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Topography of Olivo-Cortico-Nuclear Modules in the Intermediate Cerebellum of the Rat

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ABSTRACT

This study provides a detailed anatomical description of the relation between olivo-cortico-nuclear modules of the intermediate cerebellum of the rat and the intrinsic zebrin pattern of the Purkinje cells. Strips of climbing fibers were labeled using small injections of biotinylated dextran amine into either the medial or dorsal accessory olives, while, in some cases, simultaneous retrograde labeling of Purkinje cells was obtained using gold-lectin injections into selected parts of the interposed nuclei. Our data are represented in a new, highly detailed, cortical surface reconstruction of the zebrin pattern and in relation to the collateral labeling of the climbing fibers to the cerebellar nuclei. We show that the somatotopic regions of the dorsal accessory olive behave differently in their projections to essentially zebrin-negative regions that represent the C1 and C3 zones of the anterior and posterior parts of the cortex. The rostral part of the medial accessory olive projects to zebrin-positive areas, in particular to the P4+ band of the anterior lobe and lobule VI and to the P5+ band of the posterior lobe, indicating that C2 has two noncontiguous representations in the SL and crus 1. By relating the areas of overlap that resulted from the injections in the accessory olives, i.e., labeling of climbing fiber strips and patches of climbing fiber nuclear collaterals, with the results from the injections in the interposed nuclei, i.e., retrograde labeling of Purkinje cells and of inferior olivary neurons, direct verification of the concept of modular cerebellar connections was obtained. J. Comp. Neurol. 492:193–213, 2005.

Indexing terms: zebrin; Purkinje cells; modules; climbing fiber; inferior olive; cerebellar nuclei

Although the structure of the cerebellar cortex is uniform, it exerts its actions on quite different functional systems. This functional heterogeneity is probably based on specific in- and output relations (Llinás and Sasaki, 1989; Bloedel, 1992; Voogd and Ruigrok, 1997; Jörntell et al., 2000; Brown and Bower, 2001). The organization of the afferent and efferent connections of the cerebellar cortex allows the distinction of a number of parasagittal units or modules (Voogd and Bigaré, 1980; Buisseret-Delmas and Angaut, 1993). One of the biochemical markers supporting this organization pattern of the rodent cerebellar cortex is the distribution of zebrin II (MaBQ 113, aldolase-C) in alternating bands of zebrin-positive and -negative Purkinje cells (Ahn et al., 1994; Hawkes and Leclerc, 1987). This pattern is highly reproducible between animals and can therefore be used as a reference pattern (Voogd et al., 2003; Sugihara and Shinoda, 2004).

In the past, extensive research has been done on the distribution and branching patterns of the climbing fibers, which arise from the inferior olive. Different subnuclei of this nuclear complex have been shown to project in a specific pattern of 50–1,000-μm wide zones in the contralateral cerebellar cortex and to provide a collateral innervation of the cerebellar target nuclei of the Purkinje cells located in these zones (Buisseret-Delmas and Angaut, 1993; Ruigrok and Voogd, 2000). In addition, the GABAergic nucleo-olivary pathway connects the cerebel-

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lar nuclei with the appropriate subnuclei of the inferior olive (Ruigrok and Voogd, 1990; Ruigrok, 1997; De Zeeuw et al., 1998). These olivocerebellar and corticonuclear connections and recurrent loops together form a series of cerebellar modules, subserving the output of the cerebellar cortex. Some of these modules, which receive a somatosensory input from the periphery, have been shown to be constructed of narrower functional units in the cat and ferret (Andersson and Oscarsson, 1978; Ekerot and Larson, 1980; Garwicz, 1997). A microzone consists of a narrow strip of Purkinje cells innervated by climbing fibers sharing the same peripheral receptive field. Microzones in the somatosensory zones of the cerebellum have been demonstrated to be arranged in somatotopical patterns (Brown and Bower, 2002; Jörntell and Ekerot, 2003). Recently, Sugihara et al. (2001) showed that the terminal axonal arborizations of individual olivary neurons were confined to narrow ~200–300-μm wide strips in the anterior and posterior cerebellum, which may represent the anatomical substrate of the microzones in the rat. Indeed, it proved possible to correlate the organization of climbing fiber zones to the patterning of zebrin bands in increasing detail (Gravel et al., 1987; Voogd et al., 2003; Sugihara and Shinoda, 2004; Voogd and Ruigrok, 2004). However, no detailed information is available on the projections of the rostral parts of the medial and dorsal accessory olives and the dorsomedial group (r-MAO, DAO, and DM, respectively), and their possible microzonal organization to the intermediate regions of the cerebellum. In this article we report on small injections of biotinylated-dextran-amine (BDA) in the r-MAO, the DAO, and in the DM of the rat. Moreover, in several cases an additional injection with wheat-germ-agglutin bovine-serum-albumin complex conjugated to gold particles (WGA-BSA-gold, hereafter referred to as gold-lectin) was made in the contralateral cerebellar nuclei in order to trace the corticonuclear projections (Ruigrok et al., 1995). In this way we could compare olivocortical and olivonuclear projections with corticonuclear projections in the same and between animals, using the zebrin-banding pattern as a reference. These descriptions will be necessary in order to enable and evaluate future manipulation of selected cerebellar modules.

To present our data, a new, detailed, two-dimensional surface reconstruction of the entire cerebellar cortex displaying the zebrin pattern was used, based on reconstructions of individual lobules as originally developed by Ruigrok (2003).

The results agree well with studies done by Voogd et al. (2003) investigating the collateralization of climbing fibers to the paramedian lobule (PMD) and the copula pyramidis (COP), representing the hemispheres of lobule VII and VIII, respectively, and with those conducted by Sugihara and Shinoda (2004), who investigated climbing fiber projections to the cerebellar cortex in relation to the zebrin pattern. The present study, however, provides new insight into the interrelation of the C zones between the anterior and posterior parts of the cerebellar cortex. In addition, our study relates the zonal climbing fiber patterns to the terminal arborizations of the climbing fiber collaterals within the cerebellar nuclei and, together with the gold-lectin injections, provides anatomical evidence of the existence of interrelated olivo-cortico-nuclear modules.

Abbreviations

AIN	anterior interposed nucleus
β	subnucleus beta
BDA	biotinylated-dextran-amine
BSA	bovine serum albumin
COP	copula pyramidis
DAB	3,3′-diaminobenzidinetetrahydrochloride
DAO	dorsal accessory olive
DC	dorsal cap
df-DAO	dorsal fold of DAO
dl-LCN	dorsolateral part of LCN
DLH	dorsolateral hump
DLP	dorsolateral protuberance
dl-PO	dorsal leaf of PO
DM	dorsomedial group
DMCC	dorsomedial cell column
ICG	interstitial cell groups
i-MAO	intermediate medial accessory olive
L	left
LCN	lateral cerebellar nucleus
LVN	lateral vestibular nucleus
MCN	medial cerebellar nucleus
NHS	normal horse serum
PB	phosphate buffer
PBS	phosphate-buffered saline
PIN	posterior interposed nucleus
PMD	paramedian lobule
PO	principle olive
R	right
r-MAO	rostral medial accessory olive
SL	simple lobule
SVN	superior vestibular nucleus
TBS	Tris-buffered saline
vl-PO	ventral leaf of PO
VLO	ventrolateral outgrowth
Vm-LCN	ventromedial part of LCN
WGA	wheat germ agglutin

MATERIALS AND METHODS

Surgical procedures

Thirty male adult Wistar rats (Harlan, Horst, The Netherlands) were used in this study. All procedures adhered to the NIH *Guide for the Care and Use of Laboratory Animals* according to the principles expressed in the Declaration of Helsinki and were approved by a National Committee overseeing animal welfare.

Animals were anesthetized with a ketamin/xylazine mixture (100 mg/kg + 3 mg/kg) administered intraperitoneally (i.p.). Surgical levels of anesthesia were monitored by the absence of rhythmic whisker movements and pinch withdrawal reflex. When necessary, supplementary doses were administered to maintain surgical levels of anesthesia. During surgery, body temperature was monitored and kept within physiological limits. Postoperative analgesia was provided by a single subcutaneous dose of buprenorphine (0.05 mg/kg). All animals received at least one BDA injection (Molecular Probes, Leiden, The Netherlands, mol. wt. 10,000, 10% solution in 0.05 M phosphate buffer (PB)) aimed at the rostral part of the inferior olive. Because the olivocerebellar projections are strictly contralateral, bilateral BDA injections were made in several animals (Ruigrok and Voogd, 2000; Sugihara et al., 2001). In addition, selected cases received an injection of gold-lectin in the contralateral anterior or posterior interposed nucleus (AIN or PIN, respectively, n = 19). The gold-lectin is a strictly retrograde transported tracer (for details, see Ruigrok et al., 1995). Surgical procedures can be recapitulated as follows. All animals were placed in a stereotac-

tical head holder (Paxinos and Watson, 1986). Skin and neck musculature were cleaved in the midline, exposing the dura. In the case of an additional gold-lectin injection in the cerebellar nuclei, a small craniotomy was performed exposing cerebellar lobules IX, VIII, and VII. Subsequently, the atlanto-occipital membrane and dura were cut and the flaps were folded to the side. All injections were placed under guidance of stereotactical coordinates and extracellular recordings, using obex as a reference point. Once position was determined, an electrode (tip diameter ~8 μm) filled with 10 μl, 10% BDA was advanced into the brainstem at an angle of 45° with the vertical axis. The site of injection was chosen as judged by the depth of the pipette and the number of encountered layers showing the specific firing pattern of olivary neurons and an ensuing iontophoretic BDA injection was made (4 μA, 7 seconds on, 7 seconds off, for 10 minutes). A similar procedure was followed for injections in the interposed nuclei except that a double-barrel electrode was used (tip diameter ~15 μm). The sealed part of the electrode was filled with 4.0 M NaCl to obtain extracellular recordings to distinguish between Purkinje cell layer, white matter, and cerebellar nuclear neurons. The open barrel was filled with the conjugate and connected to a pressure injection device (Ruigrok et al., 1995). Alignment of the gold-lectin injection site with the placement of the olivary injection was attempted by knowledge of the relation of the organization of olivary projections to the cerebellar nuclei (Ruigrok and Voogd, 2000). Nevertheless, only 5 out of 19 cases with double injections could be used in the present study. When the typical cerebellar nuclear firing pattern was recorded at the appropriate depth, a small pressure injection of 10–50 nl was made, after which the electrode was withdrawn. Subsequently, the dura was replaced, overlying layers were sutured, and the animals were allowed to recover.

After a 7-day survival period the animals were sacrificed under deep anesthesia with pentobarbital (240 mg/kg i.p.). After an initial transcardial flush of 500 ml saline (0.9% NaCl in 0.05 M PB), 1 L of fixative was used (4% paraformaldehyde, 0.05% glutaraldehyde and 4% sucrose, in 0.05 M PB, pH 7,4). The brains were collected and postfixed for an additional 3 hours, after which they were stored overnight in 0.05 M PB, containing 10% sucrose at 4°C. Subsequently, the brains were embedded in 11% gelatin, 10% sucrose. The blocks were hardened in 30% formalin, 30% sucrose solution for 3 hours, and were stored overnight in 0.05 M PB containing 30% sucrose at 4°C.

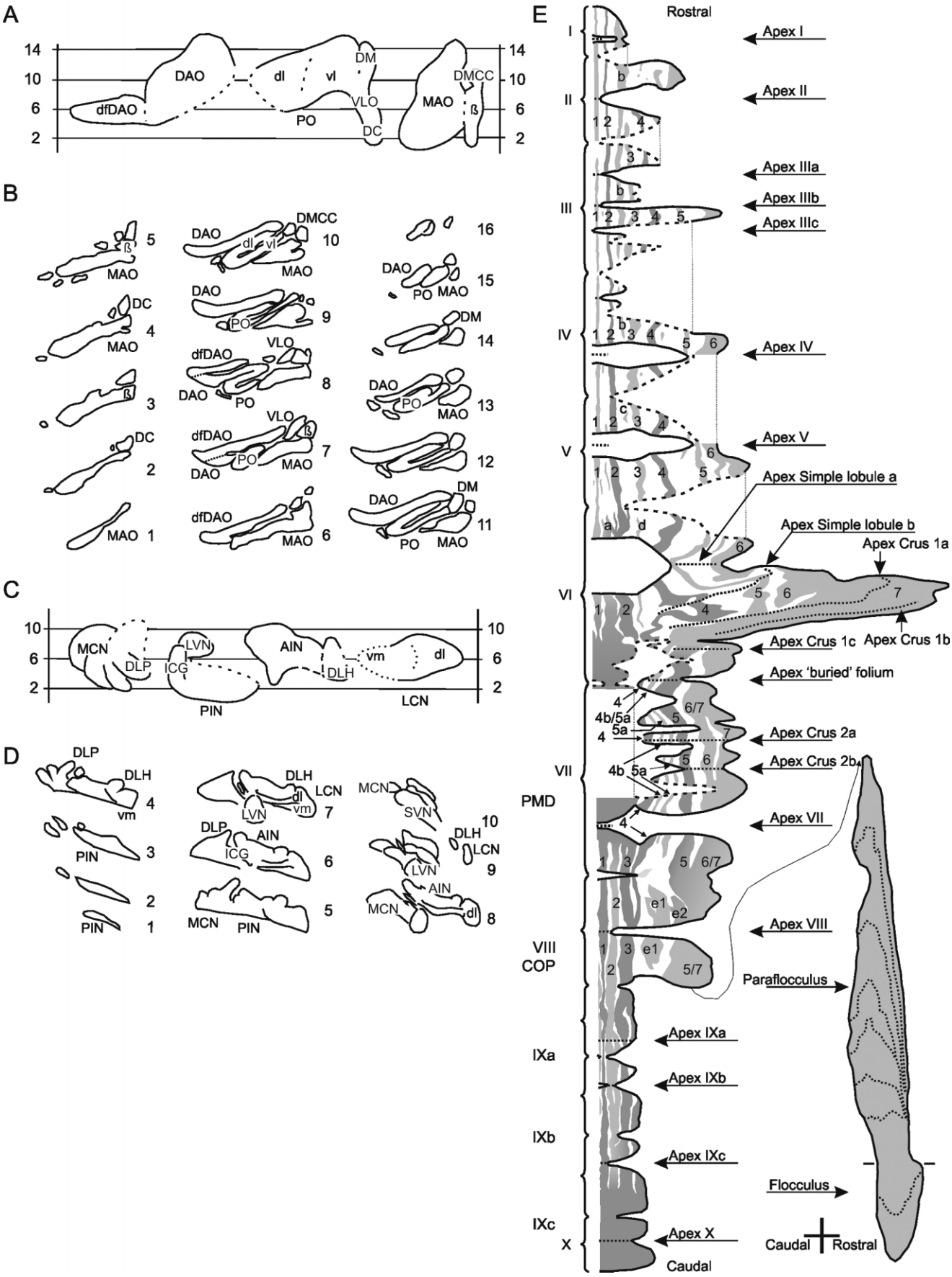
Histochemical procedures

Transverse sections were cut at 40 μm on a freezing microtome and collected serially in eight glass vials containing 0.05 M PB. For each animal, two vials were processed in a BDA protocol and two additional vials were processed for a combined BDA-zebrin-immunostaining, thus yielding a complete one out of four series of sections. All four vials were rinsed with phosphate buffered saline (PBS). The sections were incubated, freefloating, overnight at 4°C in ABC-elite solution (Vector Laboratories, Burlingame, CA; PK6100 1 drop A and 1 drop B in 10 ml PBS containing 0,5% Triton X-100). Subsequently, sections were rinsed in 0.05 M PB. The two vials for BDA staining only were incubated in 3,3′-diaminobenzidine tetrahydrochloride (DAB: 37.5 mg DAB, 25 μl H_2O_2 30%, in 150 ml 0.05 M PB) for 20 minutes. The other two vials were incubated in DAB-cobalt (1.5 ml $CoSO_4$ 1% and 1.5 ml $NiSO_4$ 1% added to the DAB protocol). All vials were rinsed in 0.05 M PB. Next, the vials processed for zebrin staining were rinsed in Tris-buffered saline (TBS, 0.05 M Tris, 0.9% NaCl, pH 7,6) and the sections were incubated, free floating, for 48–72 hours in zebrin II (antibody kindly provided by Dr. R. Hawkes, Calgary, 1:150 in Tris-buffered high saline, 0.05 M Tris, 0.5 M NaCl, 0.05% Triton X-100 (TBS+) containing 2% normal horse serum (NHS)) at 4°C. After rinsing in TBS+, sections were incubated for 2 hours in rabbit antimouse HRP (p260 Dako, Carpinteria, CA; 1:200 in TBS+, containing 2% NHS). Subsequently, sections were thoroughly rinsed in 0.05 M PB and incubated in a second DAB staining for 15–20 minutes and rinsed in 0.05 M PB. In case of an additional gold-lectin injection, the sections were processed, 2 × 10 minutes, for silver intensification of the gold label (Aurion, Wageningen, The Netherlands; 1:1, R-Gent enhancer and R-Gent developer). All sections were mounted on slides in a chromic alum solution, air-dried, and counterstained with thionin. Subsequently, slides were dehydrated in graded alcohol and xylene and coverslipped with Permount.

Analysis

In all cases, injection sites were assessed in the non-zebrin-stained sections by examining the inferior olive and contralateral cerebellar nuclei. Some cases had injections into the right (R) and left (L) olivary complex, and are referred to by their case number and the addition of R or L, respectively. However, for convenience, all injections are represented in standardized diagrams representing the left inferior olive and its projection in diagrams of the right cerebellar nuclei and a reconstruction of the right cerebellar cortical surface (Ruigrok, 2003; Fig. 1A–D). Terminology of divisions of the cerebellar nuclei was adapted from Korneliussen (1968; also see Voogd, 2004), and for the inferior olive it was based on descriptions by Bernard (1987; also see Azizi and Woodward, 1987; Ruigrok, 2004).

A general map of the zebrin-banding pattern was reconstructed from three animals. The reconstruction was performed along the lines of earlier cerebellar cortex reconstructions made by Ruigrok (2003). Figure 1E shows a cortical surface reconstruction indicating the unfolded and outstretched Purkinje cell layer with the zebrin-banding pattern superimposed. Roman numbers indicate individual lobules and arrows are used to indicate their apices. The zebrin-positive bands are presented in gray and the numbers and letters in these bands refer to the zebrin-positive compartments from the nomenclature of Hawkes and Leclerc (1987) and modified by Voogd and Ruigrok (2004) and by Sugihara and Shinoda (2004). The corresponding negative compartments lie immediately lateral to the positive compartments. In the rostral half of lobules II, IV, VI, and VIII, the caudal half of lobule VII and in lobule V some indistinct zebrin-positive compartments were observed that were not named because they were not clearly present in all our cases. Some of these bands were identified by Sugihara and Shinoda (2004), who used a different zebrin marker. All other bands were highly reproducible between animals. Hence, this reconstruction could be reliably used to accurately indicate the climbing fiber labeling resulting from the various BDA injections (see below). We have used the term "buried folium" for a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VI
VII
PMD
VIII
COP
IXa
IXb
IXc
X
Apex I
Apex II
Apex IIIa
Apex IIIb
Apex IIIc
Apex IV
Apex V
Apex Simple lobule a
Apex Simple lobule b
Apex Crus 1a
Apex Crus 1b
Apex Crus 1c
Apex 'buried' folium
Apex Crus 2a
Apex Crus 2b
Apex VII
Apex VIII
Paraflocculus
Apex IXa
Apex IXb
Apex IXc
Flocculus
Apex X
Caudal
Caudal
Rostral

Figure 1

folium that is completely covered by crus 1c (Welker, 1987). Sugihara and Shinoda (2004) indicated this folium as crus 1c.

Based on the size of the injection and verification of olivo-nuclear (BDA injections) or nucleo-olivary (gold-lectin injections) projections, the location of tracer uptake was determined (Ruigrok and Voogd, 1990, 2000). From the outcome of these results, animals were either included or excluded from the study. Inclusion criteria were: injection site restricted to the rostral parts of the inferior olive (level 8 or higher); injection site restricted to only one subnucleus or only slight involvement of a second. All zebrin sections of two series were photographed with a digital camera (Leica DC300) and printed on A-4 paper, while preserving relative size. In these prints the zebrin pattern, labeled climbing fiber collaterals, and in several cases gold-lectin labeled Purkinje cells were depicted using sections from all four series and a Leica-microscope (objectives 20× and 40×). The intersection interval was therefore 80 μm. To indicate data of individual cases into our standard diagram of the zebrin pattern, the photographs of all sections were matched and subsequently labeling was drawn into the unfolded cerebellar surface reconstruction. Photo panels were constructed in CorelDraw 11.0 using digitally obtained microphotographs that were saved in TIF format. Some correction for brightness and contrast was performed in Corel Photopaint 11.0.

RESULTS

The effective BDA injection site in the inferior olive was found to be limited to the dark-stained core as defined by Ruigrok and Voogd (1990, 2000). This view was supported by the distribution of anterograde-labeled terminal arborizations within the cerebellar nuclei. In most of our cases the effective injection site had an approximate diameter of 200–400 μm. Figure 2A shows the injection in a case where it was centered on the rostral part of the medial accessory olive (r-MAO) and resulted in a fine varicose plexus of terminal arborizations of collaterals in the medial part of the contralateral posterior interposed nucleus (PIN: Fig. 2B). In addition to the collateral labeling in the cerebellar nuclei, all cases resulted in anterogradely labeled climbing fibers, which were usually arranged in longitudinal strips in multiple lobules in both the anterior and posterior parts of the contralateral cerebellar cortex (Fig. 2C–F).

Injections centered on the rostral part of the medial accessory olive

Nine cases were selected in which the BDA injection was mostly or completely confined to the r-MAO. In two of these cases an additional injection with gold-lectin was centered on the PIN (Fig. 6D). They are divided into four sets of surface reconstructions, which are illustrated in Figure 3. The first set consists of case 877L only, with an injection at the rostral border of the intermediate MAO (i-MAO; olivary levels 8 and 9; Fig. 3A,B1); its cortical labeling is shown in Figure 3E. In the cerebellar nuclei, varicose terminal labeling was found in the interstitial cell groups (ICG: Buisseret-Delmas et al., 1993) and in the rostrolateral part of PIN (Fig. 3C1,D1). Climbing fiber labeling was located in two main strips located in lobules V and VI, i.e., one directly adjacent to the P2+ band within the P2- band, and the other in the P3- band, adjacent to and, at times somewhat within, the P4+ band. In the posterior lobe climbing fiber labeling was present in the P3- and P5a- bands of the caudal paramedian lobule (PMD), where it extended into the rostral part of the copula pyramidis (COP). In contrast to all other cases with r-MAO injections, no labeled climbing fibers were observed in the paraflocculus.

The injections of the next three sets of cases, illustrated in Figure 3, were located at successively more rostral levels of the r-MAO. The first of these set (cases 883R and 881L) were centered at levels 9 and 10 of the r-MAO (Fig. 3A,B1). The more caudal injection (case 883R) produced collateral labeling in the caudomedial PIN (Fig. 3C1,D1) and climbing fiber labeling in the zebrin-positive anterior P4+ and the posterior P5+ bands (Fig. 3F). The labeling in P4+ extends from lobule IV into the simple lobule (SL); P5+ labeling is present in the COP and the caudal PMD but also involves crus 1 and SL. Both in P4+ and P5+, the labeled climbing fibers occupy a medial position. Case 881L forms a transition with the next set of three cases in which the injections extended more rostrally into levels 11–13 of the r-MAO (003R, 880R, 891R: Fig. 3A,B2,C2,D2,G). The collateral projections to the PIN now occupied central and more caudolateral portions of the PIN. In the cerebellar cortex one main band of labeling can be recognized located in the P4+ zebrin-band of the anterior lobe and the SL and in the P5+ band of the posterior lobe from the crus 1a to the PMD and extending in P5/7+ of lobule VIII (Fig. 3G). Labeled climbing fibers generally took up more lateral positions compared to case 881L. Like cases 881L and 883R, labeling was also seen in the caudal part of the paraflocculus.

Injections in the rostralmost tip of MAO form the last set of cases (879R, 001R and 871R; olivary levels 12–15, Fig. 3A,B3) and gave rise to collateral labeling in largely overlapping areas in the caudolateral part of the PIN (Fig. 3C3,D3). Note that in case 871R there was some involvement of the ventral leaf of the principal olive (vl-PO), resulting in collateral labeling in the ventromedial part of the lateral cerebellar nucleus. In the cerebellar cortex (Fig. 3H), labeled climbing fibers predominated in the SL and the crura of the ansiform lobule. Here, in cases 879R

Fig. 1. Standardized diagrams of the inferior olive, cerebellar nuclei, and cerebellar cortex with zebrin pattern. These diagrams were used to indicate injections (solid contours) into the inferior olive (BDA) and cerebellar nuclei (gold-lectin) and projections (stippled contours in the inferior olive and/or cerebellar nuclei, and colored lines in the cortex). **A:** The "unfolded" and flattened dorsal view of the left inferior olivary complex; numbers refer to the level of the corresponding transverse sections (see B). **B:** Transverse diagrams of sections through the left inferior olivary complex, numbered from caudal (1) to rostral (16) at 160-μm intervals. **C:** The "unfolded" and flattened dorsal view of the right cerebellar nuclei, based on the transverse series shown in D. **D:** Transverse diagrams of sections through the right cerebellar nuclei, numbered from caudal (1) to rostral (10) at 160-μm intervals. A–D are adapted from Ruigrok and Voogd (2000). **E:** Standardized diagram of the unfolded and flattened right half of the cerebellar cortex in which the zebrin-positive bands are indicated. Note that the orientation of the paraflocculus and flocculus is rotated 90° clockwise and both lobules are separated from the hemispheral part of lobule VIII (copula). At the midline vermal lobules I–X are indicated, hemispheral lobules are indicated to the right. The apexes of the lobules are indicated with fine-hatched lines. For the unfolding method, see Materials and Methods and Ruigrok (2003). For abbreviations, see list.

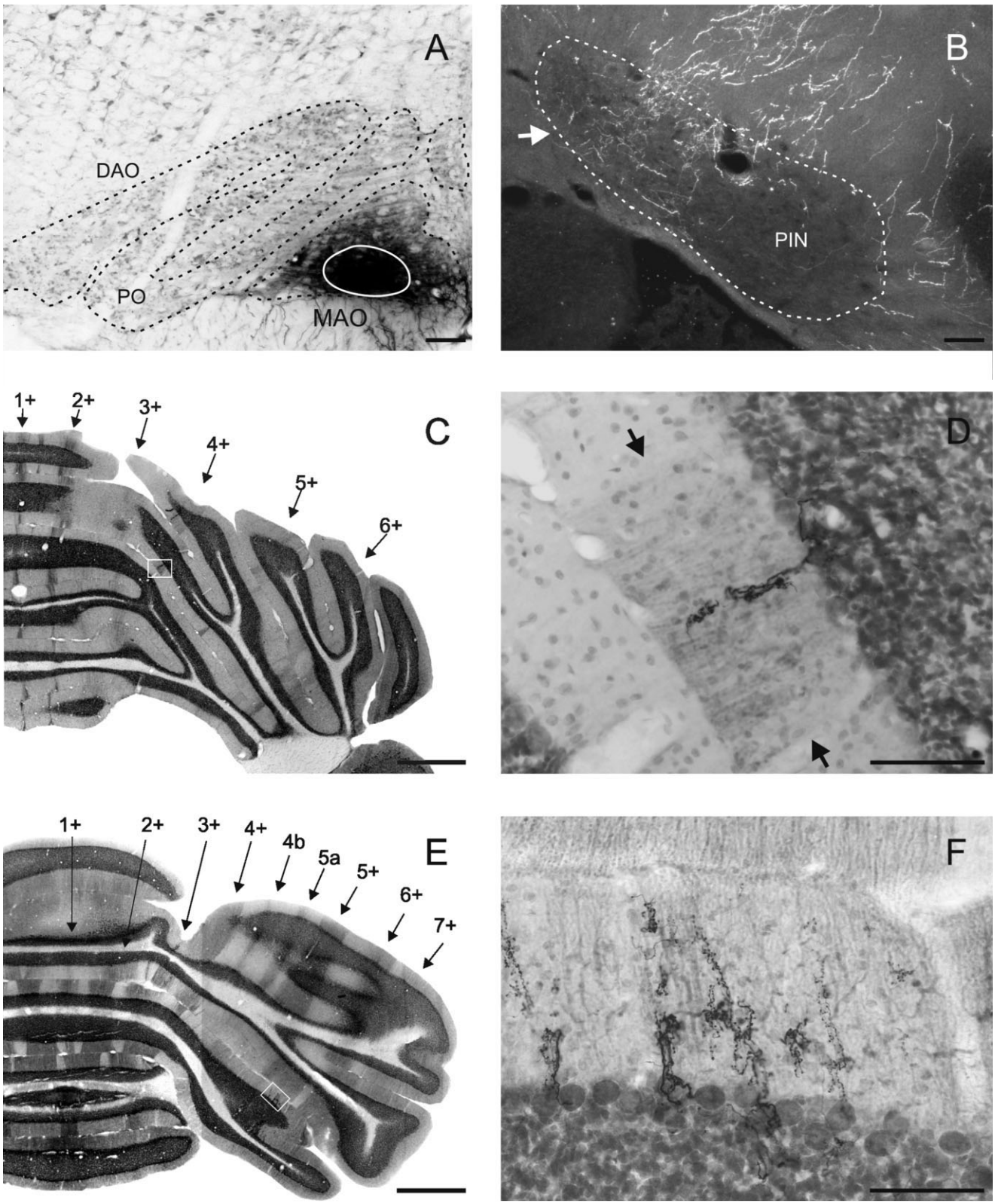

Fig. 2. Microphotographs showing examples of BDA injection site, terminal labeling in the cerebellum, and the zebrin pattern. **A:** BDA injection site in the rostral MAO of case 883R. The presumed area from which tracer uptake and transport originated is indicated by the white oval. **B:** Resultant fine varicose terminal labeling in the medial part of the PIN (arrow). **C:** Overview of a zebrin-immunostained section through the anterior part of the cerebellum; the zebrin-positive bands are indicated and the boxed-in area is enlarged in D. **D:** Double labeling of BDA-labeled climbing fiber (black) and zebrin-positive Purkinje cells (between arrows) belonging to the P4+ band of lobule IV. **E,F:** As in C and D for the posterior part of the cerebellum. Note that the labeled climbing fibers are found within the Pe2+ zebrin band. For abbreviations, see list. Scale bars = 100 μm in A,B,D,F; 1 mm in C,E.

and 001R, labeling was restricted to P5+, where they took up a lateral position. Climbing fiber labeling in case 871R was more widespread and included both zebrin-negative compartments flanking P5+ and the zebrin-positive P6+ and P7+ band in the posterior lobe. Climbing fiber labeling in the central lobules of the paraflocculus had shifted to a more caudolateral position.

In Figure 6A–D,K, two of the above-described cases are shown. However, here the BDA injection was combined with a gold-lectin injection in the cerebellar nuclei (cases 891L and 001L). The rather small injection in case 891L gave rise to Purkinje cell labeling in the anterior P4+ and posterior P5+ bands, which overlapped with the climbing fiber labeling in these bands (Fig. 6K). The gold-lectin injection in case 001L covers a large portion of the PIN and resulted in labeling of the P3+, P4+, P4-, and P5+ bands in the anterior lobe, as well as some labeling lateral to and within the P2+ band. Within the posterior lobe, most retrograde-labeled Purkinje cells were confined to P4+ and P5+, and incorporated patch e of the COP. Overlap with the climbing fiber labeling was restricted to P5+ in the posterior lobe. Both injections gave rise to retrogradely labeled neurons in the r-MAO, which were largely (case 891) or only partially (case 001) coexisting with the BDA injection site. In case 001L, labeled cells were also found in the vl-PO and within the dorsomedial group (DM) of the PO, suggesting that some of the gold-lectin tracer had spread beyond PIN into the lateral cerebellar nucleus (LCN) and the dorsolateral hump (DLH). The involvement of these nuclei may be responsible for the additional Purkinje cell labeling in the uvula and lateral nodulus in case 001L (Voogd et al., 1996).

In summary, five key observations can be derived from these results: 1) injections located at the border of i-MAO and r-MAO label two stripes of climbing fibers, mostly within zebrin-negative territory, immediately lateral to the P2+ and medial to P4+ in lobules V and VI and lateral to P3+ and medial to P5+ in PMD and COP; 2) injections into the r-MAO label the anterior P4+ and posterior P5+ bands; 3) more rostral injections result in shifts of climbing fiber labeling to more lateral positions within the P4+ and P5+ bands and, in SL and crus 1, also from P4+ to P5+; 4) a rostral shift in r-MAO results in lateral shift in the position of collaterals to PIN; 5) Purkinje cells in anterior P4+ and posterior P5+ project to the PIN.

Injections centered on the dorsal accessory olive

Seventeen cases were selected in which the BDA injection was mostly or completely restricted to the DAO (Fig. 4). In three of these cases an additional gold-lectin injection in the anterior interposed nucleus (AIN) was made (Fig. 6E-I,L). In some cases there was a slight involvement of the dorsal leaf of the PO.

All cases were grouped in three sets of injections. The first set incorporates five cases with injections centered on the caudal half of the DAO (954L, 016R, 881R, 015L, and 875L: olivary levels 6–10; Fig. 4A1,A3). In the cases with involvement of the dorsal fold of the DAO (df-DAO; all cases except 875L), collateral labeling of climbing fibers was present in the lateral vestibular nucleus (LVN) and resulted in climbing fiber labeling in the anterior P2- band of lobules I–VI and in the posterior P4- band of the COP and caudal PMD. This typical projection pattern is particularly evident in case 954L, where the injection was restricted to the df-DAO. Close inspection of the labeling in the anterior P2- revealed that it remained separated from P2+ in lobules IV–V by a narrow, zebrin-negative space. Climbing fibers originating from the i-MAO occupy this space (cf. Fig. 3E; also see Voogd and Ruigrok, 2004). P2- labeling in cases 015L, 016R, and 881R was overlapping with those seen in case 954L (Fig. 4D) and, thus, was taken to originate from the df-DAO also. Therefore, additional labeling in these cases and in case 875L must have resulted from the involvement of the vf-DAO. The vf-DAO climbing fiber labeling in this group of experiments shares several features. They all provided a collateral projection to the caudomedial part of the AIN (Fig. 4A2,A4). Furthermore, they invariably supplied, possibly collateral, labeling to the two zebrin-negative bands that flanked the anterior P4+ (i.e., P3- and P4-) as well as the posterior P5+ bands (P4-/P5a- and P5-). Moreover, labeling in anterior P4- always extended more rostrally compared to the labeling in anterior P3- (to lobules II and IV, respectively). Similarly, in the posterior lobe labeling from the two medialmost injection sites (cases 875L and 881R) expanded further rostrally in P5- than in P4-. Finally, only scant labeling was observed in the caudal SL and both crura.

The second set involves five cases where the injection was centered on the rostrolateral part of the vf-DAO (884R, 950R, 001L, 950L, and 009R: olivary levels 11–16, Fig. 4B1,B3). In the AIN labeling had shifted to its rostromedial tip (Fig. 4B2,B4). Climbing fiber labeling extended into lobule II, but mostly spared the crura and rostral PMD (Fig. 4E). Again, labeling was distributed at both sides of the anterior P4+ band and extended more anteriorly in P4- (most clearly seen in cases 001L and 884R). The two rostromedial-most cases of this group (950L and 009R) were remarkable because labeled climbing fibers were situated on both sides of anterior P5+ in the zebrin-negative bands P4- and P5- and, in the posterior lobe, focused on the buried folium, flanking P5+. A potential origin of the labeled climbing fibers from the principal olive, which is slightly involved in both experiments, seems unlikely, because the principal olive is known to project to the zebrin-positive anterior P5+ and P6+, and the posterior P6+ and P7+ bands (Voogd et al., 2003; Sugihara and Shinoda, 2004).

The final set of injections includes seven cases, which were centered on the medial aspect of the vf-DAO (002L, 003L, 884L, 868L, 880L, 871L, and 896R: olivary levels 8–13; Fig. 4C1,C3). Their respective cortical projections are visualized in two diagrams (Fig. 4F,G). This set resulted in collateral labeling in the caudolateral part of the AIN, bordering on the dorsolateral hump (DLH: Fig. 4C2,C4). Note that the rostralmost injections projected to the rostralmost part of the lateral AIN. Climbing fiber projections to the cerebellar cortex were most abundantly found within the P4- of the caudal half of lobule V and the SL, where they took up more lateral positions compared to the previously described injections. Several cases also showed some labeling in P5- (884L, 868L, 871L: Fig. 4F,G), but this projection may, at least partly, be due to involvement of the DM group into the injection site (Fig. 4C1–4). Spread of the injection into the principal olive in cases 009R, 950L, 871L, and 884L is consistent with the presence of labeled climbing fibers in the P6+ and P7+ bands of the posterior lobe. When comparing all sets of cases, and apart from the particulars described above, a general tendency was noted that climbing fibers labeled

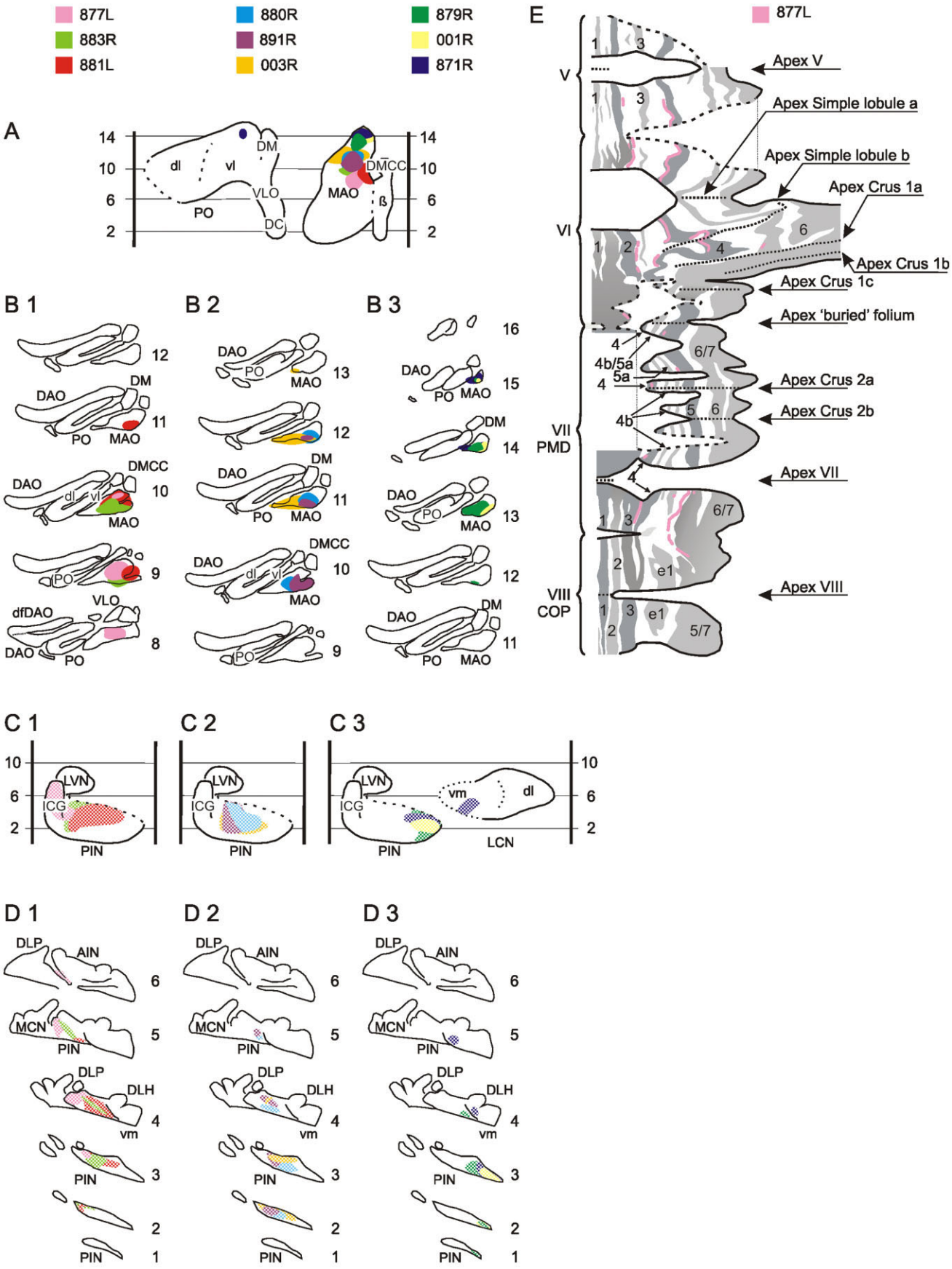

Figure 3 (Continued)

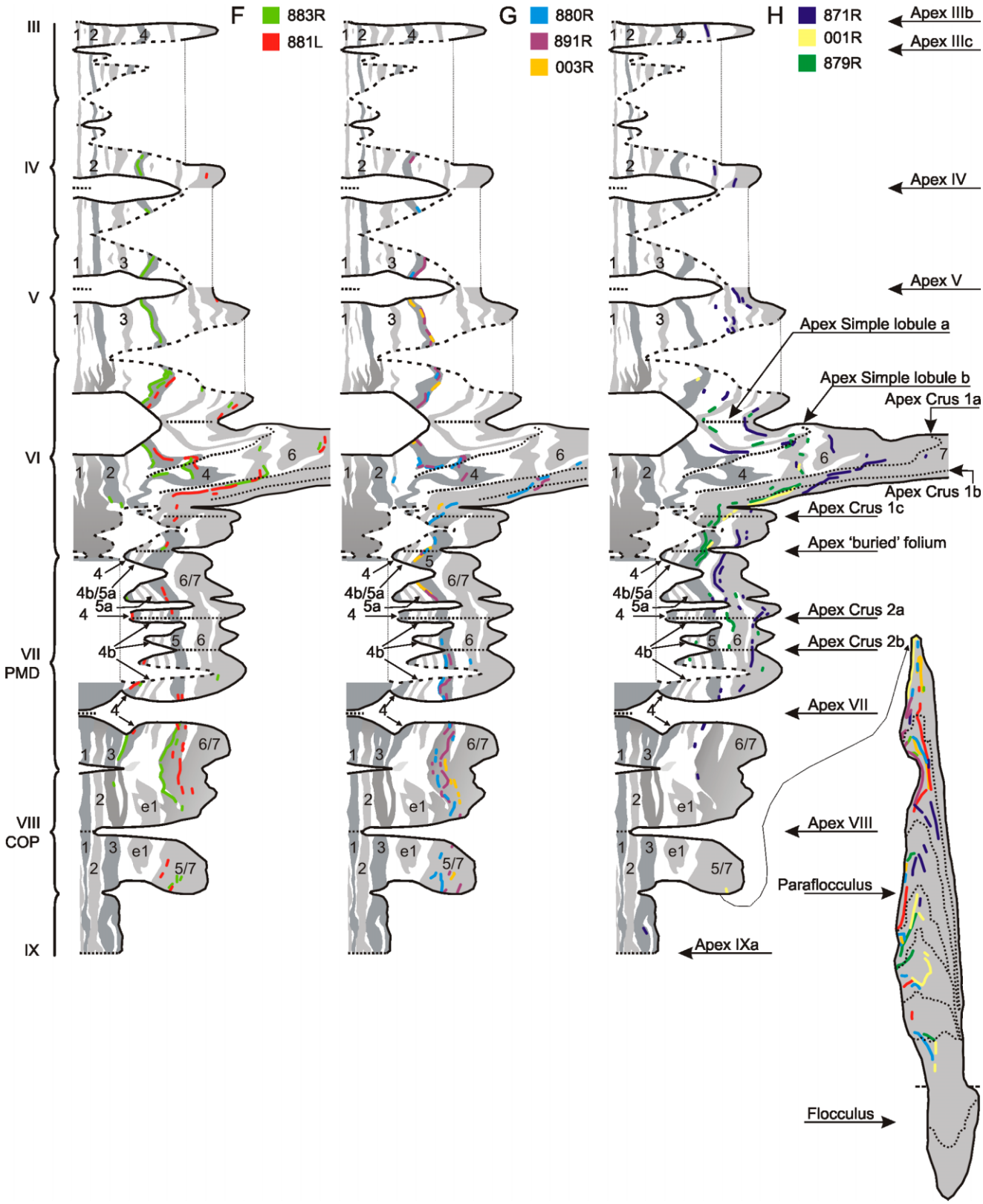

Fig. 3. Diagrams showing the results of nine cases with injections centered on the r-MAO. Case numbers and corresponding colors are indicated above each panel. **A,B:** Location of the injection sites (solid contours) shown in flattened and transverse olivary diagrams, respectively. Note that in A all cases are grouped together but are indicated in three panels of diagrams in B. **C,D:** Location of the labeled collaterals (stippled contours) in the cerebellar nuclei shown in flattened and transverse diagrams, respectively. For grouping and color-coding of cases see A,B. **E–H:** Distribution of labeled climbing fibers indicated relative to the zebrin pattern in the unfolded and flattened diagram of the cerebellar cortex (see Fig. 2). For all cases labeling to the paraflocculus has been entered in H. Note that the rostralmost r-MAO injections give rise to varicose terminal labeling in the lateral half of the PIN and labeled climbing fibers are mostly distributed within or directly adjacent to the P5+ band of lobules III–VI. See text for further explanation. For abbreviations, see list.

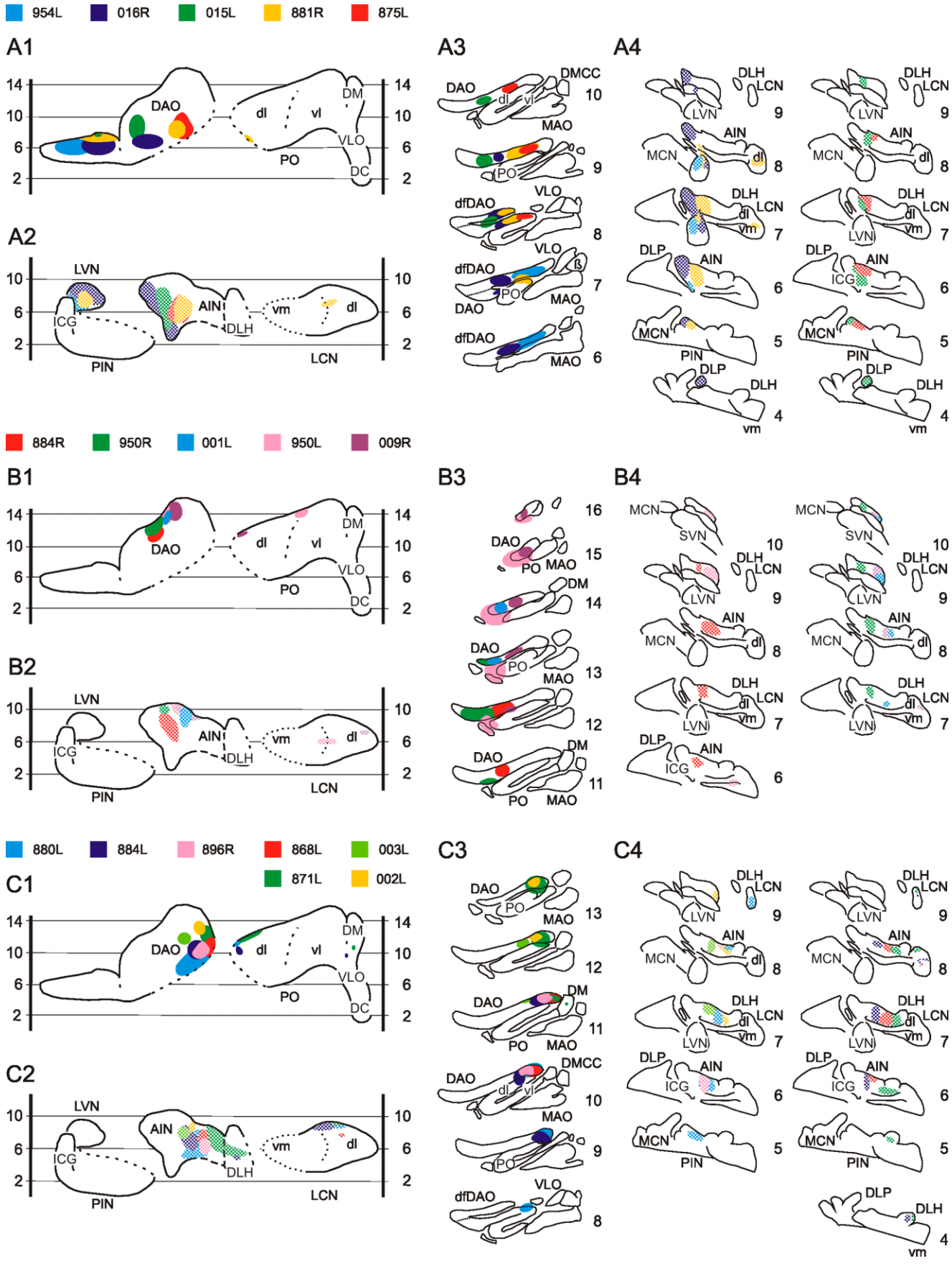

Figure 4 (Continued)

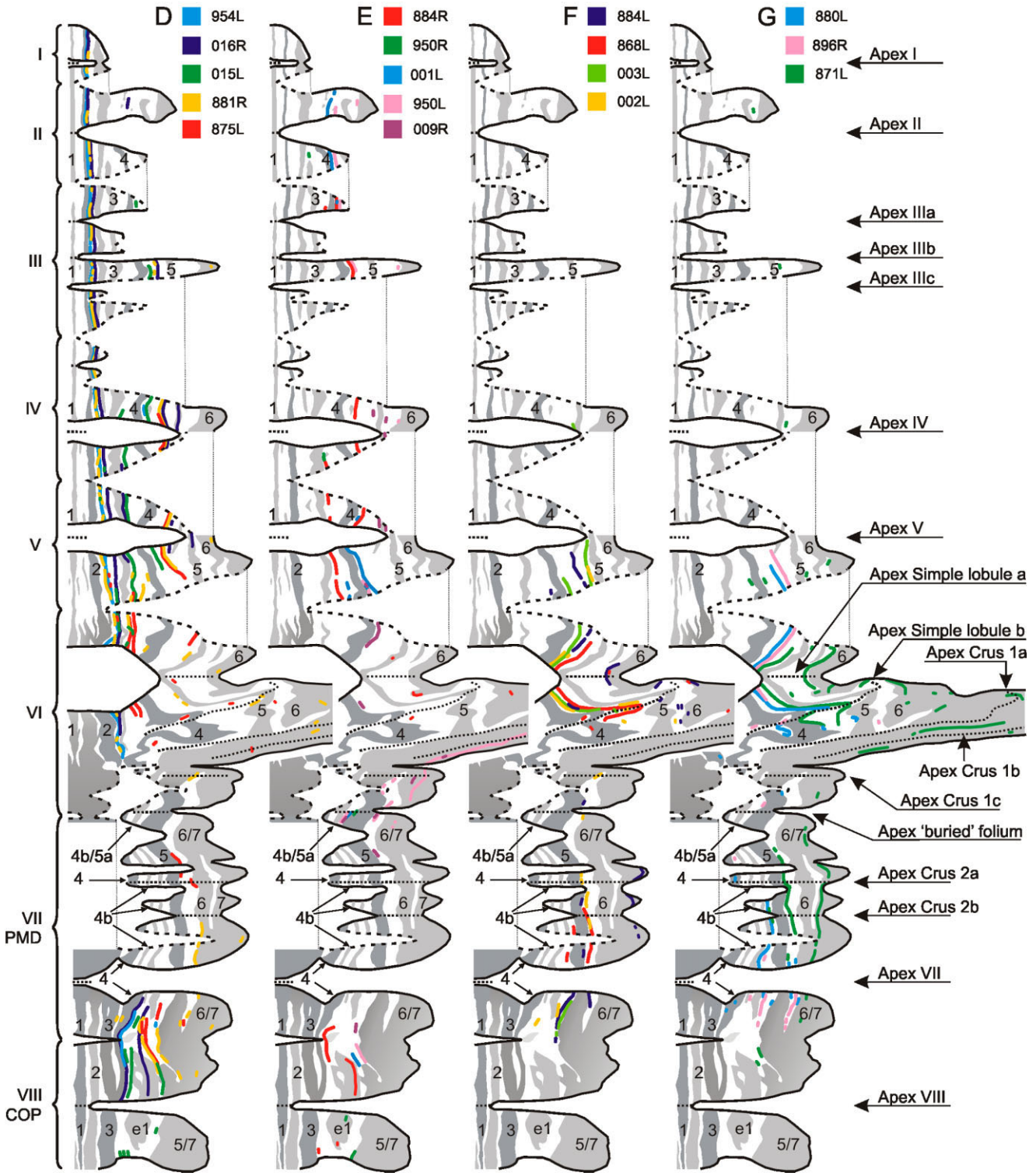

Fig. 4. Diagrams showing the injections of 17 cases with injections centered on the DAO. Case numbers and corresponding colors are indicated above each panel. **A1–C1** and **A3–C3:** Location of the injection sites shown in flattened and transverse olivary diagrams, respectively. **A2–C2** and **A4–C4:** Location of labeled collaterals in the cerebellar nuclei shown in flattened and transverse diagrams, respectively. For grouping of cases see A–C. **E–H:** Distribution of labeled climbing fibers indicated relative to the zebrin pattern in the unfolded and flattened diagram of the cerebellar cortex (see Fig. 2). A and E represent the caudal group, B and F represent the rostrolateral group, and C and G and H represent the rostromedial group. Note that between these three groups varicose terminal labeling generally shifts from medial to rostrolateral AIN and corresponds with a shift from medial positions in rostral and caudal cortex to more lateral positions in the central cerebellar cortex. See text for further explanation. For abbreviations, see list.

from more medial injection sites took up more lateral positions within the anterior P3- and P4- bands and the posterior P5- band than more lateral injection sites (compare Fig. 4E–G).

In Figure 6E–I,L, the results of three cases with gold-lectin injections that were centered on the AIN are shown (cases 001R, 002R, and 896L), as well as their accompanying BDA injections placed in the vf-DAO (cases 001L, 002L, and 896R, respectively). In all three cases zebrin-negative Purkinje cells were retrogradely labeled in P3- and P4-, flanking anterior P4+. The gold-lectin injection of 002R in the rostrolateral part of the AIN and the more medial injection of 896L resulted in continuous strips of retrograde-labeled Purkinje cells which, similar to the climbing fiber bands, reached more rostrally in P4- (in case 002R into lobule III), than in P3- (in case 002R into lobule V: Fig. 6I). In the posterior cerebellum, labeled Purkinje cells were found in the P4- band of the PMD and the rostral COP. A small patch of labeled Purkinje cells was present in P5- in case 002R. In agreement with the positioning of the gold-lectin injection sites, retrograde-labeled olivary cells were found in the appropriate locations of the vf-DAO. In case 002R the gold-lectin injection site clearly did not encompass the PIN, yet a number of retrogradely labeled neurons was found throughout the r-MAO (Fig. 6I) and PO (not shown). This labeling is likely to be related to the observation that the injection also involved the fibers directly dorsal to the AIN (Fig. 6H) and, as a pressure injection, may have caused some damage to the climbing fibers that course directly around the interposed nuclei (Ruigrok and Voogd, 2000; van der Want et al., 1989; Sugihara et al., 2001), resulting in inadvertent labeling of some olivary neurons.

It will be obvious that the location of the gold-lectin injections and of the BDA injection are related. E.g., in case 001R the retrograde gold-lectin labeling in the vf-DAO overlaps with the BDA injection site of case 015L (Fig. 4A), coinciding with apparent overlap of the gold-lectin injection site with the collateral labeling of climbing fiber collaterals in the AIN in case 015L. This conforms rather well to the distribution of retrogradely labeled Purkinje cells of case 001R and to the distribution of labeled climbing fibers in case 015L. Similar parallels can be drawn between, e.g., cases 896L and 002R with gold-lectin injections and cases 001L and 003L with BDA injections of the vf-DAO, respectively.

Obviously, in this material, matching of injection sites and projections can be directly evaluated within the same animal. Hence, it is specifically relevant to notice that in case 896L partial overlap was observed between the DAO territory containing gold-lectin retrogradely labeled neurons and the BDA injection deposit (Fig. 6I). Indeed, a corresponding area in the cerebellar nuclei that fell both within the gold-lectin injection site and received labeled climbing fiber collaterals was present (Fig. 6I). Finally, in the cerebellar cortex a region of overlap was found between the strips of gold-lectin-labeled Purkinje cells and strips of labeled climbing fibers (Fig. 6G,L). In cases 001L-BDA and 001R-gold-lectin no overlap in injection and resultant labeling was present either in the inferior olive or in the cerebellar nuclei. Hence, no overlap in the labeling of Purkinje cells and climbing fibers could be observed in the cerebellar cortex (Fig. 6I,L). A similar observation can be made for the injection pairs 002R-gold-lectin and 002L-BDA.

The following conclusions can be drawn from these results: 1) olivary neurons of the DAO supply climbing fibers to Purkinje cells of zebrin-negative areas only; 2) for the df-DAO these zebrin-negative zones involve the P2- band from lobules I–VI and medial P4- in posterior VII and VIII, for the vf-DAO they are located medial and lateral to P4+ (P3- and P4-) in the anterior lobe and SL, but on either side of P5+ in the posterior lobe; (3) the caudal and lateral vf-DAO projects to a single band in lobule VIII (lateral to P4+) and to two bands on either side of P4+ in the anterior lobe, whereas the medial vf-DAO projects to two bands in PMD and crus 2 (located on either side of P5+) but predominantly to a single band (P4-) in caudal lobule V, SL, and rostral crus 1. (4) Double injections confirm the modular and submodular organization of olivo-cortico-nuclear connections.

Injections centered on the rostral part of the dorsomedial group

In Figure 5, three cases (002R, 008R, and 013R) in which the BDA injection was restricted to the rostral DM are presented (olivary levels 13–16; Fig. 5A,B). Collateral labeling in the cerebellar nuclei was mostly restricted to the rostral part of the DLH (Fig. 5C,D) such that the injection in the rostralmost tip of the DM (case 002R) resulted in the rostralmost labeling within the DLH (levels 5–9; Fig. 5C,D). Labeled climbing fiber collaterals were found in the anterior P5- band in lobules II, III, IV, V, SL, crus 1a, and in the posterior P5- and P6- of the caudal half of buried folium and the rostral half of crus 2a (Fig. 5E). In cases 884L and 871L (Fig. 4A) the injection sites encroached upon the DM. Collateral labeling was found in small patches in caudal DLH. In the cortex strips of labeled climbing fibers were present in the zebrin-negative area medial to the P6/7+ band of SL and in case 871L in the posterior P6- compartment. It should be noticed that climbing fiber labeling in the medial anterior P5- was also present in cases 009R and 950L (Fig. 4B).

Summary

The projections of the accessory olives to the cerebellar nuclei and cortex, as established in this study, are summarized in Figure 6J,M. Projections from the i-MAO (green) are located in the ICG of the cerebellar nuclei and in the cortical X and CX zones that border the anterior P2+ and P4+ bands and posterior P3+ and P5+ bands. Projections from the r-MAO (blue) include the PIN and the C2 zone, which corresponds to the anterior P4+ band and the posterior P5+ band and extends in the caudal paraflocculus. Projections from successively more rostral levels of the r-MAO are indicated in lighter shades of blue. Note that both P4+ and P5+ receive climbing fibers in SL and crus 1. The projection of the df-DAO to the LVN and the B zone is indicated in yellow. Projections from the vf-DAO to the AIN and to the C1 and C2 zones occupy the zebrin-negative areas flanking anterior P4+ and posterior P5+. Different shades of red and orange indicate the topographical relations between the vf-DAO and its projection area. Arrows provide additional gradients in the relation between DAO and AIN. The projection of the DM to the DLH and the anterior P5- and posterior P6- bands (corresponding to the D0 zone) are indicated in pink. Projections from the vf-DAO to this zone have not been included in the diagram, and will be considered in the discussion.

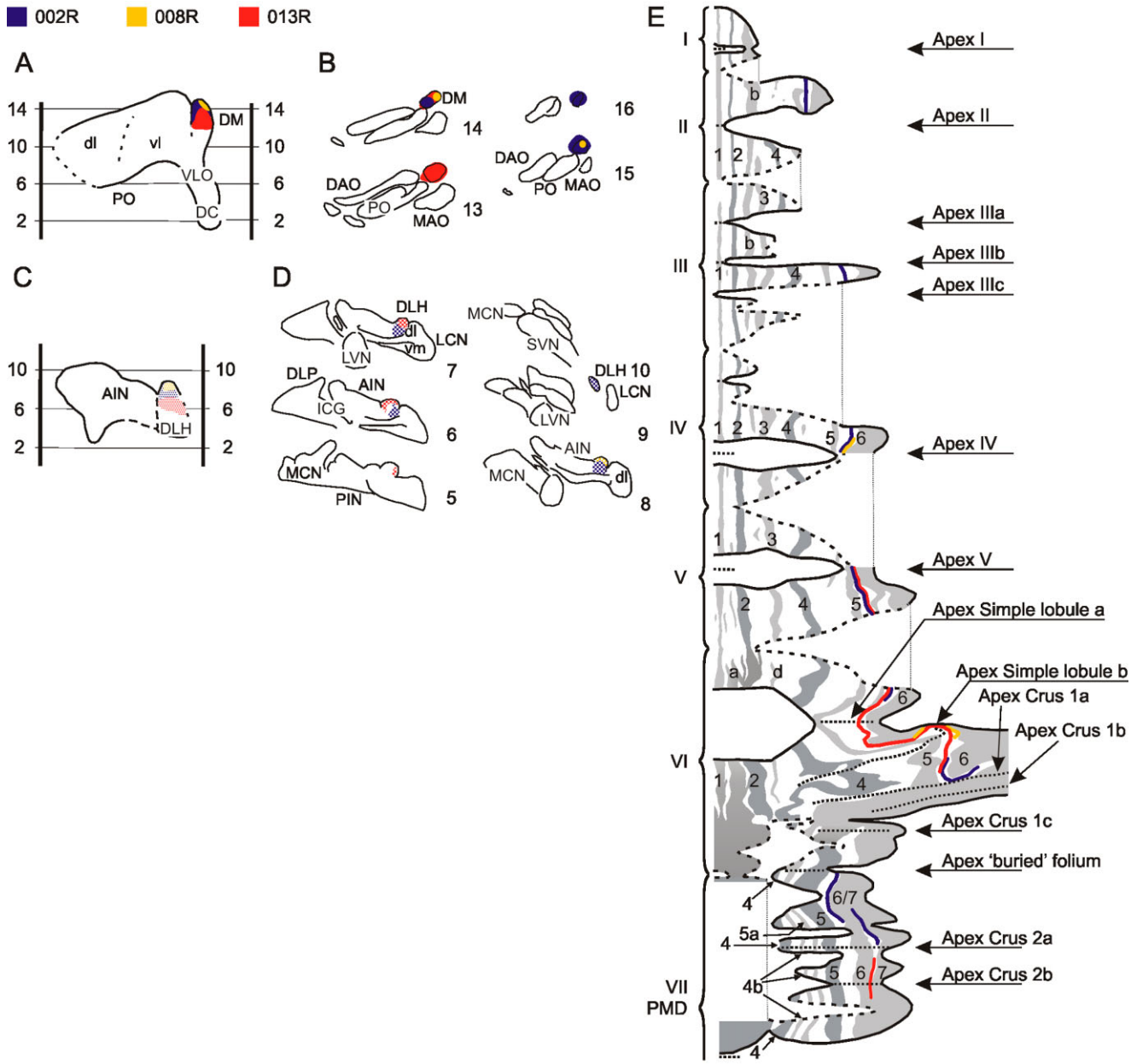

Fig. 5. Diagrams showing the injections of three cases with injections centered on the rostral DM group of the principal olive. Case numbers and corresponding colors are indicated above each panel. **A,B:** Location of the injection sites shown in flattened and transverse olivary diagrams, respectively. **C,D:** Location of labeled collaterals in the cerebellar nuclei shown in flattened and transverse diagrams, respectively. **E:** Distribution of labeled climbing fibers indicated relative to the zebrin pattern in the unfolded and flattened diagram of the cerebellar cortex (see Fig. 2). See text for further explanation. For abbreviations, see list.

DISCUSSION

The present study extends and integrates data from our previous studies in the rat on the topography of climbing fiber branching (Ruigrok, 2003; Voogd et al., 2003; Voogd and Ruigrok, 2004) and the interrelation between connections of the inferior olive and the cerebellar nuclei (Ruigrok and Voogd, 1990, 2000) by providing a detailed account of the projections of the accessory olives to the intermediate cerebellum. The cortical topography of climbing fiber projections in the rat has recently been the subject of a study by Sugihara and Shinoda (2004), but they did not consider the collateral innervation of the cerebellar nuclei, which provides important additional information on the organization of the olivocerebellar system, nor did they consider the relationship with the corticonuclear projection. Moreover, their experimental material on the connections of the rostral MAO and DAO was incomplete.

The zebrin pattern proved to be essential as a reference frame for the identification of zonal climbing fiber pat-

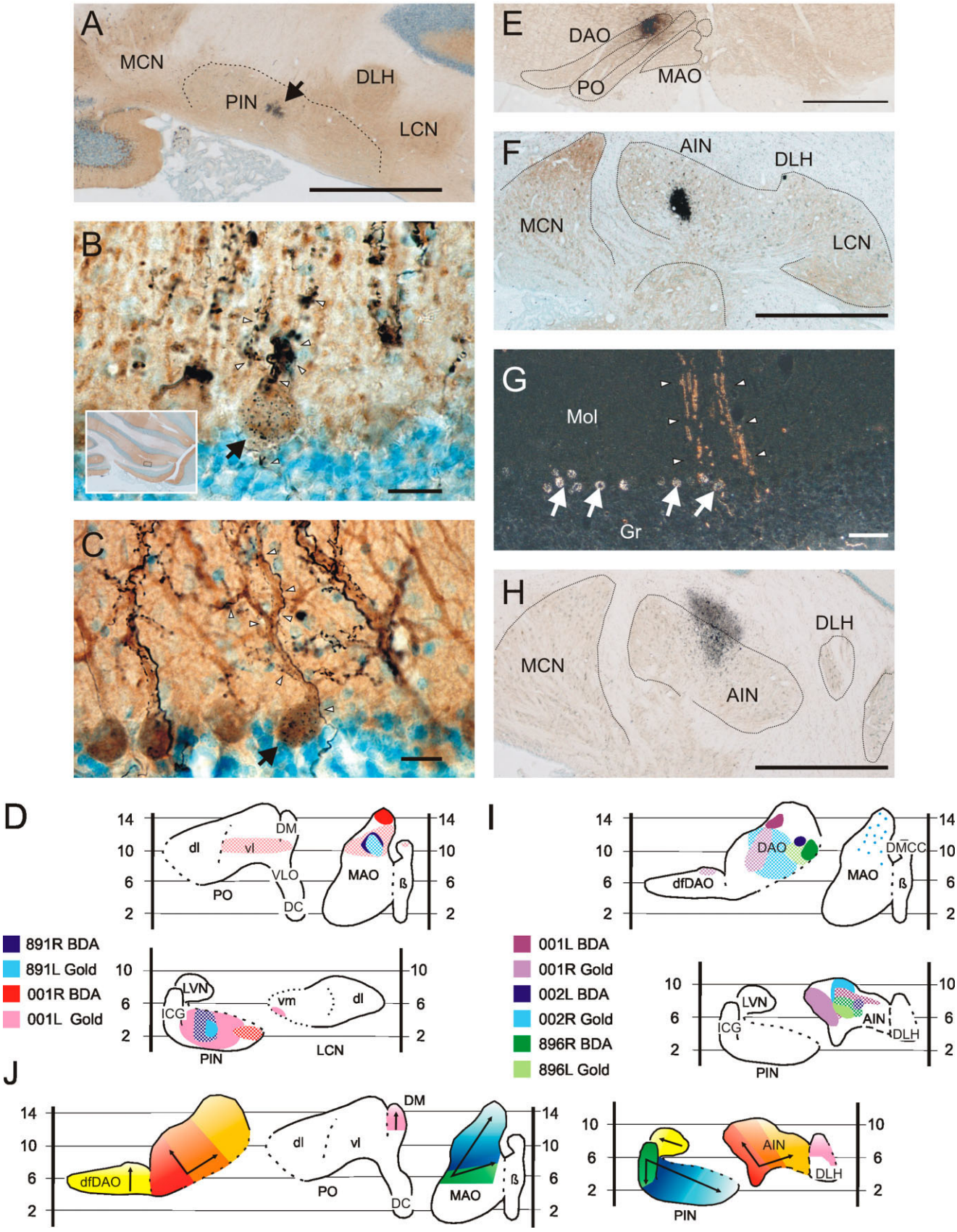
A
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Figure 6

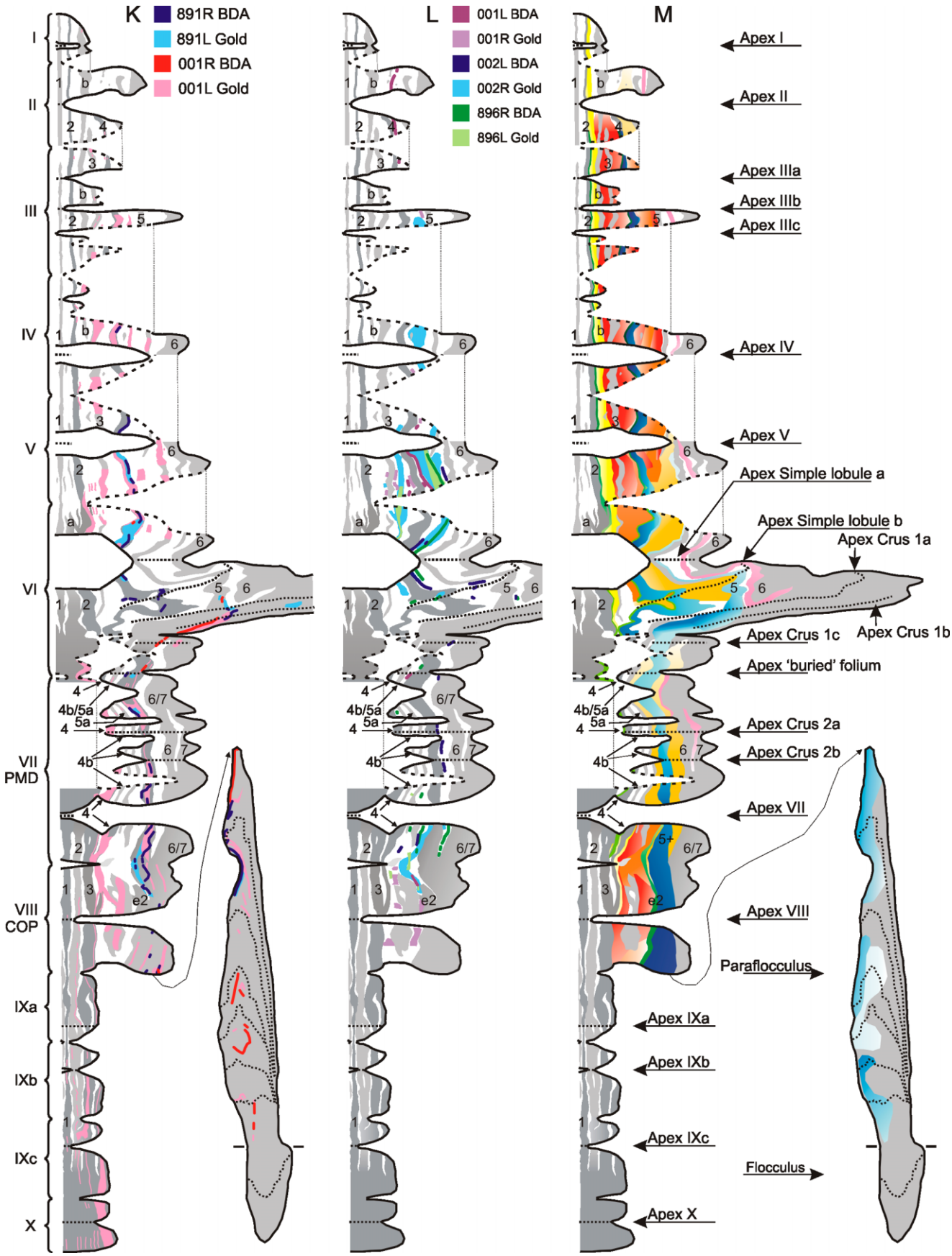

Figure 6 (Continued)

terns (also see Voogd and Ruigrok, 2004; Sugihara and Shinoda, 2004). Obviously, the zebrin pattern does not reveal the borders of all topographical or functional units. Indeed, Armstrong and Hawkes (2000) showed that expression patterns of various other antigens (HNK-1, GP65, Map-1a, and α-dystrophin) revealed higher levels of complexity in the organization of the cerebellar cortex. Therefore, input originating from a specific subnucleus of the inferior olive is not necessarily restricted to either a zebrin-positive or -negative band and within each band further subdivisions can be made. In our study this could be noted by labeled strips of climbing fibers, located at the border of a zebrin band, and that seemingly shifted from positions within the band to adjacent zebrin-negative areas (e.g., case 877L, Fig. 3E).

Topography of the X and CX zones

The relatively simple subdivision of the vermis and the intermediate cerebellum into the A, B, C1, C2, and C3 zones, established in the anatomical and electrophysiological studies of Voogd (1964; Voogd et al., 1969) and Oscarsson (1969), was changed by the identification of the x- and cx zone[1] in the anterior lobe of the cat (Ekerot and Larson, 1982; Campbell and Armstrong, 1985). The X zone is located between the A and B zones, whereas the CX zone is situated between the C1 and C2 zones. Like the C1 and C3 zones, these zones receive short-latency somatosensory information. However, in contrast to the C1 and C3 zones, this information is not relayed via the DAO, but through intermediate levels of the MAO (Trott and Armstrong, 1987; also see Pardoe and Apps, 2002). The X and CX zones have been found to be innervated by branching climbing fibers, but the amount of this collateralization was considered not to be extensive (Apps et al., 1991). Later, Buisseret-Delmas et al. (1993) found evidence for the existence of an X and CX zone of the rat, which, for the anterior lobe, was partially confirmed electrophysiologically by Jörntel et al. (2000). The X zone was found to extend from lobule IV to lobule VIa and to be wedged between the A and B zones. The CX zone runs from lobule V to VIa lateral to the C1 zone, but the presence of X and CX zones in the posterior cerebellum is still controversial (see below). The origin of the climbing fiber afferents of these zones was found as an oblique band in the i-MAO. The X and CX zones zone have been proposed to project to the interstitial cell groups (ICG), which includes small groups of neurons that are located between the interposed nuclei and the medial cerebellar nucleus (Trott and Armstrong, 1987; Buisseret-Delmas et al., 1993).

In the present study, as in previous studies, the identification of the X- and CX-zones in relation to the zebrin pattern was based on the localization of the climbing fiber projections of the i-MAO in combination with climbing fiber collateral or corticonuclear projections to the ICG (Voogd et al., 2003; Voogd and Ruigrok, 2004). The X and CX zones were located in the medial part of the zebrin-negative P2- band and in the lateral P3- in lobules IV, V, and the SL, immediately adjacent to P2+ and P4+, respectively. In the posterior lobe the X zone occupies P3- in the lobules VII and VIII, whereas CX is located laterally in the COP, adjacent to P5+. In case 877L the posterior CX zone was found to extend more rostrally into the PMD. It should be noted that, since the i-MAO and r-MAO, which projects to the adjacent zebrin-positive P4+ or P5+ bands (see below), as well as the ICG and medial PIN are directly adjacent, it is impossible to indicate an absolute border between CX and C2 based on a pure anatomical signature.

Sugihara and Shinoda (2004) found labeled climbing fibers in the position of the X and CX zones, stemming from injections of the rostral pole of their medial and lateral subnuclei b of the caudal MAO, respectively, but the collateral projection to the ICG was not verified and a projection to both populations from a single injection site,

[1]Lowercase lettering indicates that the zones were identified by electrophysiological techniques, whereas uppercase lettering refers to anatomically defined zones. Although many of these zones have been shown to be identical, in some cases the relationships between both types of zones are still discussed (e.g., see Pardoe and Apps, 2002; Voogd et al., 2003).

Fig. 6. (*Overleaf*) Double tracing and triple labeling experiments and summary figure. A, B, C, D, and K represent two cases with injections involving the r-MAO and PIN. E, F, G, H, and L represent three cases involving DAO and AIN injections. J and M represent the summary diagrams. **A:** detail of the gold-lectin injection site in PIN in case 891L (black arrow). **B,C:** High-magnification photographs of triple-labeled Purkinje cells in the cerebellar cortex (PMD); zebrin II immunoreactivity (DAB: brown) of the P5+ band, silver-intensified gold particles in the Purkinje cell body (black arrow), and labeled climbing fiber collaterals (DAB-cobalt: black, white arrow heads). The inset (box) in B shows a lower magnification of the PMD indicating exposed area in black box. **D:** Plots demonstrating location and the results of two cases with an injection of BDA centered on the rostral half of the MAO (solid colors) and a simultaneously placed injection with gold-lectin in the contralateral PIN (solid colors). Respective anterograde labeling in the PIN and retrograde labeling in the MAO are shown in color-matched hatching pattern. **K:** Distribution labeled climbing fibers and Purkinje cell bodies indicated relative to the zebrin pattern in the unfolded and flattened diagram of the cerebellar cortex (see Fig. 2). Note the areas of overlap between the two tracers. **E:** Photograph of the BDA injection in the medial DAO at level 11 (case 896R). **F:** photograph of the gold-lectin injection in the medial part of AIN at level 7 (case 896L). **G:** High-magnification photograph (darkfield illumination) of lobule V showing silver-intensified gold particles in the Purkinje cell body (white arrows), labeled climbing fiber collaterals (white arrow heads). **H**: Gold-lectin injection site in case 002R. Note that in this case the injection site incorporates the fibers directly dorsal to the AIN. Here, many climbing fibers course over and towards the interposed nuclei. Damage to these fibers most likely caused the retrograde uptake by scattered olivary neurons in the rostral MAO as seen in I. **I:** Diagrams demonstrating location and the results of the three cases with an injection with BDA centered on the rostral half of the DAO (solid colors) and a simultaneously placed injection with gold-lectin in the contralateral AIN (solid colors). Respective anterograde labeling in the AIN and retrograde labeling in the DAO are shown in color-matched hatching pattern. Note that the gold-lectin injection in case 002R was centered on the central part of the rostral AIN and did not involve the PIN. Nevertheless, scattered retrograde labeling was noted throughout the rostral MAO (also see H and text). **L:** Distribution labeled climbing fibers and Purkinje cell bodies indicated relative to the zebrin pattern in the unfolded and flattened diagram of the cerebellar cortex (see Fig. 2). Note the areas of overlap between the two tracers. **J:** Summary figure of projections within the flattened inferior olive reconstruction and its relation with the flattened cerebellar nuclei reconstruction. **M:** Summary figure of projections to the cerebellar cortex of the r-MAO, DAO, and DM relative to the zebrin pattern. See text for further explanation. For abbreviations, see list. Scale bars = 1 mm in A,E,F,H; 25 μm in B,C; 100 μm in G.

as observed in our experiments, was not observed. The presence or absence of climbing fiber branching to the X and CX zones may depend on the localization of the injection site in the transverse zone in the intermediate MAO. In the cat this zone consists of lateral and medial portions, which project to the X and CX zones, respectively, and a central portion with double-projecting cells (Apps et al., 1991).

Topography of the B zone

The B zone is located in the lateral vermis of the anterior lobe and SL. It was defined in the cat by its projection to the LVN (Voogd, 1964). The physiological equivalent of the B zone is characterized by short latency bilateral climbing fiber activation upon stimulation of peripheral nerves (Oscarsson and Sjölund, 1977). Its climbing fibers were shown to be derived from the caudal part of the DAO, which, in the rat, is known as the dorsal fold of the DAO (df-DAO: Voogd et al., 1969; Azizi and Woodward, 1987; Groenewegen and Voogd, 1977; Buisseret-Delmas and Angaut, 1993; Voogd and Ruigrok, 2004). Recently, the B zone was related to the zebrin pattern and was shown to occupy the zebrin-negative region lateral to anterior P2+ in lobules I–VI (Voogd and Ruigrok, 1997; 2004; Sugihara and Shinoda, 2004). In the posterior lobe the B zone is located in the COP (lobule VIII), where it forms a narrow zone within the P4- band directly adjacent to the P4+ (Voogd and Ruigrok, 2004). Sugihara and Shinoda (2004) designated the anterior zebrin band b+, innervated by the lateralmost aspect of the df-DAO (and directly bordering on the main body of the DAO), as the lateral border of the B zone. However, since they did not identify the collateral projections of these climbing fibers, they were unable to define the precise border of the B with the C1 zone. Both zones receive a projection from the DAO, but C1 emits collaterals to the AIN. Our data on the B zone are in agreement with Voogd and Ruigrok (2004) and exclude the b+ band from the B zone. When the injection site included the caudal vf-DAO (e.g., 016R and 881R), additional strips of climbing fibers were labeled lateral to P3+ and collateral labeling appeared in the AIN. In the posterior lobe the B zone was found to extend somewhat more rostrally into lobule VII, than reported by Voogd and Ruigrok (2004).

Topography of the C1, C3, and D0 zones

Some of the features that characterize the C1 and C3 zones as established in the original studies in the cat can also be recognized in our experiments in the rat. These zones project to the AIN, receive climbing fibers from the rostral DAO, which, in the rat, is termed the ventral fold of the DAO (vf-DAO: Azizi and Woodward, 1987). Through this relay they receive somatotopically organized sensory information. For the rat, and based on retrograde tracing of cortical injections, the C1 and C3 zones were described by Buisseret-Delmas (1988). In accordance with her schemes, our material also indicates a C1 zone running from the anterior lobe to the COP, whereas the C3 zone is found from anterior lobe to PMD. Both zones are interrupted in crus 1. With regard to the corticonuclear projections there is some discrepancy between the scheme proposed by Buisseret-Delmas and Angaut (1993) and the classical studies in the cat. In particular, their claim that C1 (as well as C2) project to both the AIN and the PIN was not supported by Voogd et al. (2003) and by the experiments in the present study, which document an exclusive projection of C1 and C3 to the AIN. Moreover, this relationship is supported by the collateral projection of climbing fibers from the vf-DAO to the AIN reported by Ruigrok and Voogd (2000) and in the present study and by the termination of the nucleo-olivary pathway from the AIN in the vf-DAO (Ruigrok and Voogd, 1990).

Buisseret-Delmas and Angaut (1989) described another zone, D0, in the lateral cerebellum of the rat. D0 projects to the dorsolateral hump (DLH), a subnucleus located between the AIN and the lateral cerebellar nucleus, and which receives its climbing fibers from the dorsomedial group (DM) of the principal olive. Originally Buisseret and Angaut (1989, 1993) located the D0 zone between C3 and D1. However, Sugihara and Shinoda (2004) recently showed that the D0 is located between the D1 and D2 zones, as suggested earlier by Voogd et al. (1993, 2003). The present study was in accordance with this notion and supports the identification of a D0 climbing fiber zone from the DM group by the presence of collateral labeling within the rostral DLH. Like the C1 and C3 zones, D0 is interrupted in crus 1.

Recently, Voogd et al. (2003) and Sugihara and Shinoda (2004) related the C1, C3, and D0 zones in the rat to the zebrin pattern. Our results on the topography of the C1, C3, and D0 zones are in general accordance with their results. However, with respect to the possibility and the topography of interzonal branching of climbing fibers and the topical relations between the vf-DAO, the AIN and the C1 and C3 zones, our data differ somewhat from those reported by Sugihara and Shinoda (2004). All three zones occupied zebrin-negative bands. In the anterior cerebellum, C1 and C3 flank the P4+ zebrin band; however, caudal of crus 1, they are found medially and laterally to P5+, respectively. The position of the usually only vague zebrin-positive anterior P3+ band remains enigmatic. It received exclusive collateral labeling from climbing fibers terminating in the zebrin-positive patch e of the COP, originating from the junction of the df- and vf-DAO (Voogd et al., 2003). An injection located in this region of the DAO, published by Sugihara and Shinoda (2004), also produced labeling in anterior P3+ and patch e. Purkinje cell labeling in P3+ was present after injections of retrograde tracers in the ICG and/or the PIN (Voogd and Ruigrok, 2004; this study), whereas Purkinje cell labeling was restricted to zebrin-negative zones and never included P3+ in our cases with gold-lectin injections in the AIN. The D0 zone was located in the P5- band of the anterior lobe extending from lobule II well into crus 1. Leaving the rest of crus 1 devoid of labeled climbing fibers, the labeling of D0 appeared again in the lateral half of the P5- band in the dorsal leaf of buried folium. In crus 2 and the PMD, labeling is present in P6-.

The anterior D0 zone is suggestive of the electrophysiologically defined y zone in the anterior lobe of the cat (Ekerot and Larson, 1979, 1982). Both are separated from the C3 zone by a zone projecting to the lateral cerebellar nucleus and that receives climbing fibers from the principal olive. A characteristic feature of the y zone relates to the branching of climbing fibers between the c3 and y zones. Indeed, some of our injections in the medial and rostral vf-DAO resulted in labeling of climbing fibers in P5- of the anterior lobe (e.g., cases 009R and 871L of Fig. 4E,G, respectively). We therefore presume that the rostral continuation of the D0 zone in P5- involves a rat equiva-

lent of the cat y zone and will, at least partly, receive its climbing fibers from the vf-DAO in addition to those of the DM group. In accordance with the data of Sugihara and Shinoda (2004), this y-part of D0 may be located within a thin medial strip in P5- band of lobules III to crus 1a. Although a D0 zone has been noted in P6- of the posterior cerebellum, a DAO contribution was not observed and a y component in the posterior cerebellum of the cat, likewise, has not been reported.

Single injections of the vf-DAO resulted in combinations of labeled climbing fiber strips belonging to C1, C3, and D0 zones in anterior and posterior parts of the cerebellum. Similar combinations of Purkinje cell labeling in the different zones were present in cases with gold-lectin injections in the AIN. The question can be raised whether climbing fiber labeling in rostral and caudal compartments of the same zone or in different zones is due to branching of individual climbing fibers. Although our material does not allow a definite answer, rostrocaudal branching within a single zone seems highly likely in view of the small size of our injection sites, the original observations in the cat of Armstrong et al. (1971, 1973) and Oscarsson and Sjölund (1977), and the rostrocaudal branching patterns of individual climbing fibers (cat: Rosina and Provini, 1983; rat: Sugihara et al., 2001; Voogd et al., 2003). With respect to collateralization to different zones, our injections in the caudal and lateral parts of the vf-DAO usually resulted in simultaneous labeling of the C1 and C3 zones (i.e., P3- and P4, respectively) in the anterior lobe and rostral SL and of C1 (P4-) in COP and caudal PMD. In contrast, medial injections invariably resulted in labeling of C3 (i.e., P4-) of SL and of C1 and C3 (i.e., P4- and P5-, respectively), in PMD and crus 2. These results are in line with early observations in the cat by Ekerot and Larson (1982; also see Apps et al., 1991; Voogd et al., 2003). Sugihara and Shinoda (2004), however, only rarely reported mediolateral branching between C1 and C3 with their injections of the vf-DAO (also see Sugihara et al., 2001).

Topography of the C2 zone

Both in the rat and other species the C2 zone extends over the entire cerebellum, from lobule II to the flocculus, projects to the PIN, and receives climbing fibers from the r-MAO. (Voogd, 1964; Voogd et al., 1969; Voogd and Bigaré, 1980; Buisseret-Delmas, 1988; Buisseret-Delmas and Angaut, 1993; Ruigrok et al., 1992; Groenewegen et al., 1979; Apps, 1990). Here we could confirm the correspondence between the C2 zone and the anterior P4+ and posterior P5+ bands (Voogd and Ruigrok, 1997, 2004; Voogd et al., 2003; Sugihara and Shinoda, 2004). However, the connections of the r-MAO and the intrinsic organization of the C2 zone were underexposed in the latter studies. The rostrocaudal extent of the climbing fiber labeling with injections at the caudal and middle levels of the r-MAO is very similar. However, with successively more rostral injections the labeling shifts laterally in P4+ and P5+. This translocation coincides with a caudolateral shift in the collateral projection to the PIN (cf. Ruigrok and Voogd, 2000). The lateral displacement of the projection in these rostral injections ultimately results in the labeling of anterior P5+ in the SL and the anterior crus 1. When P5+, representing the posterior C2 zone, was followed into crus 1 it proved to be continuous with anterior P5+ of crus 1a and the SL (cf. case 879R and 871R of Fig. 3). Indeed, in some folia both P4+ and P5+ contained labeled climbing fibers that originating from the r-MAO (see cases 883R and 881L of Fig. 3). Hence, rather than joining each other within crus 1, we propose that the anterior and posterior C2 zone are discontinuous and exist next to one another in SL and crus 1 (see Fig. 6M). Labeling of C2 in the paraflocculus is limited to its caudal half, which is in accordance with Sugihara and Shinoda (2004).

Functional considerations

A universal feature of the topography of projections from the presently studied regions of the inferior olive to the cerebellar cortex is that single olivary regions are mapped in multiple, noncontiguous zones in the cerebellar cortex. However, in each cerebellar target nucleus only a single map of the collateral projections from these olivary nuclei is produced. Apparently, multiple cortical maps converge upon a single representation in the nuclei (Garwicz and Ekerot, 1994; Apps and Garwicz, 2000). Within the zones that receive somatosensory information from the periphery through the olivocerebellar system, the olive is mapped in long and narrow strips of climbing fibers, which share the same receptive field: the microzones. Together, the microzones in each of the cat's B, C1, C3, and y zones seem to produce continuous body maps. Rostrocaudal branching of climbing fibers would duplicate these maps in the anterior and posterior C1 and C3 zones (Oscarsson and Sjölund, 1977; Andersson and Oscarsson, 1978; Ekerot and Larson, 1979, 1982; Garwicz, 1997). The longitudinal strips of climbing fibers terminal arborizations, originating from individual or from small groups of olivary neurons, described by Sugihara et al. (2001, 2004) and in this study, probably are the substrate of these microzones. They occur in all olivocerebellar projection areas and allow the distinction of mediolateral and rostrocaudal topical patterns in the projection of accessory olives. In the projection of the df-DAO to the anterior P2-band (the B zone) the localization of the different climbing fiber strips is a mediolateral one; no rostrocaudal shift in their localization is apparent. This would be in accordance with the mediolateral somatotopy in the B zone, as described for the cat (Oscarsson and Sjölund, 1977).

More distinct rostrocaudal gradients in the projection of the vf-DAO can be discussed when considering the somatotopical relations of different parts of the vf-DAO. Unfortunately, no complete map of the somatotopical organization of the vf-DAO is available for the rat, but the detailed map of Gellman et al. (1983) for the cat may be applied, since it seems to be in accordance with available data in the rat (Atkins and Apps, 1997; Pardoe and Apps, 2002). In Gellman's scheme, the caudal and lateral vf-DAO (i.e., our first group of vf-DAO injections, Fig. 4A) would represent the hindlimb; the rump and tail region would be located in the rostrolateral vf-DAO (our second group: Fig. 4B), while the forelimb and face area are represented in the medial vf-DAO (our third group: Fig. 4C). It can be noted that mediolateral branching (i.e., simultaneous labeling) between the C1 and C3 zones in the anterior lobe and SL mainly occurred in the putative tail, rump, and hindlimb regions of these zones, but not in their forelimb representation. In contrast, branching in the caudal aspects of the C1 and C3 zones, mediolateral branching between C1 and C3, may occur in PMD and crus 2 for the forelimb/face representations but not, or only sparsely, for the hindlimb/rump/tail regions in COP and posterior

PMD. According to Jörntell et al. (2000) the forelimb was represented in the c1 zone of lobules V and VI. The presumed absence of c3 and y zones in the anterior cerebellum of the rat was interpreted by these authors as a reflection of differences in the organization of motor systems in the rat as compared with the cat. Neither of these observations could be confirmed in our material. The absence of a forelimb representation (i.e., resulting from our injections in the medial vf-DAO) in C1 of the SL (i.e., in P3-) is at odds with physiological data from Pardoe and Apps (2002). Future research will have to determine whether their recordings were made within P3- or P4-. The representation of hind- and forelimb in, respectively, the C1 zone of the COP and the PMD (Atkins and Apps, 1997) was confirmed in our study, although the border between these representations was less distinct. A tail representation to the medial C1 zone of COP might be represented by injections 884R and 015L (Fig. 4A,D and 4B,E, respectively) and is also in line with the anatomical data provided by Atkins and Apps (1997).

It is possible to transpose the somatotopical map of the vf-DAO to the AIN by evaluating the climbing fiber collateral labeling. Hence, the hindlimb region would be represented in the caudomedial AIN, rump/tail region in its rostromedial tip, whereas forelimb/face receptive areas are found in the caudolateral part of the AIN. This general scheme is in line with known projections from the AIN to the spinal cord via the magnocellular red nucleus (Daniel et al., 1987; Teune et al., 2000; for review, see Ruigrok, 2004).

Subdivisions of the C2 zone have not been reported before. However, with rostrally shifting injections of the r-MAO we have noted a mediolateral shift of the labeling in the P4+ and labeling within the P5+ band of the SL and the crus 1 becomes more prominent. The electrophysiological equivalent of the C2 zone (i.e., the c2 zone) has been shown to receive long latency input from the ipsi- and contralateral forelimb in both cat and rat (Ekerot and Larson, 1979: Atkins and Apps, 1997; Pardoe and Apps, 2002), but the functional meaning of the topical patterns in the projection of the r-MAO remains unknown (also see description of afferents to their group I by Sugihara and Shinoda, 2004).

As yet, it is not known if the breaking up of the cortical zones is related to developmental (e.g., Hashimoto and Mikoshiba, 2003) or to functional aspects (or both). Indeed, it is not known if zones in the anterior and posterior cerebellum that receive climbing fiber input from the same olivary cells (Sugihara et al., 2001; Voogd et al., 2003) function in a similar way. Observations by Apps and collaborators (Apps and Lee, 1999; Apps, 2000; also see Pardoe and Apps, 2002) have indicated that gating of climbing fiber excitability of C1 zones in SL and PMD behave differently. However, as outlined above, our results show that the olivary regions supplying C1 of PMD preferably reach C3 of the SL rather than C1 (also see Voogd et al., 2003). Furthermore, in order to understand the functional implications of the multiple and essentially discontinuous organization of the olivocortical projections it will be important to gain more detailed information on the identity and patterning of the specific mossy fiber inputs to these regions (Pijpers et al., 2003, Voogd et al., 2003).

Zones and modules

The notion of matching olivocortical and corticonuclear, as well as of olivonuclear and nucleo-olivary connections, has resulted in the concept of the modular organization of the cerebellum. However, a detailed study correlating the collateral projections of the olive to the cerebellar nuclei directly with the corticonuclear projection is still lacking. In the present study combinations of injections of anterograde tracers within the inferior olive and retrograde tracers in the cerebellar nuclei of the same animal were used to study this problem. In this way, by analyzing the topography of retrogradely labeled Purkinje cells and anterogradely labeled climbing fibers, we were able to directly verify the complementary nature of corticonuclear and olivocortical projections. The modular nature of the interconnectivity of the injected areas was specifically highlighted by the observation that overlap of injection site and transported label in olive coincided with overlap in the cerebellar nuclei. Only in these instances was it possible to find congruence of retrogradely labeled Purkinje cells and BDA-labeled climbing fibers. Although, as yet, it cannot be excluded that some mismatch between parts of the entities of olivocortical, olivonuclear, corticonuclear, and nucleo-olivary projections may exist, the present data strongly suggest that the modular extension of cerebellar cortical zonal organization presents a key feature of cerebellar functioning.

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LITERATURE CITED

Ahn AH, Dziennis S, Hawkes R, Herrup K. 1994. The cloning of zebrin II reveals its identity with aldolase C. Development 120:2081–2090.

Andersson G, Oscarsson O. 1978. Climbing fiber microzones in cerebellar vermis and their projection to different groups of cells in the lateral vestibular nucleus. Exp Brain Res 32:565–579.

Apps R. 1990. Columnar organization of the inferior olive projection to the posterior lobe of the rat cerebellum. J Comp Neurol 302:236–254.

Apps R. 2000. Gating of climbing fibre input to cerebellar cortical zones. Prog Brain Res 124:201–211.

Apps R, Garwicz M. 2000. Precise matching of olivo-cortical divergence and cortico-nuclear convergence between somatotopically corresponding areas in the medial C1 and medial C3 zones of the paravermal cerebellum. Eur J Neurosci 12:205–214.

Apps R, Lee S. 1999. Gating of transmission in climbing fibre paths to cerebellar cortical C1 and C3 zones in the rostral paramedian lobule during locomotion in the cat. J Physiol 516:875–883.

Apps R, Trott JR, Dietrichs E. 1991. A study of branching in the projection from the inferior olive to the x and lateral c1 zones of the cat cerebellum using a combined electrophysiological and retrograde fluorescent double labeling technique. Exp Brain Res 87:141–152.

Armstrong CL, Hawkes R. 2000. Pattern formation in the cerebellar cortex. Biochem Cell Biol 78:551–562.

Armstrong DM, Harvey RJ, Schild RF. 1971. Distribution in the anterior lobe of the cerebellum of branches from climbing fibers to the paramedian lobule. Brain Res 25:203–206.

Armstrong DM, Harvey RJ, Schild RF. 1973. Branching of the inferior olivary axons to terminate in different folia, lobules or lobes of the cerebellum. Brain Res 54:365–371.

Atkins MJ, Apps R. 1997. Somatotopical organisation within the climbing fibre projection to the paramedian lobule and copula pyramidis of the rat cerebellum. J Comp Neurol 389:249–263.

Azizi SA, Woodward DJ. 1987. Inferior olivary nuclear complex of the rat: morphology and comments on the principles of organization within the olivocerebellar system. J Comp Neurol 263:467–484.

Bernard J-F. 1987. Topographical organization of olivocerebellar and corticonuclear connections in the rat — an WGA-HRP study. I. Lobules IX, X and the flocculus. J Comp Neurol 263:241–258.

Bloedel JR. 1992. Functional heterogeneity with structural homogeneity: how does the cerebellum operate? Behav Brain Sci 15:666–678.

Brown IE, Bower JM. 2001. Congruence of mossy fiber and climbing fiber tactile projections in the lateral hemispheres of the rat cerebellum. J Comp Neurol 429:59–70.

Brown IE, Bower JM. 2002. The influence of somatosensory cortex on climbing fiber responses in the lateral hemispheres of the rat cerebellum after peripheral tactile stimulation. J Neurosci 22:6819–6829.

Buisseret-Delmas C. 1988. Sagittal organization of the olivocerebellonuclear pathway in the rat. II. Connections with the nucleus interpositus. Neurosci Res 5:494–512.

Buisseret-Delmas C, Angaut P. 1989. Anatomical mapping of the cerebellar nucleo-cortical projections in the rat: a retrograde labeling study. J Comp Neurol 288:297–310.

Buisseret-Delmas C, Angaut P. 1993. The cerebellar olivo-cortico-nuclear connections in the rat. Prog Neurobiol 40:63–87.

Buisseret-Delmas C, Yatim N, Buisseret P, Angaut P. 1993. The X zone and CX subzone of the cerebellum in the rat. Neurosci Res 16:195–207.

Campbell NC, Armstrong DM. 1985. Origin in the medial accessory olive of climbing fibers to the x and lateral c1 zones of the cat cerebellum: a combined electrophysiological/WGA-HRP investigation. Exp Brain Res 58:520–531.

Daniel H, Billard JM, Angaut P, Batini C. 1987. The interpositorubrospinal system. Anatomical tracing of a motor control pathway in the rat. Neurosci Res 5:87–112.

De Zeeuw CI, Simpson JI, Hoogenraad CC, Galjart N, Koekkoek SKE, Ruigrok TJH. 1998. Microcircuitry and function of the inferior olive. Trends Neurosci 21:391–400.

Ekerot CF, Larson B. 1979. The dorsal spino-olivocerebellar system in the cat. II. Somatotopical organization. Exp Brain Res 36:219–232.

Ekerot C-F, Larson B. 1980. Termination in overlapping sagittal zones in cerebellar anterior lobe of mossy and climbing fiber paths activated from dorsal funiculus. Exp Brain Res 38:163–172.

Ekerot C-F, Larson B. 1982. Branching of olivary axons to innervate pairs of sagittal zones in the cerebellar anterior lobe of the cat. Exp Brain Res 48:185–198.

Garwicz M. 1997. Sagittal zonal organization of climbing fibre input to the cerebellar anterior lobe of the ferret. Exp Brain Res 117:389–398.

Garwicz M, Ekerot C-F. 1994. Topographical organization of the cerebellar cortical projection to nucleus interpositus anterior in the cat. J Physiol 474:245–260.

Garwicz M, Jorntell H, Ekerot CF. 1998. Cutaneous receptive fields and topography of mossy fibres and climbing fibres projecting to cat cerebellar C3 zone. J Physiol 512(Pt 1):277–293.

Gellman R, Houk JC, Gibson AR. 1983. Somatosensory properties of the inferior olive of the cat. J Comp Neurol 215:228–243.

Gravel C, Eisenman LM, Sasseville R, Hawkes R. 1987. Parasagittal organization of the rat cerebellar cortex: direct correlation between antigenic Purkinje cell bands revealed by mabQ113 and the organization of the olivocerebellar projection. J Comp Neurol 265:294–310.

Groenewegen HJ, Voogd J. 1977. The parasagittal zonation within the olivocerebellar projection. I. Climbing fiber distribution in the vermis of cat cerebellum. J Comp Neurol 174:417–488.

Groenewegen HJ, Voogd J, Freedman SL. 1979. The parasagittal zonation within the olivocerebellar projection. II. Climbing fiber distribution in the intermediate and hemispheric parts of cat cerebellum. J Comp Neurol 183:551–602.

Hashimoto M, Mikoshiba K. 2003. Mediolateral compartmentalization of the cerebellum is determined on the "birth date" of Purkinje cells. J Neurosci 23:11342–11351.

Hawkes R, Leclerc N. 1987. Antigenic map of the rat cerebellar cortex: the distribution of parasagittal bands as revealed by monoclonal anti-Purkinje cell antibody mabQ113. J Comp Neurol 256:29–41.

Jörntell H, Ekerot CF. 2003. Receptive field plasticity profoundly alters the cutaneous parallel fiber synaptic input to cerebellar interneurons in vivo. J Neurosci 23:9620–9631.

Jörntell H, Ekerot CF, Garwicz M, Luo XL. 2000. Functional organization of climbing fibre projection to the cerebellar anterior lobe of the rat. J Physiol (Lond) 522(Pt 2):297–309.

Korneliussen HK. 1968. On the morphology and subdivision of the cerebellar nuclei of the rat. J Hirnsforsch 10:109–122.

Llinás R, Sasaki K. 1989. The functional organization of the olivocerebellar system as examined by multiple Purkinje cell recording. Eur J Neurosci 1:587–603.

Oscarsson O. 1969. Termination and functional organization of the dorsal spino-olivocerebellar path. J Physiol 200:129–149.

Oscarsson O, Sjolund B. 1977. The ventral spino-olivocerebellar system in the cat. I. Identification of five paths and their termination in the cerebellar anterior lobe. Exp Brain Res 28:469–486.

Pardoe J, Apps R. 2002. Structure-function relations of two somatotopically corresponding regions of the rat cerebellar cortex: olivo-corticonuclear connections. Cerebellum 1:165–184.

Paxinos G, Watson C. 1986. The rat brain in stereotaxic coordinates. Sydney: Academic Press.

Pijpers A, Pardoe J, Apps R, Voogd J, Ruigrok TJH. 2003. Partial congruence of climbing and mossy fiber projections to the rat cerebellum. Program No. 274.14. 2003 Abstract Viewer/Itinerary Planner. Washington, DC: Soc Neurosci Online.

Rosina A, Provini L. 1983. Somatotopy of climbing fiber branching to the cerebellar cortex in cat. Brain Res 289:45–63.

Ruigrok TJH. 1997. Cerebellar nuclei: the olivary connection. In: De Zeeuw CI, Strata P, Voogd J, editors. The cerebellum: from structure to control. Amsterdam: Elsevier Science. p 162–197.

Ruigrok TJ. 2003. Collateralization of climbing and mossy fibers projecting to the nodulus and flocculus of the rat cerebellum. J Comp Neurol 466:278–298.

Ruigrok TJH. 2004. Precerebellar nuclei and red nucleus. In: Paxinos G, editor. The rat nervous system, 3rd ed. San Diego: Elsevier Academic Press. p 167–204.

Ruigrok TJH, Voogd J. 1990. Cerebellar nucleo-olivary projections in rat. An anterograde tracing study with *Phaseolus vulgaris*-leucoagglutinin (PHA-L). J Comp Neurol 298:315–333.

Ruigrok TJH, Voogd J. 2000. Organization of projections from the inferior olive to the cerebellar nuclei in the rat. J Comp Neurol 426:209–228.

Ruigrok TJH, Osse R-J, Voogd J. 1992. Organization of inferior olivary projections to the flocculus and ventral paraflocculus of the rat cerebellum. J Comp Neurol 316:129–150.

Ruigrok TJH, Teune TM, van der Burg J, Sabel-Goedknegt H. 1995. A retrograde double labeling technique for light microscopy. A combination of axonal transport of cholera toxin B-subunit and a gold-lectin conjugate. J Neurosci Meth 61:127–138.

Sugihara I, Shinoda Y. 2004. Molecular, topographic, and functional organization of the cerebellar cortex: a study with combined aldolase C and olivocerebellar labeling. J Neurosci 24:8771–8785.

Sugihara I, Wu HS, Shinoda Y. 2001. The entire trajectories of single olivocerebellar axons in the cerebellar cortex and their contribution to cerebellar compartmentalization. J Neurosci 21:7715–7723.

Sugihara I, Ebata S, Shinoda Y. 2004. Functional compartmentalization in the flocculus and the ventral dentate and dorsal group y nuclei: an analysis of single olivocerebellar axonal morphology. J Comp Neurol 470:113–133.

Teune TM, van der Burg J, van der Moer J, Voogd J, Ruigrok TJH. 2000. Topography of cerebellar nuclear projections to the brain stem in the rat. In: Gerrits NM, Ruigrok TJH, De Zeeuw CI, editors. Cerebellar modules: molecules, morphology and function. Amsterdam: Elsevier Science. p 141–172.

Trott JR, Armstrong DM. 1987. The cerebellar corticonuclear projection from lobule Vb/c of the cat anterior lobe: a combined electrophysiological and autoradiographic study. I. Projections from the intermediate region. Exp Brain Res 66:318–338.

Van der Want JJL, Wiklund L, Guegan M, Ruigrok T, Voogd J. 1989. Anterograde tracing of the rat olivocerebellar system with Phaseolus vulgaris leucoagglutinin (PHA-L). Demonstration of climbing fiber collateral innervation of the cerebellar nuclei. J Comp Neurol 288:1–18.

Voogd J. 1964. The cerebellum of the cat: structure and fiber connections. Assen, Netherlands: Van Gorcum.

Voogd J. 2004. Cerebellum. In: Paxinos G, editor. The rat nervous system, 3rd ed. San Diego: Elsevier Academic. p 205–242.

Voogd J, Bigaré F. 1980. Topographical distribution of olivary and cortico nuclear fibers in the cerebellum: a review. In: Courville J, de Montigny

C, Lamarre Y, editors. The inferior olivary nucleus. Anatomy and physiology. New York: Raven Press. p 207–234.

Voogd J, Ruigrok TJH. 1997. Transverse and longitudinal patterns in the mammalian cerebellum. In: De Zeeuw CI, Strata P, Voogd J, editors. The cerebellum: from structure to control. Amsterdam: Elsevier. p 21–37.

Voogd J, Ruigrok TJH. 2004. The organization of the corticonuclear and olivocerebellar climbing fiber projections to the rat cerebellar vermis: the congruence of projection zones and the zebrin pattern. J Neurocytol 33:5–21.

Voogd J, Broere G, van Rossum J. 1969. The medio-lateral distribution of the spinocerebellar projection in the anterior lobe and the simple lobule in the cat and a comparison with some other afferent fibre systems. Psychiatr Neurol Neurochir 72:137–151.

Voogd J, Eisenman LM, Ruigrok TJH. 1993. Relation of olivocerebellar projection zones to zebrin pattern in rat cerebellum. Soc Neurosci Abstr 19:1216.

Voogd J, Gerrits NM, Ruigrok TJH. 1996. Organization of the vestibulocerebellum. Ann N Y Acad Sci 781:553–579.

Voogd J, Pardoe J, Ruigrok TJH, Apps R. 2003. The distribution of climbing and mossy fiber collateral branches from the copula pyramidis and the paramedian lobule: congruence of climbing fiber cortical zones and the pattern of zebrin banding within the rat cerebellum. J Neurosci 23:4645–4656.

Welker W. 1987. Spatial organization of somatosensory projections to granule cell cerebellar cortex: functional and connectional implications of fractured somatotopy (summary of Wisconsin studies). In: King JS, editor. New concepts in cerebellar neurobiology. New York: Alan R. Liss. p 239–280.