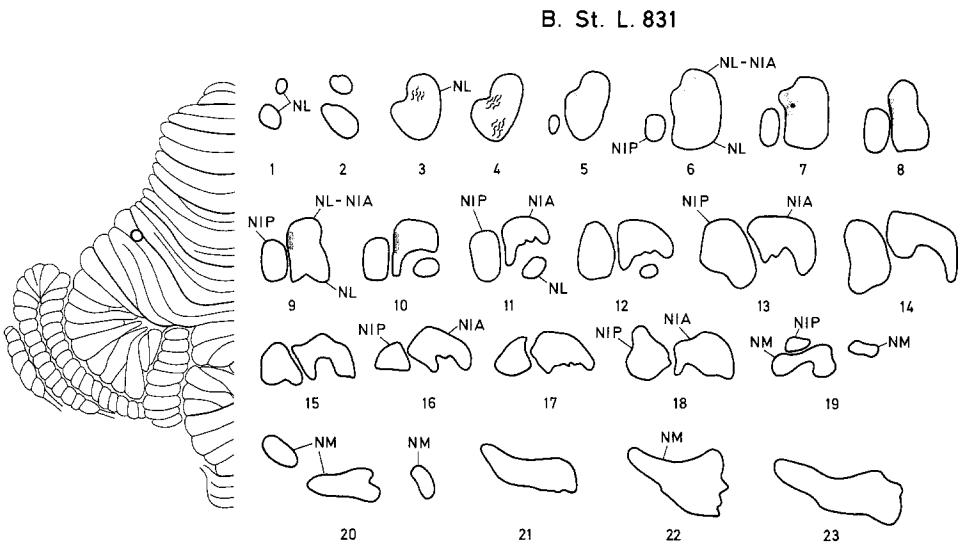

Fig. 3. Diagram showing the injection site in cat B.St.L. 831 and the distribution of HRP labelled preterminal (*wavy lines*) and terminal (*hatched area*) Purkinje fibres and retrogradely labelled nucleocortical neurons (*dots*) in the cerebellar nuclei, as seen in a series of 23 equally spaced sagittal sections (one dot in this and the following figures does not represent one labelled cell). For abbreviations, see Fig. 2. The nuclear diagrams in this figure and Figs. 5 and 6 are modified from Brodal and Courville (1973)

presumably the D_2 zone, of two folia. Anterogradely labelled Purkinje axons could be followed from the injection site into a dense terminal plexus in the rostral NL. This plexus extended a short distance caudally and ventrally. Cat B.St.L. 879 (Figs. 1A og 2) had a small injection just medial to that of the former case, in what was considered to be the D_1 zone. Terminal fibres were located dorsomedially in NL and the NL-NIA transition area, but at a more caudal level than in B.St.L. 869.

Injections in Crus I

Our findings from lateral injections in crus I correspond with the observations in our two cases with deposits in lobulus simplex. When anterogradely labelled corticonuclear fibres only emanate from what appears to be the D_2 zone, like in cat B.St.L. 870 (Fig. 1B), the terminal area extends somewhat medially, caudally and ventrally from the rostrodorsal pole of NL. The projection from the slightly more medially situated region, probably the D_1 zone, however, ends in the NL-NIA transition area. This is learnt from two other cats, B.St.L. 751 and 831 (Figs. 1A and 3), where the terminal fibres are situated in the dorsal half of this nuclear region. The latter cat had also some retrogradely labelled cells within the terminal corticonuclear plexus. Both these cats had in addition a few faintly labelled preterminal fibres which got lost more laterally and ventrally within the NL (see Fig. 3). Also in cats B.St.L. 825 and 827

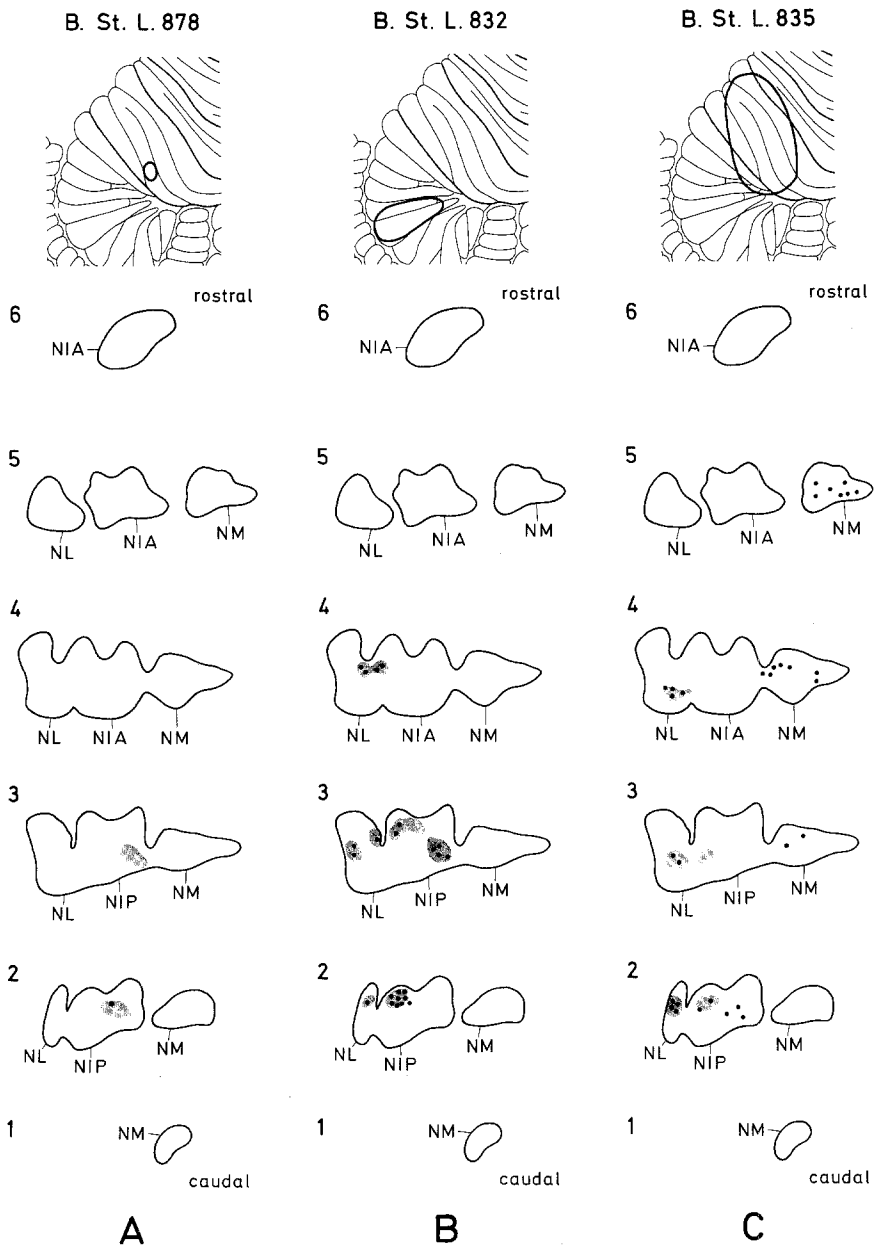

Fig. 4. Diagrams showing the injection sites in cats B.St.L. 878, 832 and 835 and the distribution of terminal Purkinje axons (*hatched areas*) and retrogradely labelled nucleocortical neurons (*dots*) in the cerebellar nuclei, as seen in three series of six equally spaced frontal sections. For abbreviations, see Fig. 2

(Fig. 1B) where anterogradely stained Purkinje axons descended from the supposed zones D_1 (the majority) and D_2, were anterogradely labelled terminal fibres found only in NL and the NL-NIA transition area, but the termination in the latter cat, which was injected more medially, extended dorsally and medially to that of the former. Both these cats had many retrogradely labelled cells among the terminal fibres, and bilaterally within NM.

A somewhat different distribution of anterogradely labelled terminal fibres was found in cat B.St.L. 779 (Fig. 1A), with a small injection medial to those of the cases described above, presumably in the C_2 zone. Only few terminal fibres occured in this cat, and these were located within the dorsolateral NIP. A number of stained preterminal fibres, however, was traced into the NL and the dorsal part of the NL-NIA transition area.

Also in cat B.St.L 878 (Figs. 1B and 4A) were terminal Purkinje axons found restricted to NIP. The cortical staining in this cat covered a small part of what is believed to be zones C_2 and C_1, and the termination area extended ventrally and medially from the middle part of NIP. A few retrogradely labelled neurons were found in the dorsal part of the terminal field.

Our results from the cases presented above were supported by the findings in cat B.St.L. 835 (Figs. 1B and 4C), where the cortical staining covered most of crus I, including all cortical zones (D_2 through C_1). The anterogradely labelled Purkinje axons in this cat emanated from all zones except the presumed C_1, and they terminated along a continuous mediolateral band, which from its origin in the dorsocaudal NL looped rostrally into the ventral NL and then turned caudally and dorsally through the NL-NIA transition area before entering the dorsolateral NIP, where it ended. The terminal plexus was most dense in the NL. Retrogradely labelled neurons occurred among the terminal fibres along the entire mediolateral band, and in the ventromedial part of the ipsilateral NIP. Many retrogradely labelled cells of all sizes were in addition found bilaterally within NM.

Our findings from cat B.St.L. 635 (Fig. 1A) were unexpected. In this case, with an injection in the medialmost part of crus I, just rostral to the first folium of the paramedian lobule, anterogradely filled fibres could be followed into the caudolateral part of NM. The terminal arborization of the Purkinje axons, however, was not visualized in this case.

Injections in Crus II

Our observations after injections in crus II are similar to those made in the previous cases. When the stained Purkinje axons emanate from the lateralmost part of the folia, from what is considered to be the D_2 zone, as in cat B.St.L. 828 (Fig. 1A), terminal fibres occurred dorsally in the lateral NL. Termination was found in this region also in cat B.St.L. 666 (Figs. 1A and 5), but in this cat the terminal plexus extended into the NL-NIA transition area. The cortical staining covered the entire mediolateral extent of crus II, but the anterogradely labelled Purkinje axons took their origin only from the lateral part of the cortically stained folia, from what appeared to be zones D_2 and D_1. Retrogradely

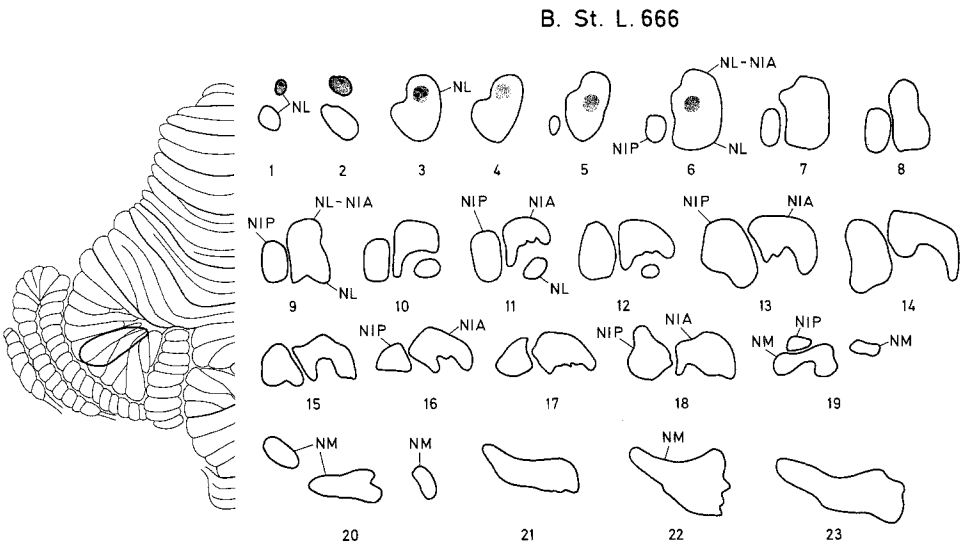

Fig. 5. Diagram showing the injection site in cat B.St.L. 666 and the distribution of anterogradely filled terminal Purkinje fibres (*hatched area*) and retrogradely labelled nucleocortical neurons in the cerebellar nuclei (*dots*), as seen in a series of 23 equally spaced sagittal sections. For abbreviations, see Fig. 2

labelled cells were found in the lateral part of the termination area, within the NL.

Only very few terminal fibres were filled with HRP in cat C.Co.L 197 (Fig. 1 A), with a small injection in the middle part of one of the folia, presumably in the D_1 zone. These fibres were situated within the dorsal part of the NL-NIA transition area. In addition, a few Purkinje axons could be traced into the middle part of NL, but their terminal branches were not anterogradely filled.

The terminal plexus was also in cat B.St.L. 784 (Fig. 1 A) located in the dorsal half of the NL-NIA transition area, but some anterogradely labelled terminal fibres were in addition found in the dorsolateral NIP. This cat had a small injection caudal and slightly medial to that of C.Co.L. 197. Retrogradely labelled neurons occurred within and just outside the terminal field in the NL-NIA transition area.

Cat B.St.L. 830 (Fig. 1 B) had a cortical staining which covered a large part of crus II, the presumed zones D_2 to C_1, and also included the adjacent part of the paraflocculus. Anterogradely labelled Purkinje axons could only be followed from the central part of the cortically stained area within crus II (Table 2), but a number of labelled fibres originated in addition from the parafloccular folia involved by the injection. Two terminal plexus were found in this cat, one in the NL-NIA transition area, and one in the dorsolateral NIP. Only fibres from crus II could be followed into the latter region, but the terminal plexus in the NL-NIA transition area received contributions both from crural and parafloccular Purkinje axons. Retrogradely labelled neurons occurred among the terminal fibres in both regions.

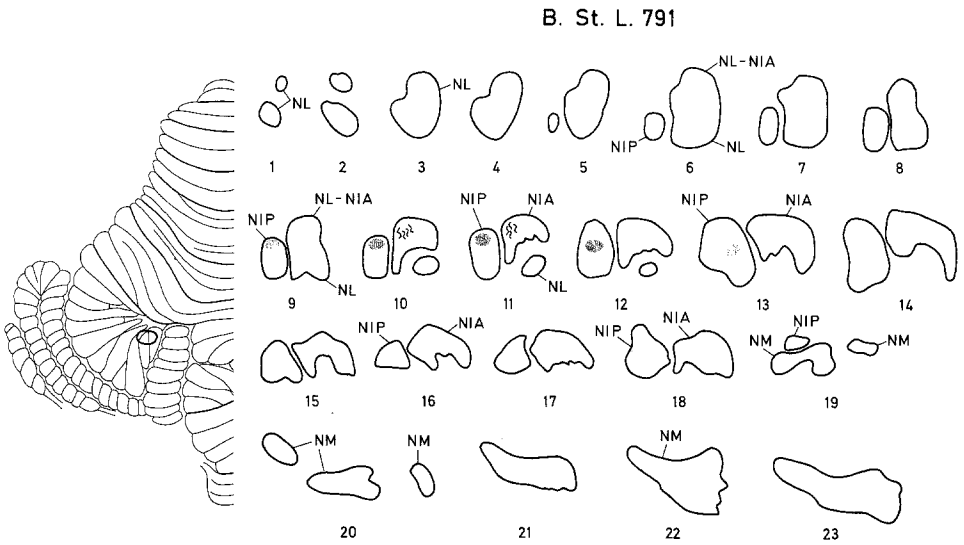

Fig. 6. Diagram showing the injection site in cat B.St.L. 791 and the distribution of anterogradely filled preterminal (*wavy lines*) and terminal (*hatched area*) Purkinje fibres in the cerebellar nuclei, as seen in a series of 23 equally spaced sagittal sections. For abbreviations, see Fig. 2

One cat with an injection in the medial half of the two caudal folia of crus II (B.St.L. 791, Figs. 1 A and 6) apparently within the C_2 zone, but possibly also involving the C_3 zone, showed a dense terminal plexus in the dorsal part of the lateral NIP. The termination extended a small distance medially and ventrally into the middle part of this nucleus. A few (pre)terminal fibres were in addition found in the lateral NIA. Also cat B.St.L. 789L (Fig. 1 B) had a medial injection, but here the cortical staining covered what is considered to be zones C_2 and C_3, with a slight involvement of zone D_1. Anterogradely labelled terminal fibres were in this cat located in the dorsal part of the NL-NIA transition area and the lateral NIA, and in the dorsolateral NIP. Preterminal fibres occurred within the ventrolateral NL and ventrally in the middle NIP. Retrogradely labelled cells were found within all these regions, but were more numerous in NIP than in other areas. The positive neurons in NIP were crowded along a narrow strip which extended medially and ventrally from the dorsolateral pole of this nucleus and into its central part.

Purkinje axons originating from all zones (D_2 through C_1) were visualized in B.St.L. 832 (Figs. 1 B and 4 B), with an injection covering the mediolateral extent of two folia in crus II. Five main terminal areas could be distinguished in this cat: one in the lateralmost part of NL, a second in the dorsal part of the NL-NIA transition area, a third in the dorsomedial NL-NIA transition area and the lateral part of NIA, a fourth in the dorsolateral NIP, and a fifth in the ventromedial NIP. Retrogradely labelled neurons were found within all five terminal regions.

Interpretation of Findings

Previous studies have given evidence that the cortex of the cerebellar hemispheres can be subdivided into five different longitudinal zones (see Introduction). These zones are in a lateromedial direction recognized as D_2, D_1, C_3, C_2 and C_1. Our material indicates that although there are no clearcut borders between the nuclear zones, each cortical zone has its own main field of termination in the cerebellar nuclei. Because of this, the nuclear region receiving Purkinje axons from one cortical zone will here be referred to as the corresponding nuclear zone. The extension of these nuclear zones is almost identical to that found in our preceding study (Dietrichs and Walberg 1979). Thus, the nuclear D_2 zone is situated laterally in the NL, the nuclear D_1 zone lies in the NL-NIA transition area, and the nuclear C_3 zone is located medially in the NL-NIA transition area and in the lateral part of NIA, while the nuclear C_2 and C_1 zones are represented in the dorsolateral and ventromedial NIP, respectively. This is a further confirmation of our suggestion (Dietrichs and Walberg 1979) that the corticonuclear fibres terminate along a continuous mediolateral band which loops through the CN.

The nucleocortical connection is mainly organized according to the same pattern as the corticonuclear projection. Retrogradely labelled neurons were observed in ten of our cats, and such cells occurred within all nuclear zones. Our material indicates that they are roughly arranged along the same mediolateral nuclear band as that described for the corticonuclear termination (see especially cat B.St.L. 789L). Most cells were found among or just adjacent to the anterogradely labelled terminal Purkinje axons, but some retrogradely labelled neurons were located in regions devoid of cortical afferents. One cat (B.St.L. 835, Fig. 1B) showed terminal Purkinje axons and labelled cells in nuclear zones D_2, D_1, C_3 and C_2 with retrogradely labelled cells also in the nuclear C_1 zone, and bilaterally in NM. Furthermore, retrogradely labelled neurons were situated bilaterally within NM also in cats B.St.L. 825 and 827 (Fig. 1B). This observation indicates that at least some cells in NM project to the presumed cortical zones D_1 and/or D_2. However, an analysis of our material does not permit decisive conclusions concerning an eventual projection from NM to the other cortical zones.

Discussion

Methodological Comments

HRP is at present routinely used as a retrograde neuronal tracer. However, although this macromolecule can be used also as an anterograde axonal marker, HRP is not commonly applied for studies of efferent fibre connections.

In a previous paper we have shown that HRP to advantage can be used for an analysis of the cerebellar corticonuclear projection, and that the Graham and Karnovsky (1966) and the Mesulam (1978) techniques give identical results

(Dietrichs and Walberg 1979). However, even if the *distribution* of the corticonuclear fibres is the same when the two methods are used, the Mesulam (1978) technique is much more sensitive. This conclusion is substantiated by the findings made in the present study.

One important feature concerning anterograde transport of HRP is that in fortunate sections axons are visualized all the way from their parent cell bodies to their terminal arborization area. Our series permit such a tracing of the Purkinje axons from their cortical orgin to their ultimate ramification in a nuclear plexus. This indicates that the stained fibres are not collaterals of retrogradely labelled mossy- or climbing fibres, but anterogradely labelled corticonuclear fibres (for a further discussion of this point, see Dietrichs and Walberg 1979).

The question of uptake and anterograde axonal transport of HRP from the injected cortical area merits some comments. As concerns the transport, our cases, in agreement with our previous observations (Dietrichs and Walberg 1979), demonstrate that axonal labelling may occur from only a small part of the cortically stained area, presumably the region around the needle tip. On the other hand, many of our cats showed HRP filled Purkinje axons descending from the greater part of the cortically stained area. One of our cases (cat B.St.L. 830, Fig. 1 B) is of special interest in this connection. This cat showed filled Purkinje axons which descended from crus II and the paraflocculus, despite the fact that the needle track was located in the medial part of crus II. This observation indicates that it is probable that the HRP concentration (and unknown biological factors) within the cortex is responsible for the width of the field from which the labelled axons take their origin (see also Dietrichs and Walberg 1979).

Another point which merits attention is that it can not be excluded that axons from an unstained cortical region passing through the white matter where HRP has been deposited, may take up the tracer. We have recently given evidence that, as concerns the olivocerebellar fibres, these may in Mesulam (1978) sections be visualized even if the HRP is deposited only in axons, and not within the cell bodies (Walberg et al., in press). Actually, several of the cases presented here had labelled efferent fibres from cortical areas that were unstained, but where the white matter below the unstained cortex was coloured (e.g. B.St.L. 779 and 831, Figs. 1 A and 3, and Table 2). In most of these cases the fibres could be traced into the nuclei, but the terminal arborizations were not stained (see sections 3–4, Fig. 3).

The majority of our TMB activated cases had retrogradely labelled cells in the CN. However, some of them failed to show retrogradely labelled neurons. These cases had no or only a slight staining of the granular layer. This finding is in keeping with our previous observations (Dietrichs and Walberg 1979) and supports the assumption that the nucleocortical fibres terminate within the granular layer (Palay and Chan-Palay 1974; Tolbert et al. 1976). Most of our cases treated according to the less sensitive DAB technique were, however, negative. Only three of these cats had intensely labelled CN neurons (see Fig. 8 A and Table 2).

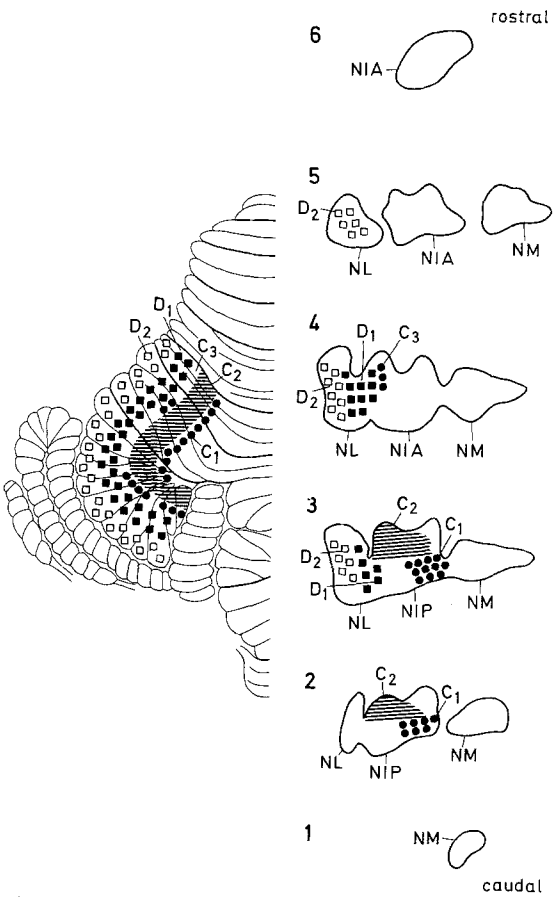

Fig. 7. Diagram giving a synopsis of our findings of the hemispheral corticonuclear projection as this appears in frontal sections through the cerebellar nuclei. Note that there is some lack of information as concerns the rostrocaudal distribution of zones C_1 and C_3. Further details are given in the text. For abbreviations, see Fig. 2

The Corticonuclear Projection

The findings presented in this study show that the nuclear zones receiving corticonuclear fibres from the simple and ansiform lobules are almost identical to those previously shown to receive terminal Purkinje axons from the paramedian lobule (see Dietrichs and Walberg 1979). It should especially be mentioned that the suggested position of the C_3 zone within the medial part of the NL-NIA transition area and the lateral NIA (Dietrichs and Walberg 1979) was supported by our present findings, especially by those in cat B.St.L. 832, where a separate terminal area was located in the region just medial to the D_1 zone, the alleged C_3 zone. Only one minor difference was observed. This small discrepancy concerns NL, where the terminal region for the hemispheral corticonuclear fibres

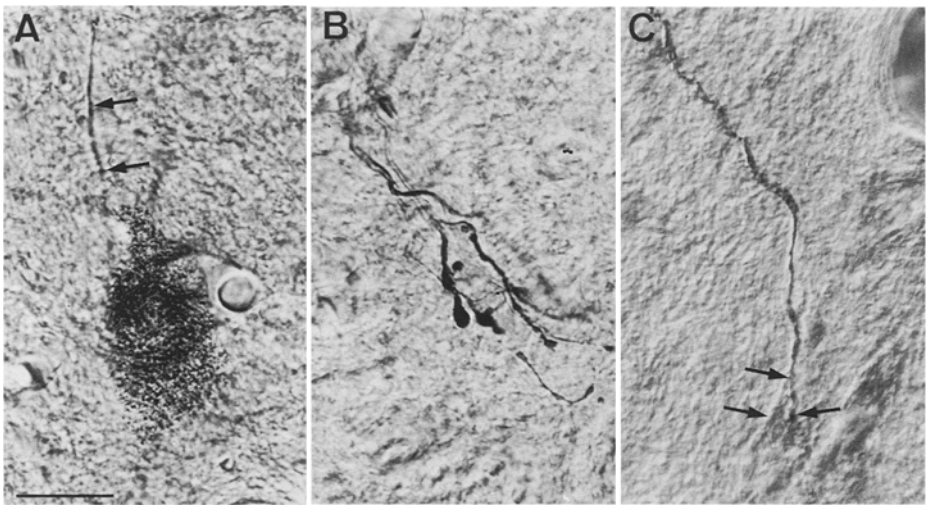

Fig. 8. A. Photomicrograph from cat B.St.L. 789L, showing a retrogradely labelled neuron in nucleus interpositus posterior in a DAB (Graham and Karnovsky, 1966) activated section. The arrows indicate an anterogradely filled thin axon (out of focus) approaching the retrogradely labelled cell. Scale line 25 µm. **B.** Photomicrograph from cat B.St.L. 789L, showing a terminal plexus (with boutons?) of thin anterogradely labelled Purkinje fibres within nucleus interpositus posterior. Scale line as in A. **C.** Photomicrograph from cat. B.St.L. 784, showing a preterminal fibre within the transition area of nucleus lateralis and nucleus interpositus anterior. The *upper arrow* points to a bifurcation, the two *lower arrows* indicate the branches. Scale line as in A

from zone D_2 extends somewhat dorsal to the area receiving the paramedian D_2 fibres (cp. sections 3–5 in Fig. 7 with those in Fig. 9 of Dietrichs and Walberg 1979). In addition, the D_1 zone can be followed somewhat more caudally. It should be noted that this observation coincides with that made in the fibre degeneration study by Courville et al. (1973, see their Fig. 1, planes 1–5).

Our delimitation of the nuclear zones D_2, D_1, C_3 and C_2 (Dietrichs and Walberg 1979, and the present study) is essentially in accordance with the findings made by other investigators (Voogd 1964, 1969; Groenewegen and Voogd 1977; Groenewegen et al. 1979; Voogd and Bigaré, in press). The C_1 zone, however, is by these authors placed within NIA; our observations indicate that this zone lies in the ventromedial NIP. In none of our paramedian or hemispheral injected cases have we been able to trace anterogradely labelled Purkinje axons from the C_1 zone into NIA. Actually, four cases in the present study showed anterogradely filled cortical efferents from what is considered to be the C_1 zone of crus I and II (see later), and all these cats showed labelled fibres within the ventromedial NIP (cats B.St.L. 789L, 791, 832 and 878 (Figs. 1, 4, 6)). We take this as a further indication that the nuclear C_1 zone is located within NIP.

We have previously demonstrated that although no clearcut borders exist between each nuclear zone receiving the fibres from the paramedian lobule, the Purkinje axons from one folium terminate along a continuous mediolateral band which loops through the CN (for diagrams showing this corticonuclear

terminal band in frontal, sagittal and horizontal views, and in stereopictures, see Figs. 10 and 11 in Dietrichs and Walberg 1979). An analysis of our present material shows that the same principle is valid for the hemispheral projection. This band loops from the ventromedial NIP through the dorsolateral part of this nucleus, and proceeds rostrally into the lateral NIA and the NL-NIA transition area before reaching the NL, where it ends. Our material does of course, not include injections restricted to each zone of each folium, but a comparison of the various cases indicates that the terminal fields from the different folia form parallel and probably overlapping nuclear bands. Although the relation between each of these bands is unclear, our material allows for some comments, since minor differences in termination occurred among cats injected in the same zone at varying rostrocaudal hemispheral locations. Thus, the terminal field in cats B.St.L. 828, 870 and 869, injected in the presumed D_2 zone of crus II, crus I and lobulus simplex, respectively (see Fig. 1), showed a successively more ventral position in the CN. The same tendency was observed in some of our other cases (cats B.St.L. 832 and 835, Fig. 4B and C). These observations are in keeping with previous findings made by Brodal and Courville (1973). These authors demonstrated that the projection from rostral[5] parts of crus II terminated ventral and lateral to that from the caudal folia (see their Fig. 8). Compatible findings were also made by Armstrong and Schild (1978, rat) who stated that the corticonuclear projection from crus I and II had an obvious mediolateral localization, and that the Purkinje axons from crus II terminated predominantly dorsal to those from crus I. All these observations make it probable that the nuclear terminal bands from the rostral parts of the cerebellar hemispheres are located slightly ventral to those from the caudal parts. Mediolateral differences were not observed in our study.

The Cortical Zonal Arrangement

The precise length and width of each longitudinal zone within lobulus simplex, crus I and II, have not been definitely established. The lack of information is most prominent regarding zones C_1 and C_3 (for details and a comparison of the various studies, see Brodal and Kawamura, in press). Because of this, we have put special emphasis on the distribution in our cases of retrogradely labelled neurons within the inferior olive. This has enabled a more accurate identification of the presumed longitudinal representation of each cortical zone.

Some of our cases (cats B.St.L. 789L, 791, 832) indicate that a C_3 zone is present within the hemisphere. It should be noted, however, that other cats with a cortical staining including the presumed C_3 zone (B.St.L. 666, 784 and 835) showed retrogradely labelled neurons within the olivary D_2, D_1 and C_2 areas, but not within the nuclear C_3 (and C_1) regions. Similar observations were also made by Kotchabhakdi et al. (1978) (five cats: B.St.L. 667, 749, 789R, 791 and C.Co.L. 201). These negative findings may either indicate that the C_3 zone is absent in certain parts of the crura, or that the C_3 zone is

[5] These authors used the terms "lateral" and "medial" for cortical regions which in this study are referred to as "rostral" and "caudal". (See Material and Methods for our nomenclature)

extremely narrow, thus in many cases preventing uptake of HRP sufficient to label cells in the olivary C_3 zone. The former suggestion appears most likely: two of our cats (B.St.L. 784 and 830, Fig. 1 and Table 2) had anterogradely labelled Purkinje axons descending from a cortical area corresponding to what would be expected to be the C_3 zone, but both cats showed terminal plexus only within the nuclear zones C_2 and D_1.

Observations by Voogd and Bigaré (in press, see their Fig. 6) indicate that a C_1 zone is present in both crura. Our material does, however, not permit definite conclusions on this point. The findings in cat B.St.L. 791 (Fig. 6) indicate that a C_1 zone may be absent in the caudalmost part of crus II. As concerns the C_2 zone, our observations indicate that this is relatively wide, and that it is present throughout the rostrocaudal extent of the hemisphere.

As mentioned above, the occurrence of retrogradely labelled olivary neurons is usually a helpful index for a delimitation of the injection area. On one point, however, there is a controversy concerning the zonal distribution of olivocerebellar fibres. Groenewegen et al. (1979) and Voogd and Bigaré (in press) thus propose that the ventral lamella of the principle olive projects to zone D_1 and the dorsal lamella to zone D_2, but Walberg and Brodal (1979) and Brodal et al. (in press) have recently indicated that the D_1 zones of the paramedian lobule and the anterior lobe receive afferent fibres from the dorsal lamella, while the D_2 zones are connected with the ventral lamella. Referring to this discrepancy it should be noted that our cat B.St.L. 879 (Fig. 2), injected in the presumed zone D_1, showed retrogradely labelled neurons only in the ventral lamella, while B.St.L. 869 (Fig. 2), with a more lateral injection within the same folium (in a presumed D_2 zone), had positive cells restricted to the dorsal lamella. The mediolateral distance between the injection sites in these cases was too broad to make individually varying zonal borders a likely explanation for our findings. A more probable explanation is that the two hemispheral D zones may receive fibres from the dorsal as well as the ventral lamellae. An alternative explanation is that there within the two lamellae is a rostrocaudal and/or mediolateral topical arrangement which is reflected in the projection to certain parts of the cortex, so that each lamella project onto one D zone in some cortical areas, and onto the other in other areas.

An analysis of our results indicates that as concerns the width of the cortical zones, the D_1 and D_2 zones remain wide throughout the rostrocaudal extent of the hemispheres, and that they occupy most of the caudal part of crus II (Fig. 7). This observation is in contrast to those made by Voogd and Bigaré (in press), who concluded that these zones reached their maximal width in crus I, and that they were reduced to narrow slits in the caudal part[6] of crus II.

Another point to mention is that the cortical A zone in the region of lobule VIIA may extend laterally beyond the border of the vermis and into the adjacent crus I. This is learnt from the unexpected findings made in cat B.St.L. 635 (Fig. 1A) where the Purkinje axons (preterminal) could be traced into NM.[7] This observation clearly invites further experiments.

[6] These authors use the term "ventral" for cortical regions which in this study are referred to as "caudal". (See Material and Methods for our nomenclature)

[7] Labelled cells were in this case found in the caudal part of the medial accessory olive, the presumed olivary A zone

Apart from what is said above, we have no comments on the lenght and width of the zones within the simple and ansiform lobules. Fig. 7 gives a diagram of the zones as they occur from an analysis of the cases shown in the present communication.

The Nucleocortical Projection

Since the pilot studies by Carrea et al. (1947) and Cohen et al. (1958), the nucleocortical projection in the cat has been reinvestigated by several authors (Gould and Graybiel 1976; Gould 1977, 1979; Tolbert et al. 1976, 1978; Dietrichs and Walberg 1979). As concerns the projection to lobulus simplex, crus I and crus II, the other HRP studies are compatible with our results, although in these only a gross topical pattern was demonstrated. Gould and Graybiel (1976) and Gould (1979) thus demonstrated a projection from the NI and NL to crus I and II, and similar observations were made by Tolbert et al. (1978), who found that the "lateral hemisphere" received nucleocortical fibres mainly from NL, while the projection from NI mainly reached the "medial hemisphere". These authors also stated that "Along the periphery of each cortical zone, the nucleocortical projection from adjacent deep nuclei overlapped slightly." (loc.cit., p. 39). This fits well with our observation that the nucleocortical projection to each folium mainly originates from a mediolateral nuclear band, similar to that receiving corticonuclear fibres from the same folium.

Since retrogradely labelled nucleocortical cells usually occur among the anterogradely stained corticonuclear fibres, our material gives further evidence for a corticonuclear-nucleocortical reciprocal relationship (Tolbert et al. 1976; Haines 1978; Gould 1979; Haines and Pearson 1979; Tolbert and Bantli 1979; Dietrichs and Walberg 1979), but it should be stressed that the occurrence of retrogradely labelled neurons bilaterally within NM after injections in crus I makes generalizations impossible (see also Tolbert et al. 1978; Tolbert and Bantli 1979; Dietrichs and Walberg 1979). It should furthermore be noted that this bilateral projection which previously has been described by Gould and Graybiel (1976) and Tobert et al. (1978), and found in three of our cases, includes neurones of varying sizes, but its functional importance is still unclear.

Contralateral nucleocortical projections from NL and NI to lobulus simplex, crus I and II, or projections from the contralateral NM to lobulus simplex and crus II, were not observed by us. Such connections have not been described in the cat, but it can not be excluded that a weak connection may have escaped recognition even in our TMB activated sections.[8] More sensitive techniques may in the future add information on this point.

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[8] Tolbert et al. (1978) mention a few fibres from NM to the contralateral hemisphere

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