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The organization of the corticonuclear and olivocerebellar climbing fiber projections to the rat cerebellar vermis: The congruence of projection zones and the zebrin pattern

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Abstract

The zonal organization of the corticonuclear and the olivocerebellar climbing fiber projections to the vermis of the cerebellum of the rat was compared to the pattern of zebrin-positive and zebrin-negative bands in material double-stained for zebrin II and for different anterograde tracers injected in subnuclei of the inferior olive, or retrograde tracers injected in the cerebellar and vestibular target nuclei of the Purkinje cells of the vermis. Projection zones A_1, A_X, X, B, C_X in the vermis and A_2 (accessory A zone) and C_2 in the hemisphere were defined by their efferent corticonuclear and their afferent climbing fiber connections, and were found to share the same topographical framework with the zebrin pattern.

Introduction

There are words that stick in your mind. Among our favorites are Sanford L. Palay's statement on synaptic vesicles, which "like chocolates, come in a variety of shapes and sizes, and are stuffed with different kind of fillings" (1967) and his remark on the "chemical idiosyncrasy" of non-synaptic monoaminergic cerebellar afferents (1982). The chemical idiosyncrasy of certain populations of Purkinje cells was the subject of a series of papers, he co-authored with Victoria Chan-Palay (Chan-Palay *et al.*, 1981, 1982a, b). These early studies on the chemical heterogeneity of Purkinje cells were followed by Hawkes and Leclerc's (1987) description of a distinct longitudinal zonal pattern in the distribution of zebrin-positive and- negative Purkinje cells in the cerebellum of the rat. The zebrin-pattern is so special, because it is representative for the distribution in the rodent cerebellum of a great number of substances in Purkinje cells, but also in the Bergmann glia (see Voogd *et al.*, 1996a; Hawkes & Eisenman, 1997, for reviews). These substances are diverse, and include the enzyme 5′-nucleotidase (Scott, 1964; Marani, 1982, Scott's publication antedated the description of the zebrin pattern for more than two decades!), aldolase C (or zebrin II: Brochu *et al.*, 1990; Ahn *et al.*, 1994), the low affinity nerve growth factor receptor protein (Dusart *et al.*, 1994), protein kinase C delta (Chen & Hillman, 1993) the glutamate transporter EAAT4 (Dehnes *et al.*, 1998) and the metabotropic glutamate receptor mGluR1b (Mateos *et al.*, 2001), to name only a few. Recently, it was shown that zebrin II-expressing Purkinje cells are more resistant to death (Sarna *et al.*, 2001). A unifying hypothesis on the functional significance of the features, which distinguish zebrin-positive and negative Purkinje cells is still lacking.

We asked the question, whether the zebrin-positive and -negative Purkinje cells also differ in their projections to the cerebellar nuclei and in their afferent climbing fiber connections from the inferior olive. The zonal organization of the corticonuclear and olivocerebellar projections has been studied extensively in carnivores and primates (reviewed by Voogd & Bigaré, 1980; Haines *et al.*, 1982). This type of zonal organization was confirmed for the rat by Buisseret-Delmas and her collaborators (Buisseret-Delmas & Angaut, 1993; see Fig. 1). In earlier attempts to correlate axonal projections with the zebrin pattern the individual olivocerebellar and corticonuclear projection zones were not

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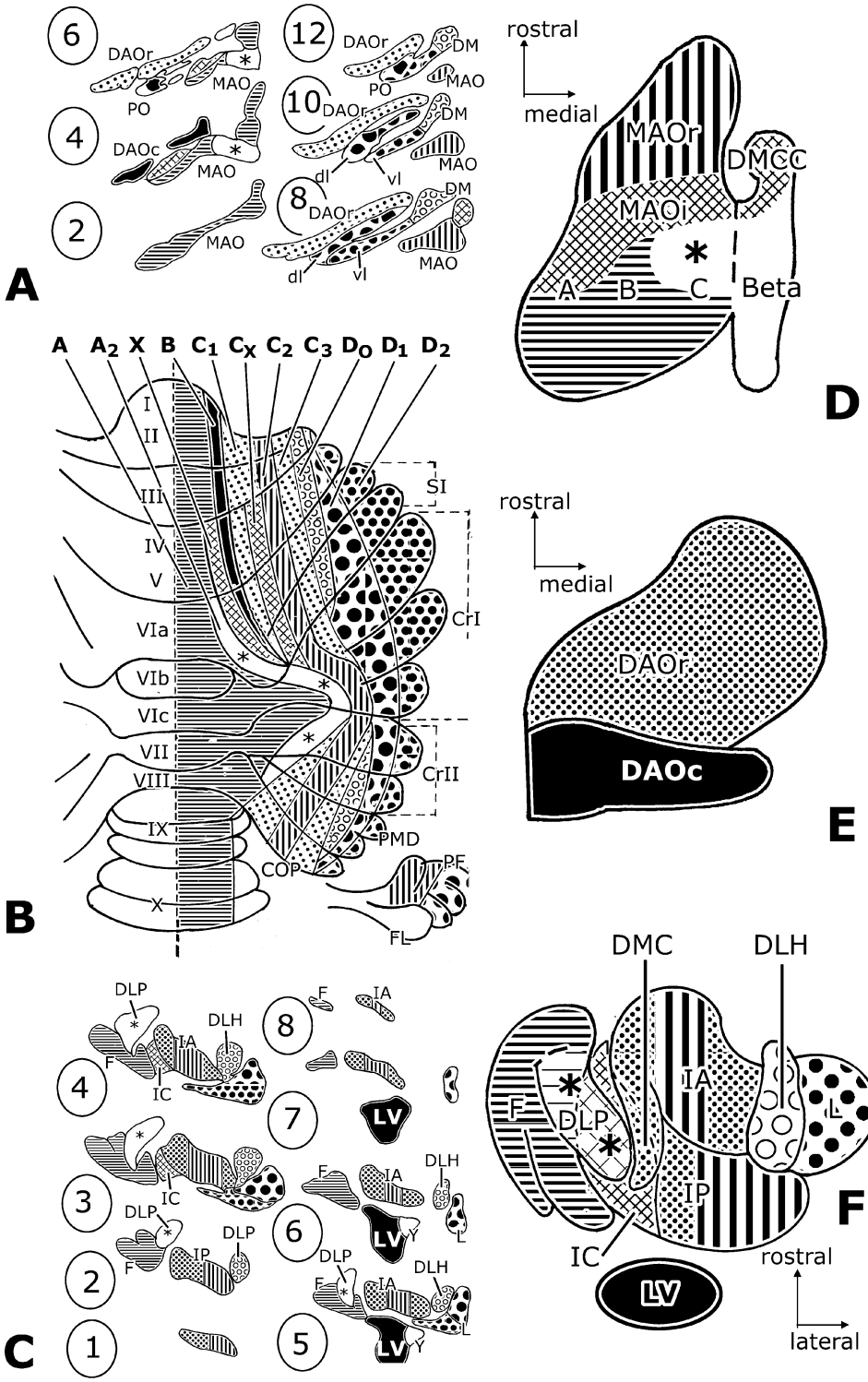
identified (Gravel *et al.*, 1987; Hawkes & Leclerc, 1989; Wassef *et al.*, 1992).

In a recent paper (Voogd *et al.*, 2003a, b) on climbing fiber collateralization from small injections of cholera-atoxin subunit B in electrophysiologically identified climbing fiber zones of the cerebellar cortex, we established that climbing fiber zones and zebrin banding reflect a common organizational scheme in the rat cerebellar hemisphere. In the present paper we review our evidence on the topographical relations of individual projection zones to the zebrin pattern in the vermis of rat cerebellum. This evidence consists of cases with combined anterograde tracing of olivocerebellar climbing fibers or retrograde labeling of cerebellar Purkinje cells from small injections in their target nuclei, with counterstaining with an antibody against zebrin II



(Brochu *et al.*, 1990). Some of our data have been published in abstract form (Voogd *et al.*, 1993) or were used in reviews (Voogd *et al.*, 1996a; Voogd & Ruigrok, 1997).

Methods

In this study we report on experiments in 29 male Wistar rats. All surgical procedures adhered to NIH guidelines and permission was obtained from the local committee overseeing animal experiments.

In most rats injections with a retrograde or an anterograde tracer were made bilaterally. In some rats (*e.g.* 548) injections with a retrograde as well as with an anterograde tracer were made bilaterally resulting in four experiments within one animal. 15 Injections made in 11 animals will be illustrated in this review. Most of our results could be confirmed in the other experiments (see Table 1).

Two different anterograde tracers (Phaseolus vulgaris leucagglutinin, Phal, and biotinylated dextran amine, BDA) and two different retrograde tracers (horseradish peroxidase conjugated to wheat germ agglutinin, WGA-HRP, and a gold-lectin conjugate consisting of gold conjugated to bovine serum albumin and wheat germ agglutinin) were used. Experimental procedures and details on the histological processing and the counterstaining with an antibody against zebrin II (Brochu *et al.*, 1990) were reported in previous papers (Ruigrok *et al.*, 1995; Ruigrok & Voogd, 1990, 2000; Teune *et al.*, 2000; Voogd *et al.*, 2003a, b).

Data on the localization of the labeled climbing fibers and the zebrin pattern are presented as graphical reconstructions of the rostral and dorsal aspects of the anterior lobe, and the rostral, dorsal and caudal aspects of the posterior lobe, made by stacking the sections, using the floor of the fourth ventricle as a guide (see Voogd *et al.*, 2003a, for details). Hatching indicates the position of the labeled climbing fibers relative to the zebrin pattern; labeled Purkinje cells are indicated by dots. Injection sites were drawn on standard diagrams of serial transverse sections through the cerebellar nuclei or the inferior olive (Ruigrok & Voogd, 2000). Injections in the inferior olive were also depicted in horizontal projections of the medial and dorsal accessory olives prepared from these sections.

Table 1. Specifying injection sites, tracers used and case numbers of similar experiments.

Case NR.	*Injection site*	*Tracer*	*Similar cases*
016	LV	WGA-HRP	10, 11, 28-r, 41
029-l	IP/ICG	WGA-HRP	
029-r	F/ICG	WGA-HRP	40-r
032	DLP	WGA-HRP	33, 577
244-r	MAOc-C	Phal	597
245	MAOc-C	Phal	see 244
415	DAOc	Phal	459-l, 954
548-l	ICG	GL	547-r
548-r	F	GL	28-l, 40l, 547-l, 954
548cf-l	MAOi	BDA	428-r, 439, 459-r, 467
548cf-r	MAOc-A/B	BDA	see 614
528	MAOr	Phal	427
614	MAOc-A/B	Phal	244-l, 559, 468, 479-l
740	LV	GL	see 016
953	DAOc	BDA	954

Abbreviations: A, group A of the caudal medial accessory olive; B, group B of the caudal medial accessory olive; BDA, biotinylated dextran amine; C, group C of the caudal medial accessory olive; DAOc, caudal dorsal accessory olive; DLP, dorsolateral protuberance; F, fastigial nucleus; F/ICG, border region of fastigial nucleus and interstitial cell groups; ICG, interstitial cell groups; IP/ICG, border region of interstitial cell groups and posterior interposed nucleus; GL, gold-lectin; LV, lateral vestibular nucleus; MAOc, caudal portion of the medial accessory olive; MAOi, intermediate portion of the medial accessory olive; MAOr, rostral portion of the medial accessory olive; Phal, Phaseolus vulgaris leucoagglutinin; WGA-HRP, wheat germ-coupled horseradish peroxidase.

Definition of zones

In the present study the individual, rostrocaudally-oriented cerebellar projection zones were defined by their inferior olive (climbing fiber) and Purkinje cell corticonuclear connections as reported by Buisseret-Delmas (1988a, b), Buisseret-Delmas and Angaut (1993) and Buisseret-Delmas *et al.* (1993) (see Fig. 1). To facilitate the description of the olivocerebellar projection, the medial accessory olive (MAO) was subdivided into

Fig. 1. Corticonuclear and olivocerebellar projection zones of the rat cerebellum. The cortical zones (B), the subnuclei of the inferior olive that innervate particular zones (A) and their cerebellar and vestibular target nuclei (C) are indicated with the same symbols. The sections in A and C are viewed in the transverse plane with sections 12 (A) and 8 (C) the most rostral. Redrawn from Buisseret-Delmas and Angaut (1993), with the addition of the A_2 zone (asterisks; Buisseret-Delmas, 1988a) and the X and C_X zones (Buisseret-Delmas *et al.*, 1993). Horizontal projections of the medial accessory olive (D), the dorsal accessory olive (E) and a diagram of the cerebellar nuclei of the rat (F) summarize the connections of the zones. In diagram F the dorsolateral protuberance partially hides the fastigial nucleus and the interstitial cell groups. Abbreviations: A-D_2, zones A-D_2; A, group A of the caudal medial accessory olive; B, group B of the caudal medial accessory olive; Beta, group Beta; C, group C of the caudal medial accessory olive COP, copula pyramidis; CrI, Crus I; CrII, Crus II; DAOc, caudal part of dorsal accessory olive; DAOr, rostral part of dorsal accessory olive; dl, dorsal lamina of the PO; DLH, dorsolateral hump; DLP, dorsolateral protuberance of the fastigial nucleus; DM, dorsomedial group of the PO; DMC, dorsomedial crest; DMCC, dorsomedial cell column; F, fastigial nucleus; FL, flocculus; IA, anterior interposed nucleus; IC, interstitial cell groups; IP, posterior interposed nucleus; I-X, lobules I-X; L, lateral cerebellar nucleus; LV, lateral vestibular nucleus; MAO(r,i,c), medial accessory olive (rostral, intermediate, caudal portions); PFL, paraflocculus; PMD, paramedian lobule; PO, principal olive; SI, lobulus simplex; vl, ventral lamina of the PO; Y, group Y.

rostral (MAOr), intermediate (MAOi) and caudal (MAOc) parts. The MAOc was further subdivided into three, latero-medially arranged cell groups, indicated as A, B and C (Gwyn *et al.*, 1977). These subdivisions cannot be readily recognized in cell-stained sections, but derive their significance from the localization of olivary neurons projecting to a particular zone. The dorsal accessory olive was divided into rostral (DAOr) and caudal (DAOc) parts, corresponding to the ventral fold and the dorsal fold of the DAO of Azizi and Woodward (1987), respectively.

Three zones, A, X and B, were distinguished in the vermis. The A and B zones receive a projection from group B of the caudal medial accessory olive (MAOc-B) and the caudal dorsal accessory olive (DAOc), and project to the fastigial nucleus (= medial cerebellar nucleus) and the lateral vestibular nucleus, respectively (Fig. 1D and E). The X zone receives its climbing fibers from an intermediate region of the medial accessory olive (MAOi), which extends rostrally into the dorsomedial cell column (DMCC), and for some distance caudally into group A, located along the lateral margin of the MAOc (Fig. 1D). The X-zone projects to the small groups of cells that are wedged between the fastigial nucleus and the interposed nuclei and which were termed the interstitial cell groups by Buisseret-Delmas *et al.* (1993).

An "accessory A zone", located lateral to the A zone in the medial hemisphere of the lobules VI and VII was distinguished in the rat by Buisseret-Delmas (1988a). This zone receives climbing fibers from the medial group C of the caudal MAO and projects to the dorsolateral protuberance of the fastigial nucleus. To simplify the description, we have indicated the original, medial A zone as A_1 and the accessory A zone as A_2 (Atkins & Apps, 1997).

We will first describe the general appearance of the zebrin zones and subsequently relate these zones to the climbing fiber and corticonuclear projection of the B-zone and to the different sets of zones innervated by various subdivisions of the medial accessory olive.

The zebrin pattern

The molecular marker zebrin is expressed in a subset of cerebellar Purkinje cells which can be revealed by immunohistochemical techniques, using antibodies against zebrin I (Hawkes & Leclerc, 1987) or zebrin II (Brochu *et al.*, 1990). Rostrocaudally oriented bands of immunoreactive (zebrin-positive) Purkinje cells alternate with cells that are zebrin-negative (see summary diagram Fig. 7A, left hand side). The nomenclature used in the present paper for these zebrin-positive and -negative bands was introduced by Hawkes and Leclerc (1987). A total of 7 zebrin-positive bands can be identified in the rat cerebellum (termed P1+ to P7+, and indicated with the numbers 1–7 in the figures). Zebrin-negative bands P1− to P6− are indicated with the number of the next-medial zebrin-positive band. With the exception of the P3+ band in the anterior cerebellum the zebrin-positive bands are distinct and clearly delineated, while some of the numberless "satellite" bands of Hawkes and Leclerc (1987) occur regularly and have been indicated in the present study with the letters a, b, d and e. In the dorsal region of the anterior lobe and in the lobulus simplex one or two satellite bands (a) usually are present in P1−. A narrow satellite band b usually can be identified lateral to P2+ in the same lobules. Ill-defined patches or bands of zebrin-positive Purkinje cells, which are present more laterally in the P2− band of the lobulus simplex are indicated with a "d". Satellite band e is located as a patch in P4− of the copula pyramidis. In lobules VIb/c, and VII, the nodulus, the caudal crus I, the flocculus and the paraflocculus all Purkinje cells stain for zebrin and no bands can be distinguished. In crus II and the paramedian lobule two additional narrow zebrin-positive bands are located between P4+ and P5+ (indicated as bands P4b+ and P5a+).

The zebrin pattern, as described here, is highly reproducible. Small deviations from the sequence are sometimes visible in the reconstructions of individual cases, although such deviations are usually due to staining artifacts, missing sections or errors in the stacking or the spacing of the sections.

The B zone: Climbing fiber and corticonuclear projections (Fig. 3)

Labeling of Purkinje cells of the B zone was obtained with injections of retrograde tracers in their target nucleus, the lateral vestibular nucleus. Climbing fibers terminating in the B zone were labeled from injections of the DAOc. The distributions of these labeled structures were very similar, irrespective of the retrograde or anterograde tracer used (compare cases 16 and 740, and 415 and 953 in Fig. 3). Purkinje cell labeling from injections of the vestibular nuclei, including the lateral vestibular nucleus (Fig. 3, cases 16, 740) resulted in the labeling of Purkinje cells in the anterior lobe, the lobulus simplex, the copula pyramidis, the uvula and the nodulus.

In the anterior lobe and the lobulus simplex the labeled Purkinje cells were found in zebrin-negative territory. They were located in two strips, on both sides of P2+, these strips terminated at the fissure separating the lobulus simplex from the crus I of the ansiform lobule. In the lobules I–III the lateral strip of labeled Purkinje cells abuts on the P2+ band (Fig. 2E, F and H). In the lobules IV and V and in the lobulus simplex, however, a narrow band of non-labeled Purkinje cells separated the labeled cells from P2+ (arrows in Fig. 3). In the lobulus simplex, and sometimes in lobule V, satellite band b formed the lateral boundary of the

labeled Purkinje cells. In the same lobules the medial strip of labeled Purkinje cells occupied a narrow, zebrin-negative compartment, bordered on its medial side by one of the satellite bands a. In the copula pyramidis labeled Purkinje cells occupied the zebrin-negative P2− compartment and a narrow zone immediately lateral to P4+. Scattered labeled cells in the uvula and the nodulus did not display a specific relation to the zebrin-pattern.

Pha-L or BDA-labeled climbing fibers appear in transverse sections as two thin parallel lines of varicose axonal fragments in the molecular layer where they delineate the dendrites of Purkinje cells (Fig. 2C and I). The distribution of climbing fibers, labeled from the DAOc, coincides with the lateral strip of Purkinje cell labeling from injections of the lateral vestibular nucleus (Fig. 3, cases 415, 953). These Purkinje cells, therefore, represent the B zone. The medial strip of labeled Purkinje cells is part of the A_1 zone, to be considered below. In the lobules I–III the climbing fibers of the B zone were located immediately lateral to P2+, in lobules IV and V and in the lobulus simplex they were separated from P2+ by an empty area (Fig. 2I; Fig. 3, arrows). In the lobulus simplex satellite band b borders them on their lateral side. In the copula pyramidis a narrow, one or two labeled climbing fiber-wide strip was present lateral to P4+.

COMMENTS

The results of our studies on the B zone are summarized in Figure 7. Its distinguishing feature is its localization in zebrin-negative territory. The B zone shares this feature with the C_1 and C_3 zones, which are innervated by the rostral DAO (Voogd *et al.*, 2003a, b). Our conclusions on the topography of the B zone are in accordance with earlier observations in carnivores (Voogd & Bigaré, 1980) and in the rat (Buisseret-Delmas, 1988a). The B zone has not been identified before in the copula pyramidis.

The A_1, A_X X, and C_X zones: Climbing fiber projections (Fig. 4)

The A_1, X and C_X zones can be defined by their afferent, climbing fiber projections. In two cases (548cf-r and 614, Fig. 4) injections of anterograde tracers were made laterally in the MAOc. The injection in case 614 was located more rostrally than the one in 548cf-r. In both cases climbing fibers were labeled in the bands P1− and P2+ of the anterior lobe and the lobulus simplex. The climbing fibers in P1− were located both in zebrin-negative spaces and among the zebrin-positive Purkinje cells of the satellite bands. The labeling in the anterior lobe in case 548cf-r was located laterally within P1−, next to the P2+ band, in a region where Purkinje cells were labeled from injections of the vestibular nuclei (compare cases 16 and 740, Fig. 4). With the more rostral injection site in case 614, the climbing fiber labeling in P1− shifted medially. A shift was also found for the labeling in P2+, but here the caudal injection in case 548cf-r labeled the ventral segment of P2+ in lobule III and the medial half of this band in the lobules IV and V and the lobulus simplex. With the more rostral injection in case 614, the labeling in P2+ shifted laterally.

Our interpretation of these findings is that both injections affect two populations of olivary neurons (Fig. 4, lower panels). One population, located in the group B of the MAOc, projects to P1−. With more rostral injection the projection to P1− shifts medially. A second population, located within group A along the lateral margin of MAOc, projects to P2+. The lateral shift of the labeling with the more rostral injection, distinguishes it from the first population. The climbing fiber projection of group B to P1− will be referred to as the A_1 zone and the projection of group A to P2+ as the A_X zone.

The A_1 and/or A_X zones were also represented in the pyramis. In both experiments labeling was present in the zebrin-negative strip P2−, located between the zebrin-positive P2+ and P3+ bands. In case 614 the P3+ and P4+ bands were also labeled. The P1± and the P2+ bands were spared.

The distribution of the climbing fiber labeling after injections of the MAOi was quite different (Fig. 4, case 548cf-l). In the anterior lobe and the lobulus simplex labeled climbing fibers occupied two zebrin-negative strips, one lateral to P2+ and another one medial to P4+. In the anterior lobe the medial strip of labeled climbing fibers was restricted to the lobules IV and V. The medial strip of climbing fibers occupied the space between P2+ (i.e. the A_X zone) and the B zone (compare Fig. 3, arrows). The lateral strip, in addition, dipped into lobule III. In our earlier study on climbing fiber collateralization these medial and lateral strips were indicated as the X and C_X zones, respectively (Voogd *et al.*, 2003a, b; also see Buisseret-Delmas *et al.*, 1993).

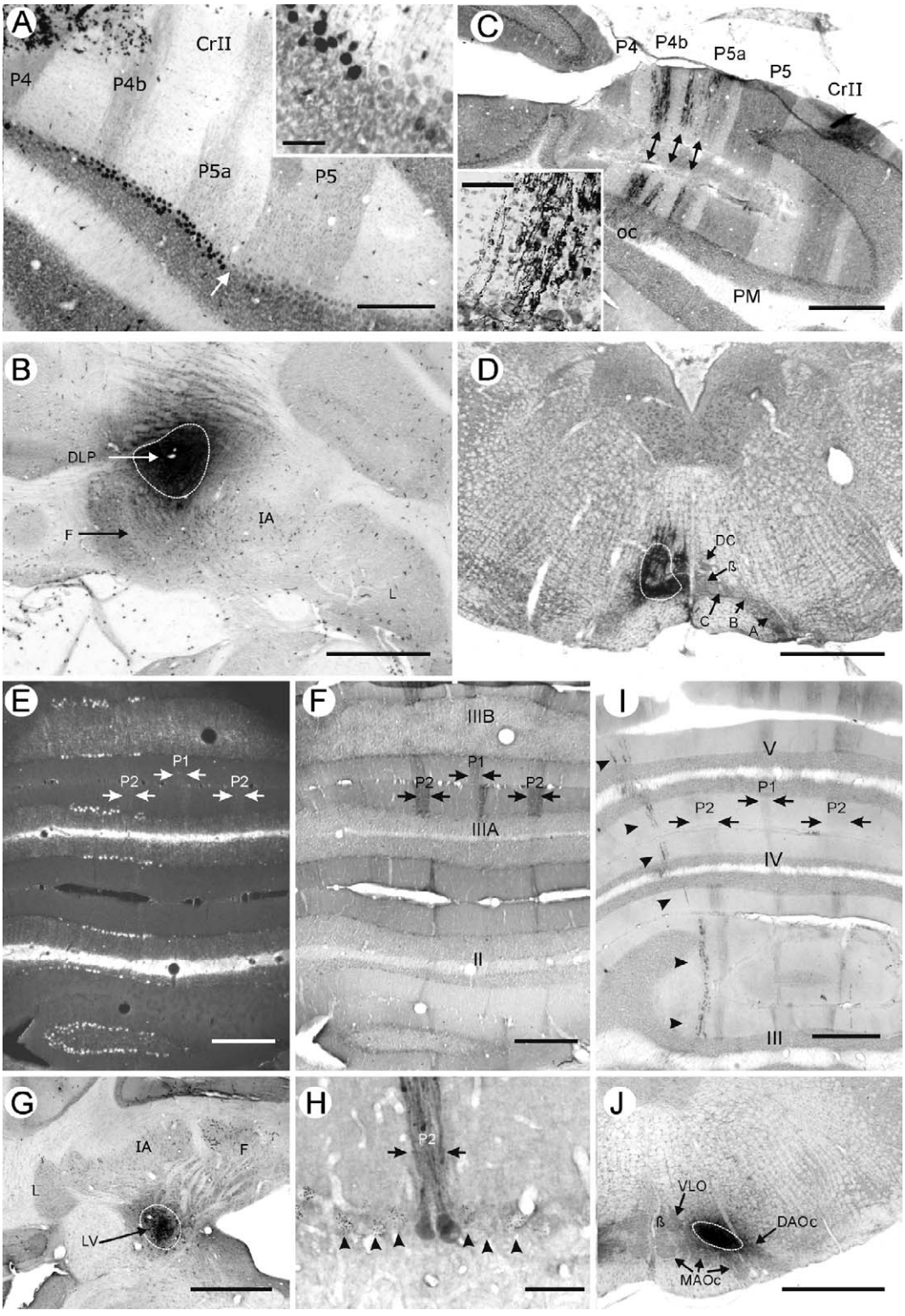
In the copula and the pyramis two strips of labeled climbing fibers were present. The labeling in P3− represents the X zone, and a more lateral strip located within or just medial to the lateral zebrin-positive pole of the copula (filled arrow in Fig. 4) corresponds to C_X.

COMMENTS

The A zone in carnivores, primates and rodents was characterized by its projection to the medial cerebellar nucleus, and its climbing fiber afferents from the caudal MAO (Voogd & Bigaré, 1980; Haines *et al.*, 1982; Buisseret-Delmas, 1988a). The A zone extends over the entire vermis, including the lobules VIb, VIc and VII (also see Apps, 1990). However, anterograde experiments on the climbing fiber projections to these lobules were not available.

The X zone was originally defined in electrophysiological experiments in the cat as a strip located between

the A and B zones which receives branches from the same climbing fibers which also project to the C_X (or lateral C_1) zone (Ekerot & Larson, 1979, 1982). The X zone was restricted to the lobules IV and V of the anterior lobe. The X and C_X zones of the cat share their electrophysiological properties with the C_1 and C_3 zones, but differ from them because they receive climbing fibers from the MAO instead of the DAO (Trott & Armstrong, 1987a, b; Pardoe & Apps, 2002). Like the rostral DAO, the intermediate MAO receives somatosensory afferents, in this case from the dorsal column nuclei (Gerrits *et al.*, 1985). It should be noted that these somatosensory



innervated zones, are all located in zebrin-negative territory. This distinguishes them from the C_2 zone, which lacks a direct somatosensory afferentiation and is located in the zebrin-positive anterior P4+ and posterior P5+ bands.

The source of the climbing fiber projections to the X and C_X zones was located in a curved band, located at the junction of the rostral and caudal halves of the MAO, both in the cat (Campbell & Armstrong, 1985; Trott & Apps, 1991) and in the rat (Buisseret-Delmas *et al.*, 1993). The projection to the X zone originates laterally in this band, the projection to C_X from its rostromedial portion. In the cat cells projecting to both zones were found in a region where the projections to the two zones overlap (Apps *et al.*, 1991). We did not include the projection of the dorsomedial cell column to the lateral uvula in the C_X zone, as proposed by Buisseret-Delmas *et al.* (1993; Fig. 1). The dorsomedial cell column, with its bilateral and branching projections (Sugihara *et al.*, 1999) to the lateral P3+ and the P4+ bands of the caudal vermis (Voogd *et al.*, 1996b) should be considered as a separate subnucleus of the inferior olive.

In an earlier review we identified the X zone in the rat as the zebrin-positive P2+ band in lobules IV and V (Voogd & Ruigrok, 1997). However, the present analysis shows that the climbing fiber projection to P2+ extends over all lobules of the anterior lobe; that it originates from caudal subnucleus A of the MAOc; and that it does not collateralize to the C_X zone. Consequently, we revised our opinion and included the climbing fiber projection to P2+ in the A zone as a specific subdivision called A_X. The zebrin-negative region, immediately lateral to P2+, displays all the properties of the X zone and now is indicated as such. In the anterior lobe it is restricted to the lobules IV and V, it receives a projection from the intermediate MAO and it shares its climbing fiber afferents with the zebrin-negative region, medial to P4+, now indicated as the C_X zone. X and C_X continue on the lobulus simplex and are represented in the pyramis and its copula.

A topical relation was found for the projection of group A of MAOc to the A_X zone. Caudal group A projects rostrally and medially within A_X, rostral group A projects more laterally within this zone. When the spinal and dorsal column afferents of the MAO are taken into account (Boesten & Voogd, 1975), this would mean that the lower and upper extremities would be localized in rostrolateral and caudomedial subzones of A_X, respectively.

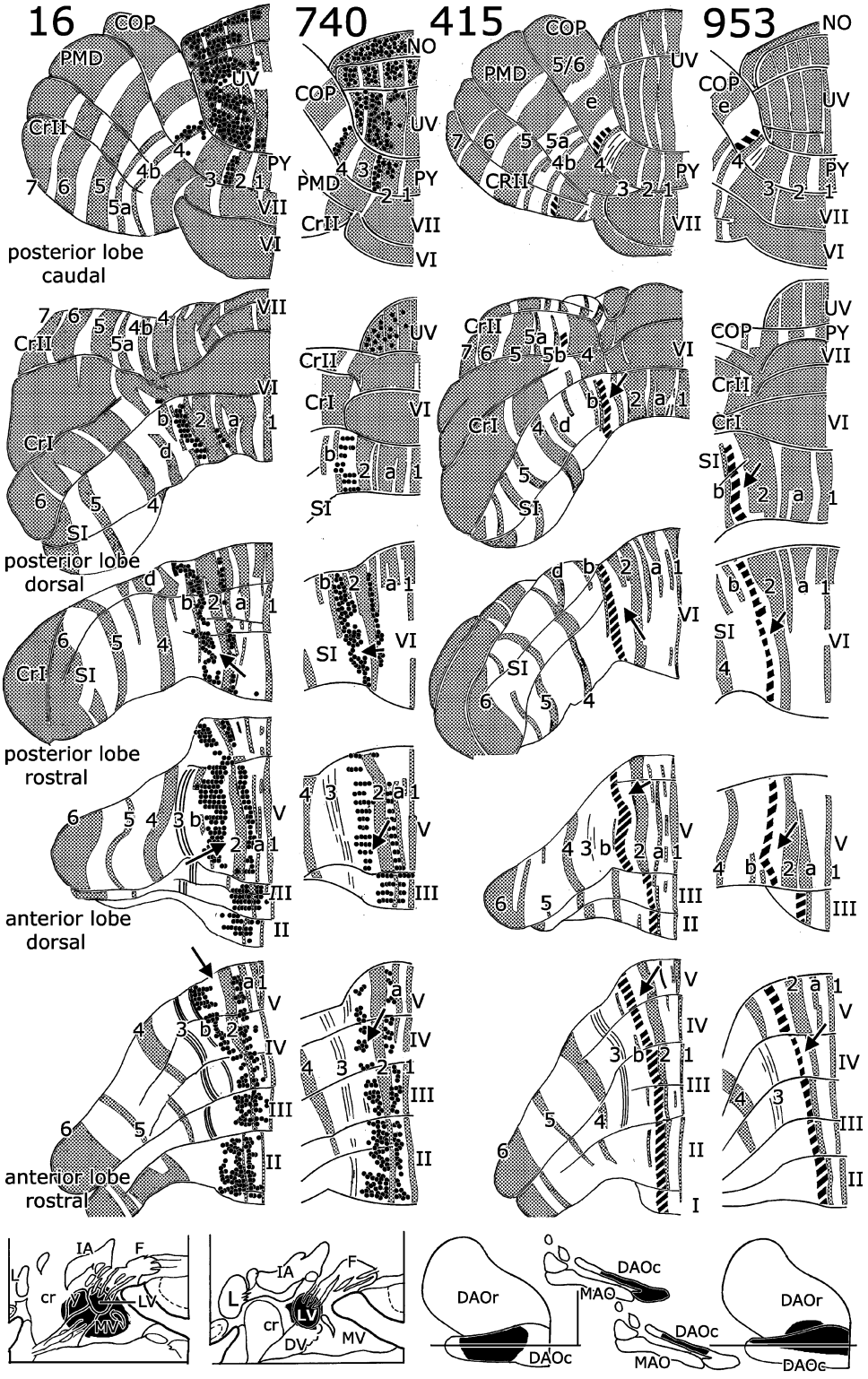
Topical relations were also evident for the projection of group B of the MAOc to the A_1 zone. However, this projection is reversed as compared to the projection to A_X, with caudal part of group B projecting more laterally and rostral group B more medially. The topical projection to A_1 and A_X is in partial accordance with the electrophysiological map, prepared by Jörntell *et al.* (2000), for the climbing fiber projection to the anterior lobe of the rat. However, these authors did not distinguish an X zone.

The C_2 zone: Climbing fiber projection (Fig. 4)

Case 528 is an injection in the MAOr. In the anterior lobe and the lobulus simplex climbing fiber

Fig. 2. (A) The Purkinje cell labeling from an injection of WGA-HRP including the dorsolateral protuberance covers the area of the P4−, P4b± and P5a± bands in the crus II. Cobalt-intensification (Lemann *et al.*, 1985) enhanced contrast between the somata of the WGA-labeled Purkinje cells and the zebrin-staining Case 577. Bar = 400 μm. Inset shows transition of bands P5a− and P5+ (arrow) at higher magnification. Bar = 100 μm. (B) WGA-HRP injection site in case 32. Bar = 1000 μm. (C) Pha-L, cobalt-intensified labeling of climbing fibers in an area overlapping with the P4b± and P5a+ bands in the right crus II, following an injection of group C of the contralateral MAOc (Fig. 2D). The bundle of labeled olivocerebellar fibers in the white matter is indicated with oc. Case 245. Bar = 1000 μm. Inset shows higher magnification of labeled climbing fibers. Note the parallel course of labeled fragments as they delineate the Purkinje cell dendrites. Bar = 100 μm. (D) Injection site of group C of the left caudal MAO. Case 245. Bar = 1000 μm. (E) Gold-lectin-labeled, silver-intensified retrograde labeling in Purkinje cells located medial and lateral to P2+ in the lobules II and III of the anterior lobe, following an injection including the lateral vestibular nucleus. In dark-field the labeled Purkinje cells appear as bright spots. The border of the P1+ and P2+ bands are indicated with arrows. Case 740. Bar = 1000 μm. (F) Same section in bright field. Bar = 1000 μm (G) Injection site of gold-lectin centered on the lateral vestibular nucleus. Case 740. Bar = 1000 μm. (H) Higher magnification of gold-lectin labeled Purkinje cells (arrowheads) on both sides of the unlabeled, zebrin immuno-reactive, Purkinje cells of P2+ (arrows). Case 740. Bar = 50 μm. (I) BDA-labeled climbing fibers in the left anterior lobe (arrowheads), following an injection of the contralateral caudal DAO (Fig. 2J). The P1+ and P2+ bands are indicated with arrows. The space between the strip of labeled climbing fibers and the P2+ band increases in the lobules IV and V. Case 953. Bar = 1000 μm. (J) Injection site of BDA in the right caudal DAO. Case 953. Bar = 1000 μm. Abbreviations: A, group A of the caudal medial accessory olive; B, group B of the caudal medial accessory olive; β, group Beta; C, group C of the caudal medial accessory olive; CrII, crus II; DAOc, caudal part of dorsal accessory olive; DC, dorsal cap; DLP, dorsolateral protuberance; F, fastigial nucleus; IA, anterior interposed nucleus; II–V, lobules II–V; L, lateral cerebellar nucleus; LV, lateral vestibular nucleus; MAOc, caudal part of medial accessory olive; oc, olivocerebellar fibers; P1–P5, zebrin-positive bands P1+–P5+; PM, paramedian lobule; VLO, ventrolateral outgrowth.

labeling was confined to P4+, with only a few labeled climbing fibers in the region of the X zone, lateral to P2+. In the posterior lobe labeling was present in P5+, medially within, and medial to the lateral, zebrin-positive pole of the copula and in the P3− band of the pyramis. In earlier studies, the climbing fiber projection of the MAOr to the anterior P4+ and the posterior P5+ bands was identified as the C_2 zone (Voogd *et al.*, 1993, 2003a, b; Voogd & Ruigrok, 1997).



The A_1, A_X, X, C_X and C_2 zones: Corticonuclear projections (Fig. 5)

Figure 5 illustrates four cases with injections of retrograde tracers in the fastigial and posterior interposed nuclei, and in an intermediate region known as the interstitial cell groups of Buisseret-Delmas *et al.* (1993). In case 548-r, with an injection of the medial cerebellar nucleus over its entire, rostrocaudal extent the Purkinje cell labeling in P1−, corresponding to the A_1 zone, was found in the anterior lobe and the lobulus simplex. The P1+ band was spared. In this case labeled Purkinje cells also were found in the zebrin-positive lobules VIb/c and VII. Labeling in these lobules was only present when the injection extended into the caudo-dorsal pole of the fastigial nucleus. In the pyramis P2+ and P2− contained labeled cells. Labeled Purkinje cells extended into the medial uvula, but spared the ventral uvula and nodulus. An injection of the lateral fastigial nucleus and the adjoining interstitial cell groups, in case 29-r labeled Purkinje cells in the P2+ (A_X) band and the satellite band a in the anterior lobe and the lobulus simplex. Labeling in the lobules VIb/c and VII was located laterally and extended far into the crura of the ansiform lobule. Labeled Purkinje cells in the pyramis and the uvula were present more laterally than in the previous case. In the pyramis they covered the bands P1− to P3−.

In cases 548-l and 29-l two strips of Purkinje cells were labeled in the anterior lobe, one lateral to P2+ and another one medial to and including P4+. These two strips of Purkinje cells correspond to the X and C_X zone, respectively. The injection site in these cases involved the interstitial cell groups, with a major extension into the posterior interposed nucleus in case 29-l. The injection of the posterior interposed nucleus in case 29-l was responsible for the Purkinje cell labeling within P4+, which extended throughout the lobulus simplex and across the crus I into P5+ of the crus II and the paramedian lobule. This projection zone terminated in the lateral, zebrin-positive, pole of the copula. The labeling in the anterior P4+ and the posterior P5+ bands corresponds to the C_2 zone, as pointed out in the previous paragraphs.

In both cases labeled Purkinje cells were present laterally within lobules VIb/c and VII. Labeling in the pyramis was located in P3− and/or P3+ and P4+. In the copula of case 548-l a strip of labeled Purkinje cells was observed medial to the zebrin-positive pole of this lobule.

In case 548-l labeling was also present in the anterior lobe, among the Purkinje cells of P3+. This labeling may have been due to spread of the tracer into the anterior interposed nucleus. Labeling was also observed in the zebrin-positive satellite band e of the copula.

COMMENTS

It can be concluded that Purkinje cell labeling in the anterior lobe and the lobulus simplex shifts laterally, with the more laterally situated injection sites. The A_1 zone (P1−) was labeled from the medial fastigial nucleus and the A_X zone (P2+) from the lateral fastigial nucleus and the adjoining interstitial cell groups. Strips of Purkinje cells, located lateral to P2+ and medial to P4+ in the lobules IV and V of the anterior lobe and the lobulus simplex, corresponding to the X and C_X zones respectively, were labeled from the interstitial cell groups. This nucleus may extend medial to the anterior interposed nucleus, into the region lateral to the medial cerebellar nucleus, which was included in the injection site in case 548-l. Purkinje cell labeling in the C_2 zone corresponding to anterior P4+ and posterior P5+, was obtained with injections of the posterior interposed nucleus. In the pyramis Purkinje cells in medial bands P1− and P2+ were labeled from injections of the fastigial nucleus, and the labeling shifted laterally, and involved the P3+, P3− and P4+ bands when the injection sites included the interstitial cell groups.

Fig. 3. Drawings of reconstructions of the rostral and dorsal surface of the anterior lobe, and the rostral, dorsal and caudal surface of the posterior lobe, showing the distribution of labeled Purkinje cells and climbing fibers of the B zone in four cases with injections of tracers in the lateral vestibular nucleus or the caudal DAO, respectively. Labeled Purkinje cells are indicated as filled circles, labeled climbing fibers as hatched strips. Zebrin-positive bands are shaded. Arrows indicate a non-labeled area, medial to the B zone and lateral to zebrin-positive band P2+, which contains the X zone (compare Figs. 4 and 5). The lower panels show the injection sites in black, in transverse sections passing through the lateral vestibular nucleus, and sections through, and in a horizontal projection of the dorsal accessory olive. Abbreviations: 1–7, zebrin-positive zones 1–7; a, satellite bands a; A, group A of caudal medial accessory olive; b, satellite band b; B, group B of caudal medial accessory olive; Beta, group Beta; C, group C of caudal medial accessory olive; COP, copula pyramidis; cr, restiform body; CrI, crus I of the ansiform lobule; CrII, crus II of the ansiform lobule; d, satellite bands d; DAOc, caudal portion of dorsal accessory olive; DAOr, rostral portion of dorsal accessory olive; dc, dorsal cap; DLH, dorsolateral hump; DLP, dorsolateral protuberance; DMC, dorsomedial crest; DMCC, dorsomedial cell column; DV, spinal vestibular nucleus; e, satellite band e; F, fastigial nucleus; IA, anterior interposed nucleus; ICG, interstitial cell groups; IP, posterior interposed nucleus; I–VII, lobules I–VII; L, lateral cerebellar nucleus; LV, lateral vestibular nucleus; MAOc, caudal portion of medial accessory olive; MAOi, intermediate portion of medial accessory olive; MAOr, rostral portion of medial accessory olive; MV, medial vestibular nucleus; NO, nodulus (lobule X); PMD, paramedian lobule; PY, pyramis (lobule VIII); SI, lobulus simplex; UV, uvula (lobule IX); VIa, b, c, lobules VIa, b and c.

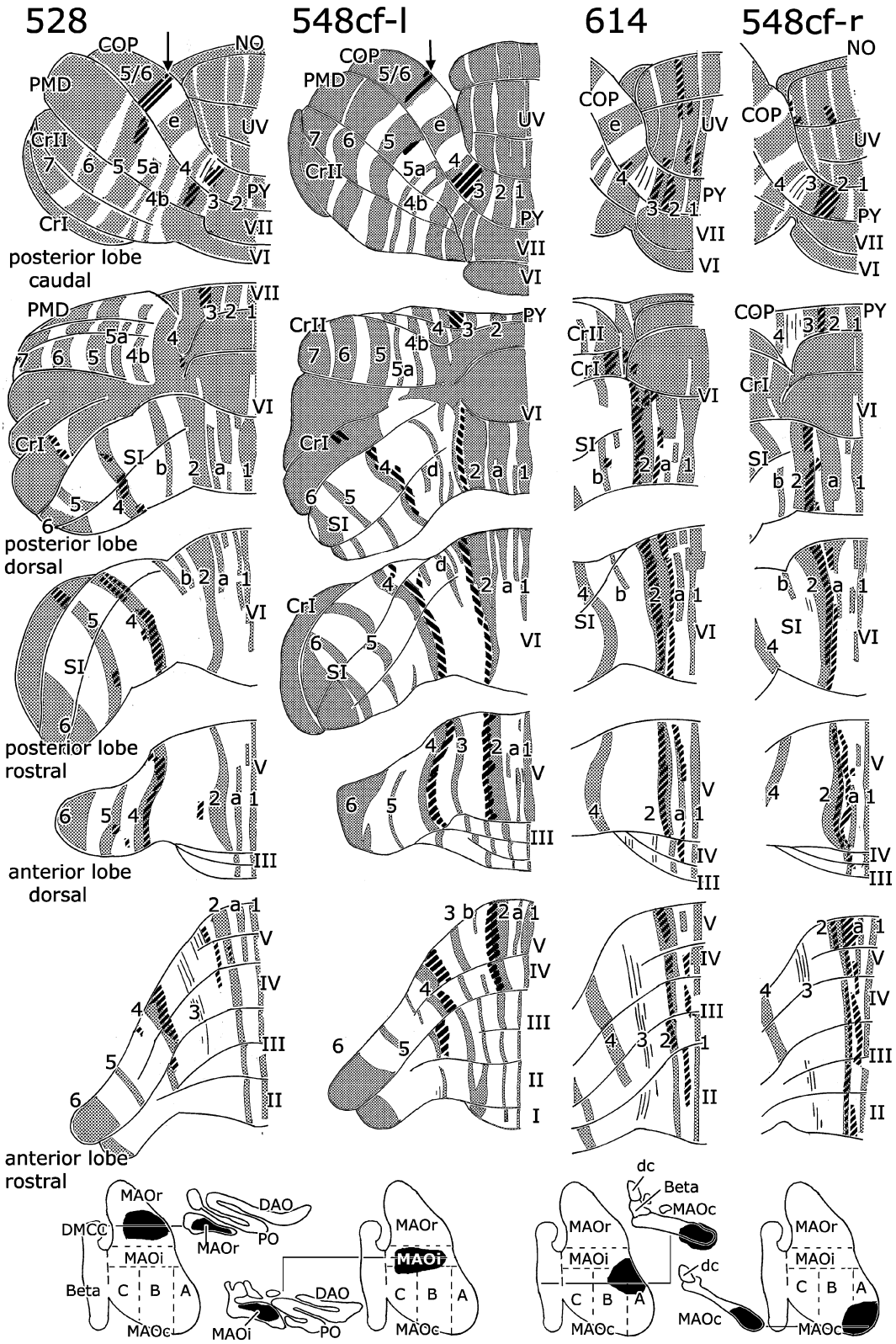


Fig. 4. Drawings of reconstructions of the rostral and dorsal surface of the anterior lobe, and the rostral, dorsal and caudal surface of the posterior lobe, showing the distribution of labeled climbing fibers in the A_1, A_X, X, C_X and C_2 zones in four cases with injections of tracers in the MAO. Conventions and abbreviations see Figure 3.

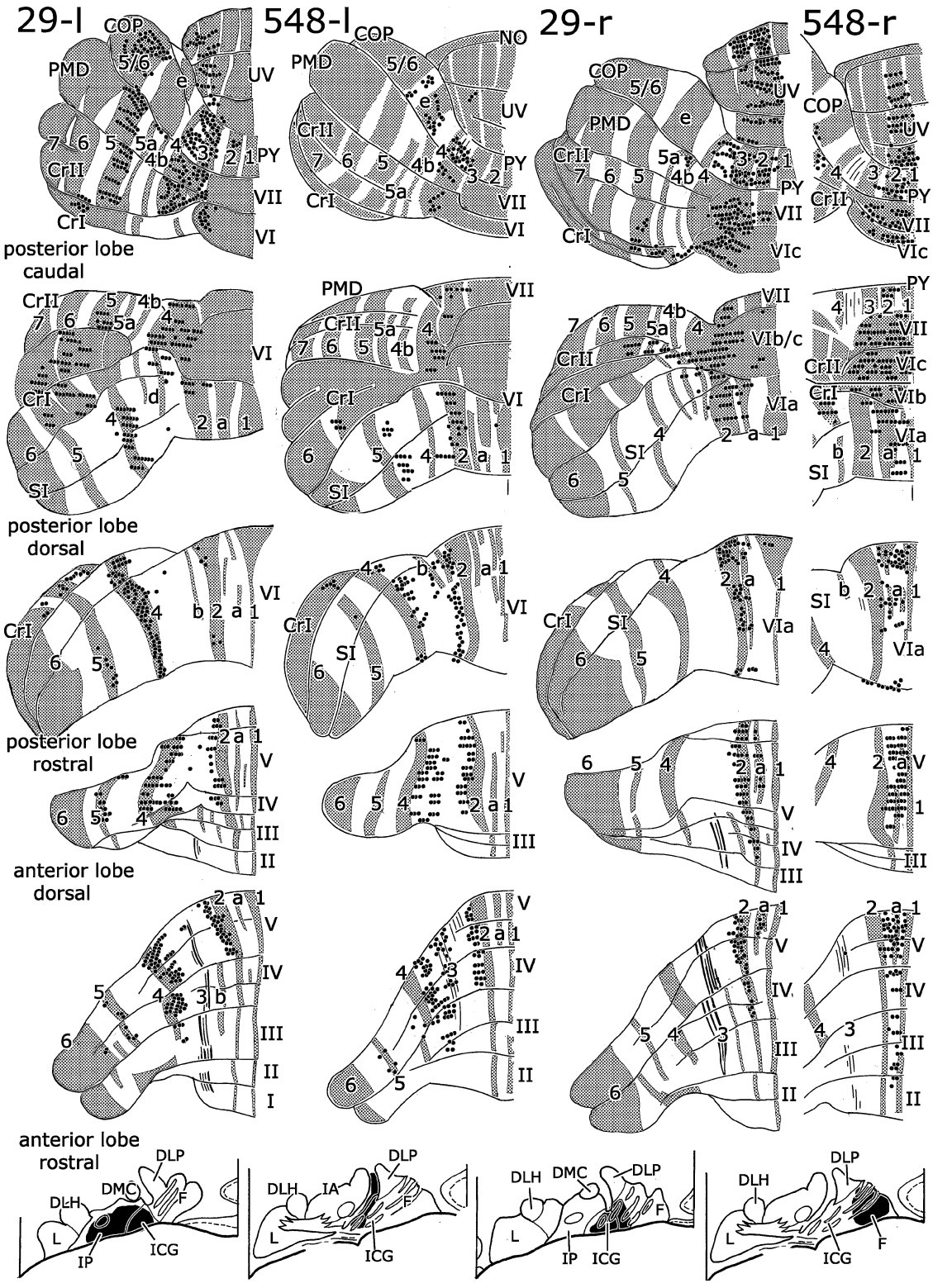


Fig. 5. Drawings of reconstructions of the rostral and dorsal surface of the anterior lobe, and the rostral, dorsal and caudal surface of the posterior lobe, showing the distribution of labeled Purkinje cells in the A_1, A_X, X, C_X and C_2 zones in four cases with injections of tracers in the fastigial nucleus, the interstitial cell groups and the posterior interposed nucleus. Conventions and abbreviations see Figure 3.

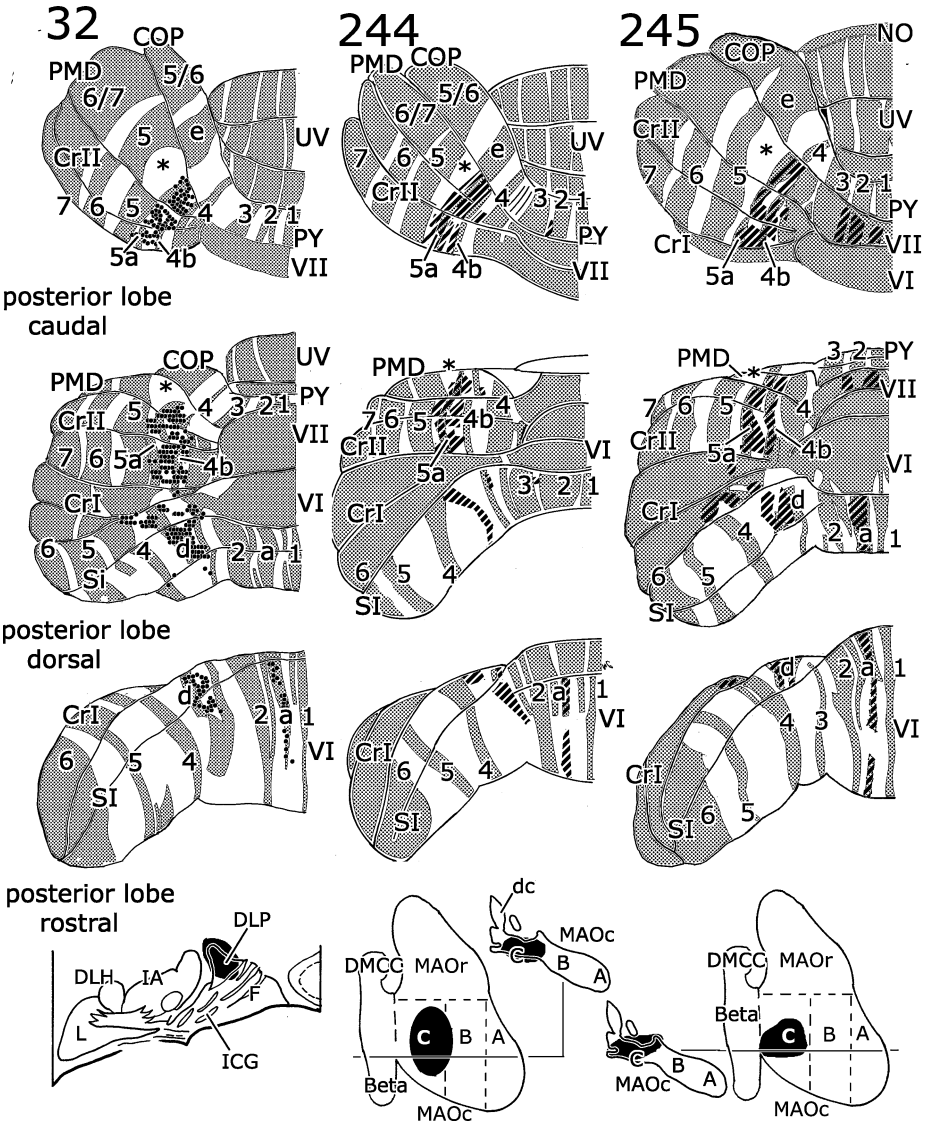


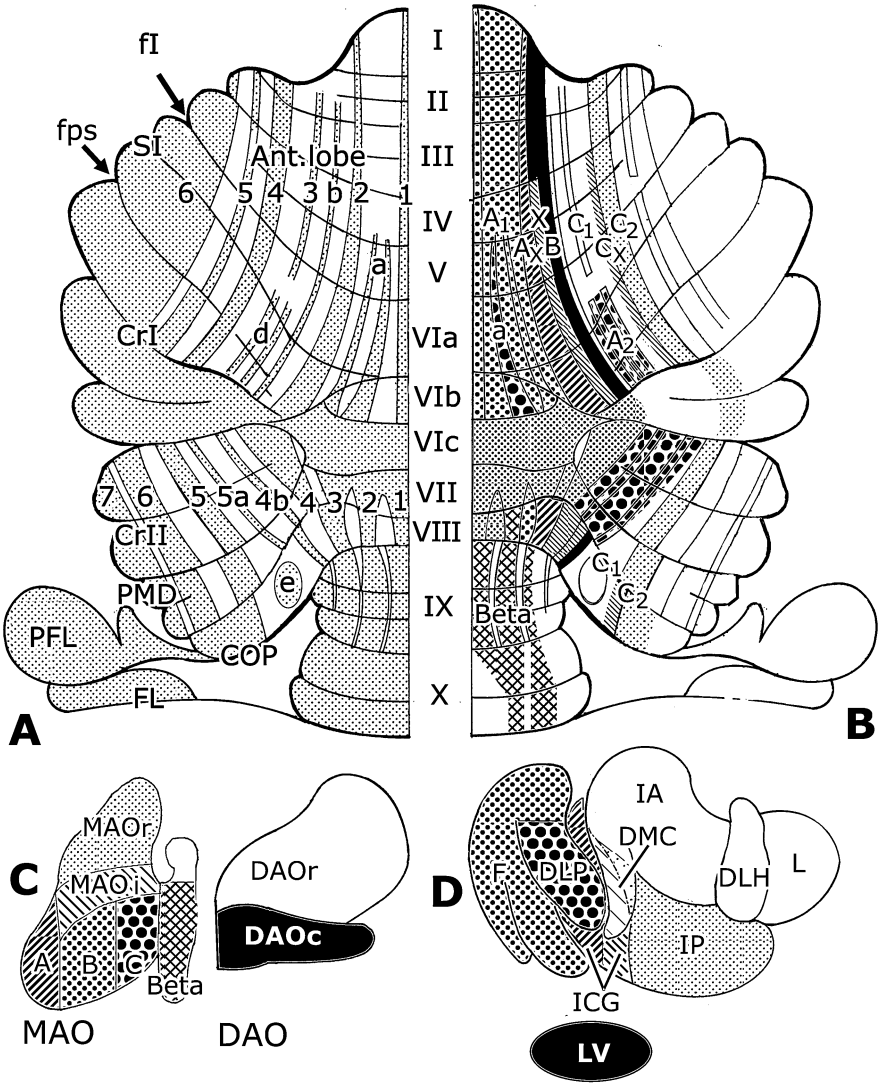
Fig. 6. Drawings of reconstructions of the rostral, dorsal and caudal surface of the posterior lobe, showing the distribution of labeled Purkinje cells and climbing fibers in the A_2 zone in three cases with injections of tracers in either the dorsolateral protuberance or group C of the caudal MAO. Asterisk indicates position of the C_1 zone in the paramedian lobule. Conventions and abbreviations see Figure 3.

Fig. 7. Diagram summarizing the localization of projection zones in the vermis of the cerebellum of the rat, their afferent and efferent connections, and their relation to the zebrin pattern. (A) Diagram of the zebrin pattern. (B) Projection zones are indicated with different symbols. The borders of the zebrin-positive zones are retained. The projection of the group Beta is based on a previous study (Voogd *et al.*, 1996b). (C) The origin of the olivocerebellar projection shown in horizontal projections of the medial and dorsal accessory olive. (D) Diagram of the target nuclei of the projection zones. (E) Key to the symbols indicating the various zones, and summary of their connections. Abbreviations: 1–7, zebrin-positive zones 1–7; a, satellite bands a; A, group A of caudal medial accessory olive; b, satellite band b; B, group B of caudal medial accessory olive; Beta, group Beta; C, group C of caudal medial accessory olive; COP, copula pyramidis; CrI, crus I of the ansiform lobule; CrII, crus II of the ansiform lobule; d, satellite bands d; DAOc, caudal portion of dorsal accessory olive; DAOr, rostral portion of dorsal accessory olive; DLH, dorsolateral hump; DLP, dorsolateral protuberance; DMC, dorsomedial crest; DMCC, dorsomedial cell column; e, satellite band e; F, fastigial nucleus; fI, primary fissure; FL, flocculus; fps, posterior superior fissure; IA, anterior interposed nucleus; ICG, interstitial cell groups; IP, posterior interposed nucleus; I–X, lobules I–X; L, lateral cerebellar nucleus; LV, lateral vestibular nucleus; MAOc, caudal portion of medial accessory olive; MAOi, intermediate portion of medial accessory olive; MAOr, rostral portion of medial accessory olive; PFL, paraflocculus; PMD, paramedian lobule; SI, lobulus simplex.

THE A_2 ZONE (FIG. 6)

The A_2 zone can be identified by anterograde labeling of climbing fibers from subnucleus C of the MAOc. Its distinguishing feature, however, is its projection to the dorsolateral protuberance of the medial cerebellar nucleus (Goodman *et al.*, 1963), which also receives a collateral climbing fiber projection from subnucleus C of the MAOc (Ruigrok & Voogd, 2000).

In case 32 (Fig. 6) an injection of the dorsolateral protuberance (Fig. 2B) resulted in labeling of Purkinje cells in the lobulus simplex, lobule VII, the crus I and II and the paramedian lobule. No labeled cells were present



E

olivary subnucleus	MAOc B	MAOc A	MAOi	DAOc	MAOc C	Beta	?	MAOr
zone	A_1	A_X	X C_X	B	A_2	Beta		C_2
target nucleus	F	F/ICG	ICG/IP	LV	DLP	F	F	IP
zebrin zone anterior	P1-	P2+	P2-/P3-	P2-	d bands			P4+
zebrin zone posterior	P2-	P3+	P3-/P4+ P4-	P4-	P4- P4b+/- P5a+/-	P1+/P3+		P5+

in the anterior lobe or the copula pyramidis. In the lobulus simplex A_2 occupied an intermediate region, containing the ill-defined satellite bands d. In the posterior lobe its labeled cells were found within and between the narrow bands P4b and P5a. The zebrin-negative band P5b− in the paramedian lobule (asterisk in Fig. 6) was spared. This region contains the C_1 zone (Voogd *et al.*, 2003a, b). In the lobulus simplex the lateral one of the satellite bands a was also labeled. Figure 2A is from case (577), with an injection of the dorsolateral protuberance extending into the fastigial nucleus. The Purkinje cell labeling extended from the P5a and P4b bands into P4+.

Two injections of group C of the MAOc (cases 244 and 245) are illustrated in Figures 2C, D and 6. In both cases the distribution of the labeled climbing fibers in an intermediate region and in one of the satellite bands a of the lobulus simplex, and in the 4b+ and the 5b± bands of the posterior lobe, closely corresponded with the Purkinje cell labeling from the dorsolateral protuberance in case 32. In addition, several narrow strips of climbing fibers were labeled in medial lobule VII, in case 245, with the more medial injection of group C.

COMMENTS

The A_2 zone (accessory A zone of Buisseret-Delmas, 1988a) originally was discovered by Goodman *et al.* (1963) in the rat, as a Purkinje cell zone in the medial hemisphere of the posterior lobe, projecting to the dorsolateral protuberance of the fastigial nucleus. A dorsolateral protuberance can be recognized in rodents, marsupials and in the rabbit, but appears to be absent in carnivores and primates. The A_2 zone, therefore, is one of the rare examples of a species-specific variation in the zonal pattern of the cerebellum (Voogd *et al.*, 1998). Its corticonuclear projection, its climbing fiber afferent projection from subnucleus C of the MAOc, and its restriction to the medial hemisphere of the lobules VI and VII are in accordance with Buisseret-Delmas' (1988) account.

The A_2 zone was identified by Akaike (1992) as one of the two tecto-recipient zones in the cerebellum of the rat. His second tecto-recipient zone is located in medial lobule VII. Both are innervated by subnucleus C, which receives a strong afferent projection from the tectum. Olivary neurons projecting to the medial (lobule VII) and lateral (A_2) tectorecipient zones represent mixed, but separate populations. The population projecting to medial lobule VII is located more medially in subnucleus C. This is in accordance with the presence of climbing fiber labeling in medial lobule VII in case 245. The A_2 zone in the paramedian lobule of the rat was characterized by Atkins and Apps (1997) by the presence of climbing fiber evoked potentials from the ipsilateral face.

Summary and general comments

Our interpretation of the experiments with double-labeling for zebrin II, and climbing fibers or Purkinje cells from discrete injections of the cerebellar nuclei or the inferior olive respectively, is summarized in Figure 7. The A_1 zone corresponds to the zebrin-negative P1− bands in the anterior lobe and the lobulus simplex, and the P2− band of the pyramis. It projects to the fastigial nucleus and receives its climbing fibers from group B of the MAOc. A lateral strip of Purkinje cells in the anterior P1− band can be labeled both from the fastigial and the vestibular nuclei. In the diagram of Figure 7 the P1+ band was included in the A_1 zone, although it was not found to be labeled in our experiments.

The A_X zone corresponds to the zebrin-positive, anterior P2+ band and the posterior P3+ band. It projects to the lateral fastigial nucleus and the adjoining interstitial cell groups and receives a climbing fiber projection from group A of the MAOc. The X and C_X zones both project to the interstitial cell groups and receive their climbing fiber innervation from the intermediate MAO. They are located in zebrin-negative territory, lateral to P2+ and medial to P4+. They are restricted to lobules IV and V of the anterior lobe and the lobulus simplex. In the pyramis and the copula, X and C_X are represented by the P3− and P4+ bands and by a strip of Purkinje cells located at the junction of the lateral, zebrin-positive, pole of the copula, and by the more medial zebrin-negative P4− band. Olivary neurons may collateralize to both zones (Voogd *et al.*, 2003).

The B zone projects to the lateral vestibular nucleus, and is innervated by climbing fibers from the caudal DAO. The B zone consists of zebrin-negative Purkinje cells. In the lobules I to III of the anterior lobe it is located next to P2+ (A_X). In the lobulus IV and V and the lobulus simplex the X zone is intercalated between P2+ (A_X) and the B zone. In the copula pyramidis, a narrow strip, lateral to P4+, represents the B zone.

The A_2 zone is confined to the lobulus simplex, the crus II and the paramedian lobule. It projects to the dorsolateral protuberance, and receives its climbing fibers from group C of the MAOc. In the lobulus simplex it is located lateral to the B zone, in a region containing the satellite bands d. In the crus II and the paramedian lobule it coincides with the P4−, P4b± and P5a± bands. In the paramedian lobule it spares the P5b− band. P5b− contains the C_1 zone, which is innervated by the rostral DAO and projects to the anterior interposed nucleus (Voogd *et al.*, 2003a, b). The C_1 zone probably extends in the crus II as a narrow strip in the medial P5a− band. The C_2 zone corresponds to the anterior P4+ and the posterior P5+ bands. It projects to the posterior interposed nucleus and receives its climbing fiber innervation from the MAOr.

The topological relations of the zones of the anterior vermis and the pyramis are very similar. However, the pattern in the pyramis is shifted laterally as compared to the anterior vermis, by a region innervated by climbing fibers of the group Beta, corresponding to the P1+ and P2+ bands of the posterior vermis (Voogd *et al.*, 1996b).

Purkinje cells of the vermal lobules VIc and VII were found to project to the fastigial nucleus, but the topography of their climbing fiber afferents is still incompletely known. In this region multiple, narrow strips of climbing fibers from group C of the MAOc may interdigitate with similar strips innervated by the group Beta and the dorsomedial cell column (Apps, 1990).

There is not a one-to-one relationship of projection zones in the vermis to the zebrin pattern. Some of the projection zones are chemically homogeneous. This is the case for the zebrin-positive A_X and C_2 zones, and for the B, X and C_X zones, which occupy zebrin-negative territory. Other projection zones, such as the A_1 and A_2 zones, consist of a heterogeneous assembly of zebrin-negative Purkinje cells and zebrin-positive (satellite) bands. Some zones share the same zebrin-negative band with other projection zones, such as he B and the X zones which share the P2− band, and the C_X zone which shares P3− with C_1 (Voogd *et al.*, 2003a, b). Sharing of the same zebrin-positive band occurs in the uvula, where P1+ and medial P2+ are innervated by caudal group Beta, lateral P2+ and medial P3+ by rostral group Beta and lateral P3+ and P4+ by the dorsomedial cell column (Voogd *et al.*, 1996b).

The conclusion that the DAO innervates zebrin-negative territory (Voogd *et al.*, 2003a) is confirmed by these observations. For the MAO the situation is far more complicated. Apparently it consists of regions innervating zebrin-positive bands (MAOr, group A of MAOc), zebrin-negative territory (MAOi) and a mixture of zebrin-positive and -negative bands (subnuclei B and C of MAOc). As pointed out before, the subdivision of the MAO is a matter of convenience, rather than a morphological fact. The morphological evidence on the subdivision of the MAO is rather meager and histochemical in nature. Marani *et al.* (1977; see also Voogd *et al.*, 1996a) published maps on the distribution of acetylcholinesterase in the MAO of the cat and the rat, which show a mediolateral, tripartitioning of the caudal MAO, and allows the distinction of the MAOr as a separate subnucleus.

Zebrin bands in the anterior and posterior cerebellum, which correspond with the same projection zone, bear a different number. The C_2 zone, for instance, corresponds with the anterior P4+ band in the anterior cerebellum and with the P5+ band posteriorly. This shift in the numbering of zebrin bands, corresponding to particular projection zones, between the anterior and posterior cerebellum, can be explained by the absence of zebrin-negative bands in the crus I. The uniform zebrin-positive appearance of this region prevented Hawkes and Leclerc (1987) from establishing their continuity.

Our conclusions on the connections of the zones of the vermis of the cerebellum of the rat generally confirm the observations of Buisseret-Delmas *et al.*, as summarized by Buisseret-Delmas and Angaut (1992) and in Figure 1. The main differences between our findings and their diagram (Fig. 1) concern the more caudal extension of our group A of the MAOc, the distinction between the A_X and X zones, and the topographical relation of the A_2 zone, which, in our opinion, is located lateral to the B zone rather than medial to it.

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