

# Projections of Purkinje Cells in the Translation and Rotation Zones of the Vestibulocerebellum in Pigeon (*Columba livia*)

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## ABSTRACT

Previous electrophysiological studies have shown that the pigeon vestibulocerebellum (ventral uvula, nodulus, and flocculus) can be divided into two parasagittal zones based on responses to optic flow stimuli. The medial zone (ventral uvula and nodulus) responds best to optic flow resulting from self-translation, whereas the lateral zone (flocculus) responds best to optic flow resulting from self-rotation. In this study we investigated the projections of the Purkinje cells in the translation and rotation zones of the vestibulocerebellum by using the anterograde tracer biotinylated dextran amine. Extracellular recording of Purkinje cell activity (complex spikes) in response to large-field visual stimuli were used to identify the injection sites. Injections into the translation zone resulted in extremely heavy terminal labeling in the cerebellovestibular process adjacent to the medial cerebellar nucleus. A moderate amount of terminal labeling was found in the medial cerebellar nucleus, the superior vestibular nucleus (laterally, dorsally, and medially), and the descending vestibular nucleus, particularly in the lateral half. Light terminal labeling was observed in the dorsolateral vestibular nucleus, the medial vestibular nucleus, the tangential nucleus, and the lateral vestibular nucleus pars ventralis. Injections into the rotation zone resulted in heavy terminal labeling in the superior vestibular nucleus (particularly dorsally and medially), the descending vestibular nucleus (particularly medially), and the medial vestibular nucleus. A moderate amount of terminal labeling was seen in the cerebellovestibular process adjacent to the lateral cerebellar nucleus, and the dorsolateral vestibular nucleus. A small amount of terminal labeling was found in the lateral cerebellar nucleus, the tangential nucleus, the prepositus hypoglossi, and the lateral vestibular nucleus pars ventralis. *J. Comp. Neurol.* 413:480-493, 1999. © 1999 Wiley-Liss, Inc.

**Indexing terms:** optic flow; flocculus; nodulus; ventral uvula; visual-vestibular integration

Motion of any object through space, including self-motion of organisms, can be described with respect to rotation relative to some frame of reference, and to translation between two points in space. Information about self-motion arises from many sensorimotor systems. The vestibular apparatus includes the semicircular canals and the otolith organs, which are sensitive to head rotation and translation, respectively (Wilson and Melvill Jones, 1979). Gibson (1954) emphasized that vision can also serve as a proprioceptive sense. Because the environment contains numerous stationary visual stimuli, self-motion induces "flowfields" or "optic flow" across the entire retina. Self-rotation results in a rotational flowfield that is opposite to the direction of one's head rotation (see Fig. 6A), whereas the flowfield resulting from self-translation consists of a

"focus of expansion," which is a point in the direction of translation from which all visual images radiate outward (see Fig. 6B). Along the axis of translation, but in the direction opposite to the translation vector, is a "focus of contraction," a point to which all visual images converge.

We have previously shown in pigeons that the vestibulocerebellum (VbC) can be divided into two parasagittal

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zones based on the complex spike (CS) activity of Purkinje cells in response to optic flow stimuli (see Fig. 6E). CS activity in the medial zone responds best to *translational* optic flow along either the vertical axis, or one of two horizontal axes oriented 45° to the midline (Wylie et al., 1993, 1998; Wylie and Frost, 1991, 1999). CS activity in the lateral zone responds best to *rotational* optic flow about either the vertical axis, or a horizontal axis oriented 45° to the midline (Wylie and Frost, 1993). Recently, by using the retrograde tracer cholera toxin subunit B, we showed that the translation and rotation zones receive climbing fiber input from the ventrolateral and dorsomedial margins of the medial column (mc) of the inferior olive (IO), respectively (see Fig. 6C,D). In the present study, we investigated the projection of Purkinje cells in the translation and rotation zones by using the anterograde tracer biotinylated dextran amine (BDA). The purpose of this study was to determine those areas of the vestibular and cerebellar nuclei involved in the processing of either rotational, translational, or translational and rotational optic flow.

## MATERIALS AND METHODS

### Surgery

The methods reported herein conformed to the guidelines established by the Canadian Council on Animal Care and were approved by the Biosciences Animal Care and Policy Committee at the University of Alberta. Experiments were performed on Silver King pigeons (obtained from the Palmetto Pigeon Plant, Sumpter NC), or Racing Homers (obtained from a local supplier), which were anesthetized with a ketamine (90 mg/kg)/xylazine (15 mg/kg) mixture (i.m.). Supplemental doses were administered as necessary during the experiments. The animals were placed in a stereotaxic device with pigeon ear bars and a beak adapter so that the orientation of the skull conformed with the atlas of Karten and Hodos (1967). A section of bone and dura was removed to expose the dorsal surface of the auricle of the cerebellum. To access the Purkinje cells responsive to translational and rotational optic flow, penetrations were made into the surface of the auricle at a 45° angle to the sagittal plane.

### Recording and injection procedures

Extracellular recordings were made with glass micropipettes (4–5 µm tip diameter) filled with 2 M NaCl. Once CSs of Purkinje cells were isolated in the molecular layer, we used stimuli described in detail elsewhere (Wylie et al.,

1998; Lau et al., 1998; Wylie and Frost, 1993, 1999) to determine whether the cells were responsive to either rotational or translational optic flow. First, we used a large (about 90 × 90°) handheld stimulus, which consisted of a pattern of dark lines and dots on a light background, moved horizontally and vertically in the central region of each visual field (i.e., along the inter-aural axis). Translation and rotation cells were easily distinguishable with this procedure. The former preferred the same direction of motion in this region of both visual fields, whereas the latter preferred the opposite directions in the two visual fields (Wylie et al., 1993). We also used a full-field planetarium projector to present rotational flowfields (Wylie and Frost, 1993), and a full-field “translator” projector to present translational flowfields (Wylie et al., 1998; Wylie and Frost, 1999). Once the flowfield preference was determined, the recording electrode was removed and replaced with a pipette (tip diameter 10–20 µm) containing BDA (Molecular Probes; 10% in 0.1 M phosphate buffer, PB; pH 7.4). The solution was iontophoretically injected (+3 µamp, 1 second ON, 1 second OFF) for 5–15 minutes.

### Processing for biotinylated dextran amine

After a survival time of 3–6 days, the animals were given an overdose of sodium pentobarbital (100 mg/kg) and then immediately transcardially perfused with PB saline (PBS; 0.9%, pH 7.4) followed by 4% paraformaldehyde in 0.1 M PB. The brains were extracted, post-fixed for 2 hours, cryoprotected in sucrose overnight (20% in 0.1 M PB), and frozen sectioned in the coronal plane at a thickness of 45 µm. The tissue was then processed to visualize BDA by using a cobalt chloride intensification of diaminobenzidine that we have described elsewhere (Wylie et al., 1997), based on the protocol described by Wild (1993) and Veenman et al. (1992). After BDA visualization, the sections were mounted onto gelatin-coated slides, dried, counterstained with neutral red, and coverslipped with Permount.

### Nomenclature

The avian cerebellum consists of a vermis, without hemispheres as is characteristic of mammalian species (Larsell, 1948; Larsell and Whitlock, 1952; Whitlock, 1952). The pigeon VbC consists of the two most ventral folia of the posterior vermis: IXc,d and X by using the nomenclature in Karten and Hodos (1967), which we use, or IXb and X according to Arends and Zeigler (1991). Generally, folia IXc,d and X are referred to as the uvula and nodulus, respectively (Larsell, 1948; Larsell and Whit-

#### Abbreviations

IXc,d	folium IXc,d	nIX	nucleus of the glossopharyngeal nerve
X	folium X	pvc/PCV	cerebellovestibular process
An	nucleus angularis	ph/PH	nucleus prepositus hypoglossi
BDA	biotinylated dextran amine	Pl	Purkinje layer of cerebellar cortex
CbL	lateral cerebellar nucleus	sa	stria acoustica
CbM	medial cerebellar nucleus	Ta	tangential nucleus
CS	complex spike	TTD	nucleus of the descending tract of the trigeminal nerve
fl/I	folium I	V	ventricle
FLM	fasciculus longitudinalis medialis	VbC	vestibulocerebellum
gl	granule layer of cerebellar cortex	VDL	dorsolateral vestibular nucleus
Inf	infracerebellar nucleus	VeD	descending vestibular nucleus
IO	inferior olive	VeLd	lateral vestibular nucleus, pars dorsalis
La	nucleus laminaris	VeLv	lateral vestibular nucleus, pars ventralis
mc	medial column (of the inferior olive)	VeM	medial vestibular nucleus
MC	nucleus magnocellularis	VeS	superior vestibular nucleus
ml	molecular layer of cerebellar cortex	wm	white matter of the cerebellar cortex

lock, 1952; Whitlock, 1952). These folia extend laterally to form the auricle of the cerebellum, which has been referred to as the paraflocculus and/or flocculus (Larsell, 1948; Larsell and Whitlock, 1952; Whitlock, 1952). Based on physiological responses of Purkinje cell CS activity (see above; Wylie et al., 1993), we have divided the VbC into a rotation zone, which we refer to as the flocculus, and a translation zone, which we refer to as the nodulus and the ventral uvula (see Fig. 6E). There is no gross morphological distinction between the zones, but our physiological recordings indicated that the border is located about 1.8–2.1 mm from the midline (Wylie et al., 1993).

For the vestibular and cerebellar nuclei, we generally use the nomenclature of Karten and Hodos (1967) with a few exceptions. According to Karten and Hodos (1967) there are two cerebellar nuclei: the medial and lateral cerebellar nuclei (CbM, CbL) although CbM can be further subdivided. Arends and Zeigler (1991) have identified a third nucleus, the infracerebellar nucleus (Inf), which resides ventral and lateral to the rostral part of CbL. In our material we could only identify Inf in a maximum of five transverse sections for each animal. Between CbM and CbL and the vestibular complex is the cerebellovestibular process (pcv). We ascribed to the pcv any terminals that resided near, but clearly outside the borders of the CbM and CbL. According to Karten and Hodos (1967) the vestibular nuclear complex consists of the medial vestibular nucleus (VeM), the superior vestibular nucleus (VeS), the descending vestibular nucleus (VeD), the lateral vestibular nucleus pars dorsalis (VeLd) and pars ventralis (VeLv), and the dorsolateral vestibular nucleus (VDL). In mammals VeLv is now referred to as the magnocellular VeM (Epema, 1990). Dickman and Fang (1996) considered VDL to be the dorsal extension of VeLv, but others consider VDL to be homologous to group  $\gamma$  in mammals (Arends et al., 1991). Dickman and Fang (1996) also identified groups A and B in pigeons, based on earlier studies in chickens (Wold, 1976). In our material, we could not reliably identify groups A and B; thus they were not included in our analysis. The tangential nucleus (Ta), although it does not receive a primary vestibular projection, does receive input from the VbC (Arends and Zeigler, 1991), as does the prepositus hypoglossi (ph).

## RESULTS

Injections of BDA were made in 23 pigeons, but we describe the data from only 13 cases. As we were interested in determining the differences in the projections of the translation and rotation zones, we only considered those cases where the injection site was clearly located on one side of the translation-rotation border. Thus, cases in which the injection site did not extend more than 1.6 mm from the midline were considered within the translation zone, and injection sites that were located at least 2.2 mm from the midline were considered to be within the rotation zone. Because we approached the VbC through the vestibular apparatus, to access the translation zone the electrode had to pass through the rotation zone. In some of these cases, which were all discarded from the analysis, there was clearly leakage along the electrode track; hence Purkinje cells within the rotation zone were (potentially) labeled. Also, we only considered cases in which the injection was clearly localized to the molecular layer (ml) and did not encroach on the granule cell layer. Terminals

from cases in which the granule cell layer was injected could represent collaterals of mossy fibers. As the injections were in the ml, it is possible that some of the terminals we observed were due to collaterals of retrogradely labeled climbing fibers. However, we never observed retrogradely labeled cell bodies in the inferior olive.

In each of the 13 cases, between 10 and 100 Purkinje cells were labeled and terminals were found throughout the vestibular and cerebellar nuclei. A "beam" of parallel fibers, which traversed the entire folia, was also labeled (see Figs. 2A,C and 5A,B). Labeled granule cells were found local to the injection site, but retrogradely labeled cells were rarely found outside the cerebellar cortex. Table 1 shows a summary of each individual case listing the folia injected, the distance of the injection site from the midline, and the location of anterogradely labeled terminals in the vestibular and cerebellar nuclei. Three, two, and one asterisk(s) denote a heavy, moderate, and small amount of observed labeling, respectively. If there was an emphasis of labeling within a particular region of a nucleus, this is indicated in parentheses.

### Translation zone injections

In six cases the injection sites were confined to the translation zone (see Table 1, cases #T1–T6). In cases #T1 and #T2 the injections were about 400  $\mu$ m in width and centered 0.8 and 0.55 mm from the midline, respectively. These injections were in dorsal X, but a few Purkinje cells were labeled in ventral IXc,d in both cases. In case #T3 the injection was about 400  $\mu$ m and centered approximately 1.2 mm from the midline. The few labeled Purkinje cells were confined to ventral IXc,d. Case #T4 was a small injection (<200  $\mu$ m wide) that labeled about 12 Purkinje cells in dorsal X. It was centered about 0.5 mm from the midline. In case #T5 the injection site was about 0.5 mm wide and centered 0.6–0.7 mm from the midline. About 20 labeled Purkinje cells were found in ventral X, and one in dorsal X was also labeled. Finally, in case #T6, the injection site was centered about 0.9 mm from the midline and was approximately 0.6 mm wide. Twelve Purkinje cells in dorsal X were labeled, but two to three times as many in ventral X were labeled.

In all six cases the pattern of terminal labeling was strikingly similar. Figure 1 shows a series of coronal sections through the vestibular and cerebellar nuclei to illustrate the location of anterogradely labeled terminals from case #T1. In all six cases the heaviest terminal labeling was seen in the pcv (Figs. 1E–K, 2E,F,H,I) in the areas between CbM and CbL, and in the area that bridges the CbM and VeS. Only a small amount of labeling was found within the borders of the CbM for this case (Fig. 1D,E). Labeling within the CbM was found in four of the other five cases (Fig. 2H,I). The fibers descended through the CbM and pcv into the rostral part of the vestibular nuclei. Some of these terminated in the VeS, which contained a moderate amount of labeling in five of the six cases, and a small amount of labeling in case #T6 (Figs. 1H–L, 2D). Although terminals were observed throughout the VeS, most of them were found dorsally (abutting on the pcv), laterally and medially in the caudal two-thirds of the nucleus. Other fibers descended through VeLd, where no terminals were ever seen, into VeD. A moderate amount of terminal labeling was seen in the VeD, particularly in the lateral half (Figs. 1A–F, 2G).

TABLE 1. Distribution of Terminal Labeling in the Vestibular and Cerebellar Nuclei After Injections of Biotinylated Dextran Amine Into Either the Rotation or Translation Zone of the Pigeon Vestibulocerebellum

Case #	Location of injection site <sup>1</sup>	pcv (CbL)	CbL	pcv (CbM)	CbM	VeS	VeM	VeD	VDL	VeLv	ph	Ta
R1	Rotation zone; 2.3–2.8 mm Lateral pole of IXcd; (dorsal IXcd; ventral IXcd)	++	++			+++ (central)	++	++	++			+
R2	Rotation zone; 2.9–3.1 mm Ventral IXcd	+	+++			+++ (dorsal)	+	++ (dorsal, medial)	+			+
R3	Rotation zone; 3.0–3.8 mm Ventral IXcd (dorsal X; dorsal IXcd; lateral pole of IXcd)	+++	++	+		++ (dorsal)	+++	+++ (medial)	++	+	+	+
R4	Rotation zone; 2.3–2.6 mm Dorsal X		+			++ (dorsal, medial)	+++	+++ (medial)	+		+	+
R5	Rotation zone; 2.9–3.1 mm Ventral IXcd; (dorsal X; dorsal IXcd)	++	+			++ (dorsal, medial)	+++	+++ (medial)	+		+	+
R6	Rotation zone; 3.2–3.5 mm Lateral pole of X (ventral X, dorsal X)	+	+			++ (dorsal, central)	+++	++ (throughout)	+	+	++	
R7	Rotation zone; 3.2–3.4 mm Dorsal IXcd	+	+			++ (dorsal, central)	+	++ (throughout)	++	+		+
T1	Translation zone; 0.6–1.0 mm Dorsal X (ventral IXcd)			+++	+	++ (dorsal, medial, lateral)	+	++ (central, lateral)	+			
T2	Translation zone; 0.2–0.7 mm Dorsal X (ventral IXcd)			+++	+++	++ (dorsal, medial, lateral)	+	++ (lateral)	+			+
T3	Translation zone; 1.0–1.4 mm Ventral IXcd		+	+++	+	++ (dorsal, medial, lateral)	+	++ (lateral)	+	+		+
T4	Translation zone; 0.4–0.6 mm Dorsal X			+++	++	++ (dorsal, medial, lateral)	+	++ (lateral)	+	+		+
T5	Translation zone; 0.3–0.9 mm Ventral X		+	+++	++	++ (dorsal, medial, lateral)	++	+++ (caudal)			+	
T6	Translation zone; 0.6–1.2 mm Ventral X (dorsal X)	+		++		++ (dorso-medial, central)		++ (caudal)				

<sup>1</sup>The lamella that received the bulk of the injection is listed first. Labeled Purkinje cells were also seen in the lamellae listed in parentheses. The distance (mm) of the injection site from the midline is also indicated. +++, heavy terminal labeling; ++, moderate amount of terminal labeling; +, small amount of terminal labeling. See list for abbreviations.

In five cases, some of these fibers continued into the VeM. In four of these cases, sparse terminal labeling was seen in VeM (Fig. 1C,L), but in case T#5, a moderate amount of labeling was seen in VeM, particularly caudally. A small amount of labeling was also seen in the VDL (four cases), Ta (three cases; Fig. 1D), VeLv (three cases), the edge of CbL (two cases), and the pcv adjacent to CbL (one case). In all cases, moderate terminal labeling was found near the wall of the fourth ventricle, ventral to the cerebellar nuclei, dorsomedial to VeS, medial to VDL, and dorsal to nucleus laminaris (La; Fig. 1E–I). These terminals were difficult to ascribe to any one nucleus. Some were ascribed to the dorsomedial portion of VeS, some to the medial margin of VDL, and some to the pcv. This is the area in which group A resides in chickens (Wold, 1976; Dickman and Fang, 1996). Figure 2 shows photomicrographs of injection sites and terminal labeling in the cerebellar nuclei from the translation zone cases.

**Rotation zone injections**

In seven cases the injection sites were confined to the rotation zone (Table 1, cases #R1–R7). In case #R1 the injection was about 500 µm wide and centered in the lateral pole IXc,d about 2.55 mm from the midline. Labeled Purkinje cells were also seen in both dorsal and ventral IXc,d. In cases #R2, #R3, and #R5 the injections were all in ventral IXc,d and centered 3.0, 3.4, and 3.0 mm from the midline, respectively. In cases #R3 and #R5 a few labeled Purkinje cells were seen in dorsal IXc,d and dorsal X. In case #R4 the injection was 300 µm wide, centered 2.55 mm from the midline and confined to dorsal X. The injection in case #R6 was 300 µm wide and centered 3.35 mm from the midline on the lateral pole of X. Finally, the injection in case #R7 was confined to dorsal IXc,d 3.2–3.4 mm from the midline.

The pattern of terminal labeling was quite similar in all cases, but not as strikingly similar as in the cases of the injections into the translation zone. Figures 3 and 4,

respectively, show series of coronal sections through the vestibular and cerebellar nuclei to illustrate the location of anterogradely labeled terminals from cases #R4 and #R3. In all cases terminal labeling was seen in within CbL and/or the white matter adjacent to CbL (pcv-CbL). Generally, this labeling was heavier after those injections that included ventral IXc,d (cases #R1–3, 5). In case #R4, dozens of fibers were seen coursing through CbL, but only a few terminals were seen (Fig. 3F–H), whereas in case #R3 and in other cases the terminal labeling was quite heavy (Fig. 4E–H). Most of the labeling was found in the lateral and dorsal margins of CbL and the pcv adjacent to the lateral edge of CbL. As with the injections into the translation zone, some fibers descended into VeS, which received a heavy (cases #R1, #R2) or moderate (cases #R3–#R7) amount of labeling (Figs. 3I,J, 4G–L, 5E). Terminals were seen throughout VeS: most were found dorsally, medially, and centrally, but labeling was not so common in the lateral VeS. In all cases light to moderate terminal labeling was also found in VDL (Figs. 3H, 4D–G). Other fibers coursed through the VeLd and branched extensively at the dorsal border of VeD. Terminal labeling was moderate to heavy in VeD (Figs. 3A–G, 4A–F, 5F–H).

Although terminals were observed throughout VeD, most were found in the medial half. Many of these fibers continued medially, and labeling was found in the VeM in all cases (Figs. 3A–D, F–L, 4A–L, 5I,J). In four of the cases the VeM labeling was heavy. In four cases a few fibers continued into the PH where a small amount of terminals were seen (Fig. 4D–H), except in case #R6 where a moderate amount of terminal labeling was seen. In six of the cases a small amount of labeling was seen in Ta (Fig. 4J,K). A small amount of labeling was also seen in the pcv adjacent to the CbM (one case) and VeLv (three cases). As with the injections in the translation zone, labeling was found near the wall of the fourth ventricle, ventral to the cerebellar nuclei, and dorsal to La (Fig. 3H–J, 4H–J). Some terminals were ascribed to the dorsomedial portion

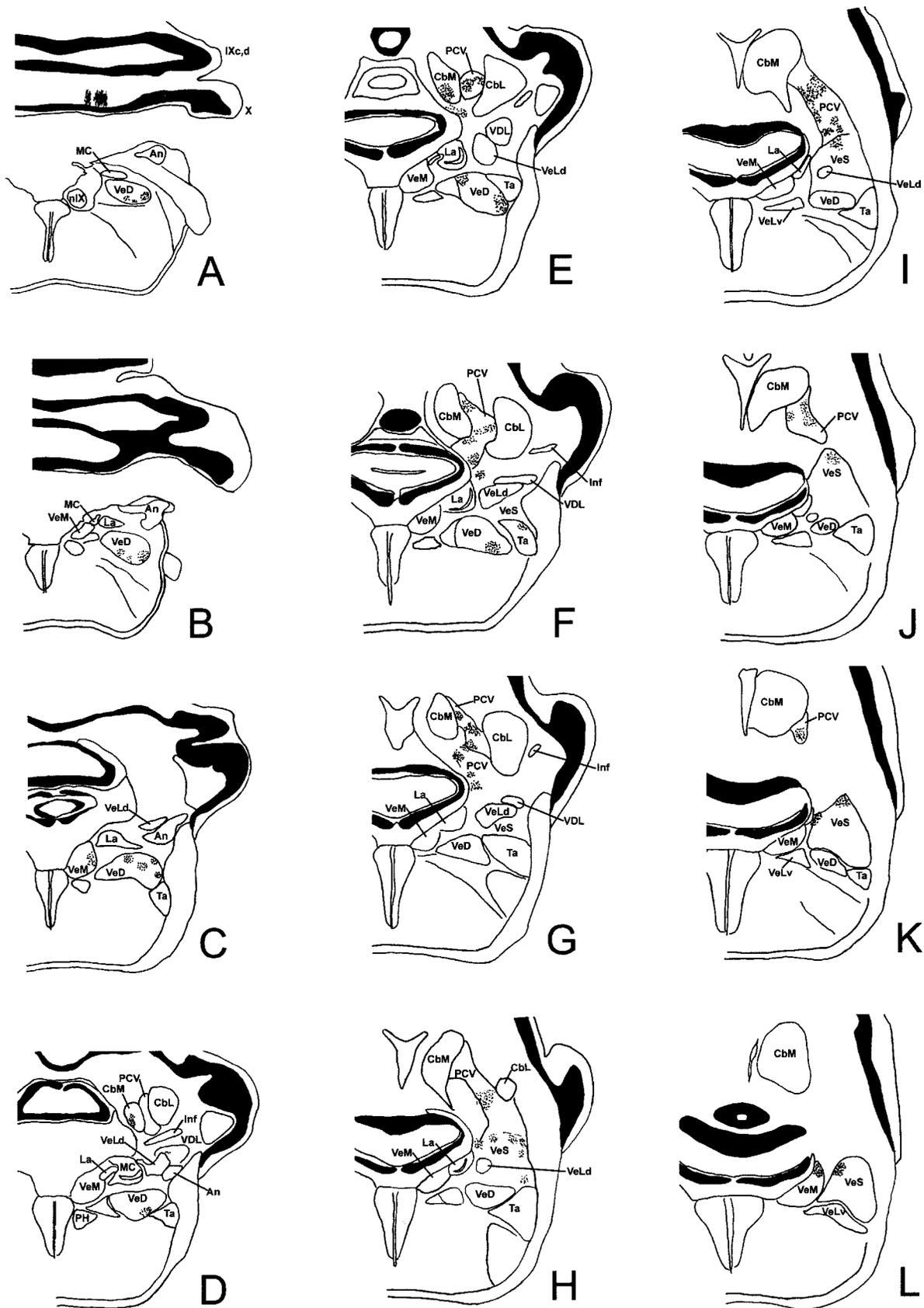


Fig. 1. Locations of the terminals of translation zone Purkinje cells. A-L: This series of coronal sections through the vestibular nuclei complex is from case #T1 (A, most caudal; L, most rostral). The injection site is shown in A by the darkened area in the molecular layer

of folium X. The stippled regions indicate areas where terminal labeling was found, but not the density of terminal labeling. See text for a detailed description. For abbreviations, see list.

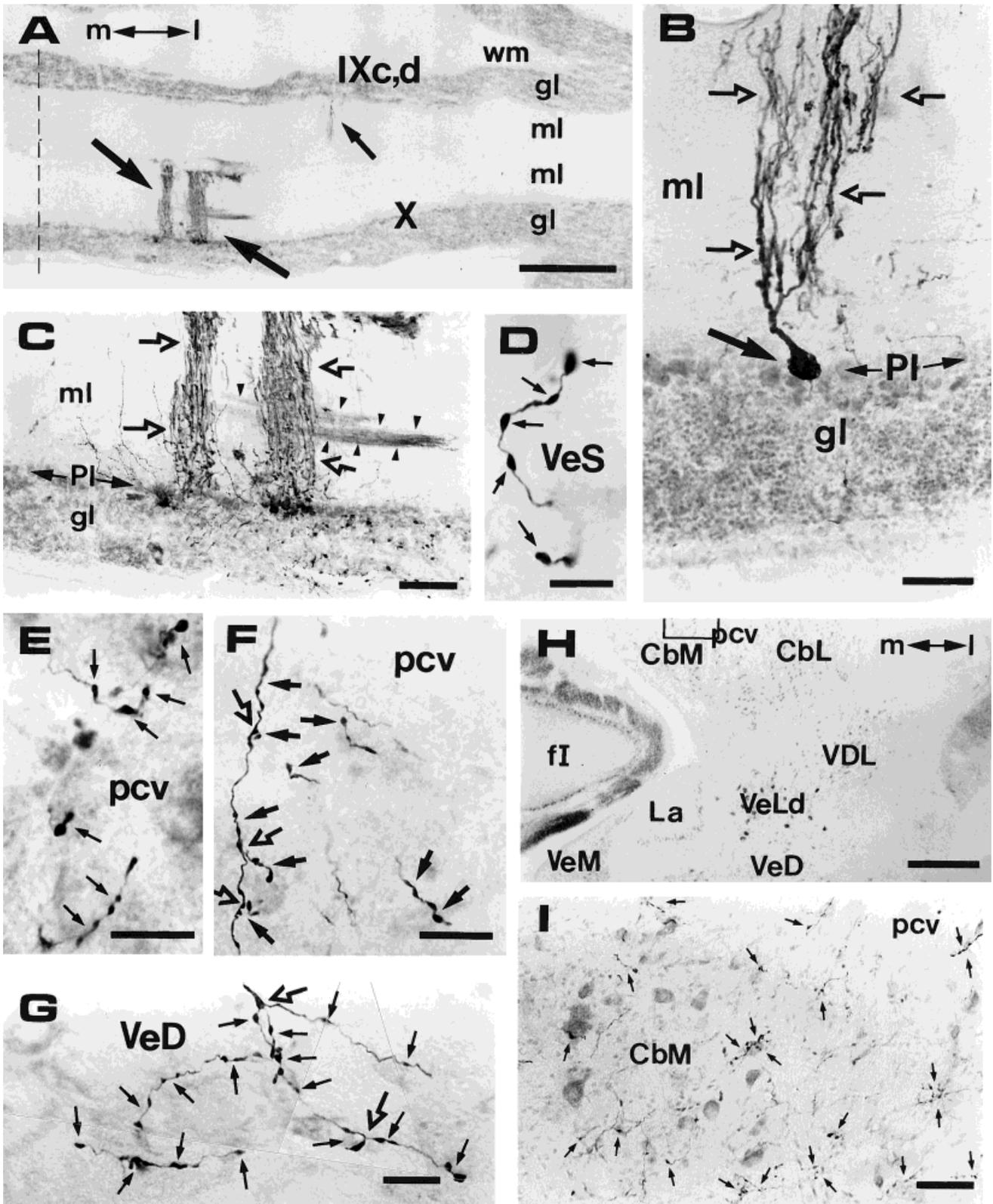


Fig. 2. Projections of Purkinje cells in the translation zone of the vestibulocerebellum (VbC). **A:** Injection site from case #T1. The bulk of the injection (large arrows) was in the dorsal lamella of folium X, 0.6–1 mm from the midline (dashed vertical line). Some of the tracer leaked and labeled a few Purkinje cells in the ventral lamella of folium IXc,d (small arrow), but they were clearly within the border of the translation zone. **B:** A single Purkinje cell from the edge of the injection site from case #T2. The cell body and the dendritic tree are respectively indicated by the large arrow and stylized arrows. **C:** Higher power photomicrograph of the same injection site. The Purkinje cell dendrites in the molecular layer (ml) are indicated by the stylized arrows. Several labeled Purkinje cells are indicated by the smaller arrows. The arrowheads (triangles) indicate the parallel fiber beam. **D:** Terminal

labeling in the superior vestibular nucleus (VeS) from case #T1. **E, F:** Terminals in the cerebellovestibular process (pcv) adjacent to the medial cerebellar nucleus (CbM) from cases #T2 and #T3, respectively. **G:** Extensive terminal field in the descending vestibular nucleus (VeD) from case #T3. **I:** Terminal fields in the CbM and adjacent pcv. The location of this area is indicated by the rectangular area in **H**. Such extensive labeling in the CbM and adjacent pcv was typical after injections in the translation zone. The stylized arrows in **F** and **G** indicate clear branch points. The other arrows in **F** and **G**, and the arrows in **D**, **E**, and **I** indicate individual terminals (varicosities) or groups of terminals. m, medial; l, lateral. Scale bars = 500  $\mu$ m in **A** and **H**; 100  $\mu$ m in **B**; 50  $\mu$ m in **C** and **I**; 10  $\mu$ m in **D**; 20  $\mu$ m in **E–G**.

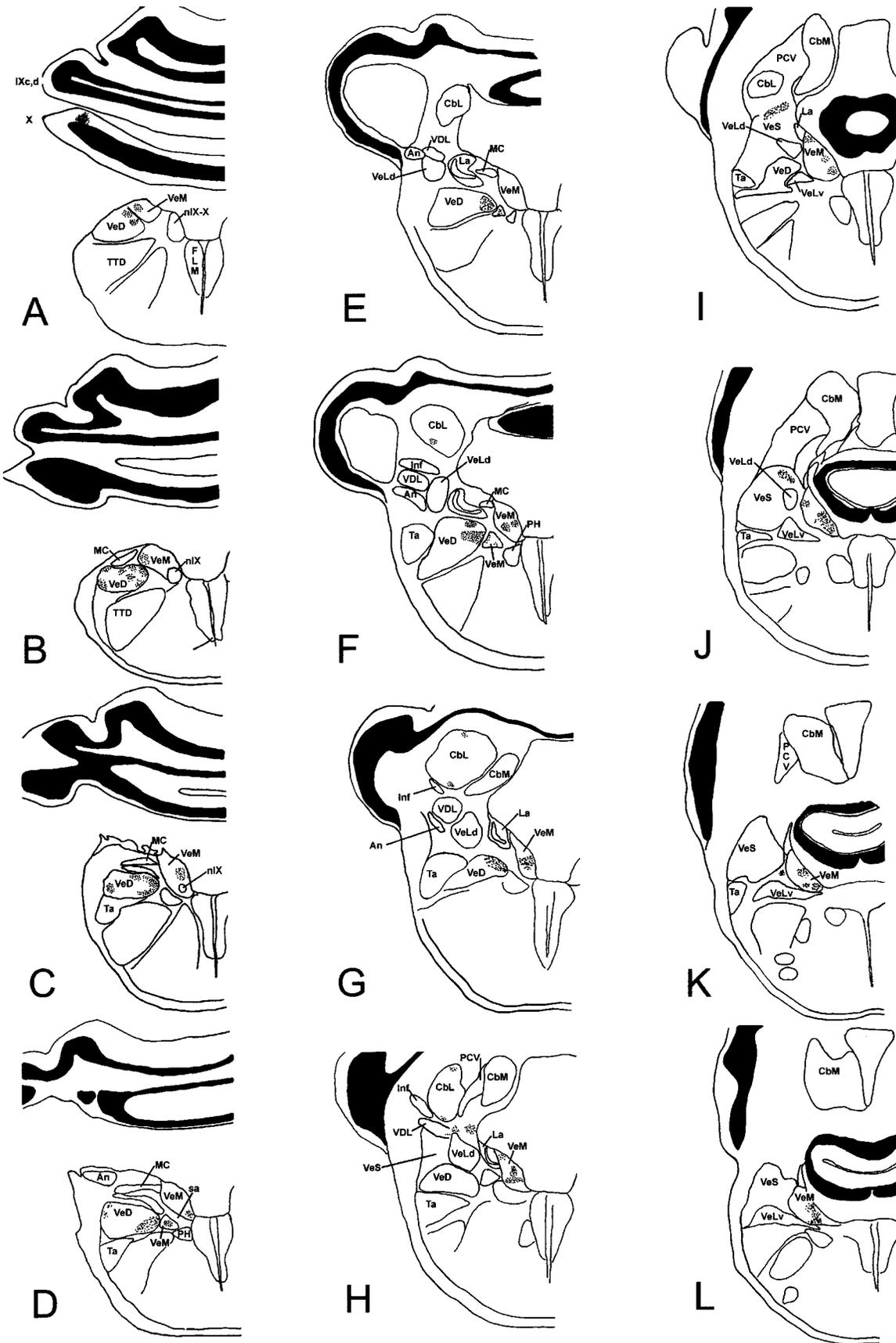


Fig. 3. Locations of the terminals of rotation zone Purkinje cells. A-L: This series of coronal sections through the vestibular nuclei complex is from case #R4 (A, most caudal; L, most rostral). The injection site is shown in A by the darkened area in the molecular layer of folium X. The stippled regions indicate areas where terminal

labeling was found, but not the density of terminal labeling. Terminal labeling found in the medial-rostral area of the VeD as in E-G was never labeled after injections in the translation zones. See text for a detailed description.

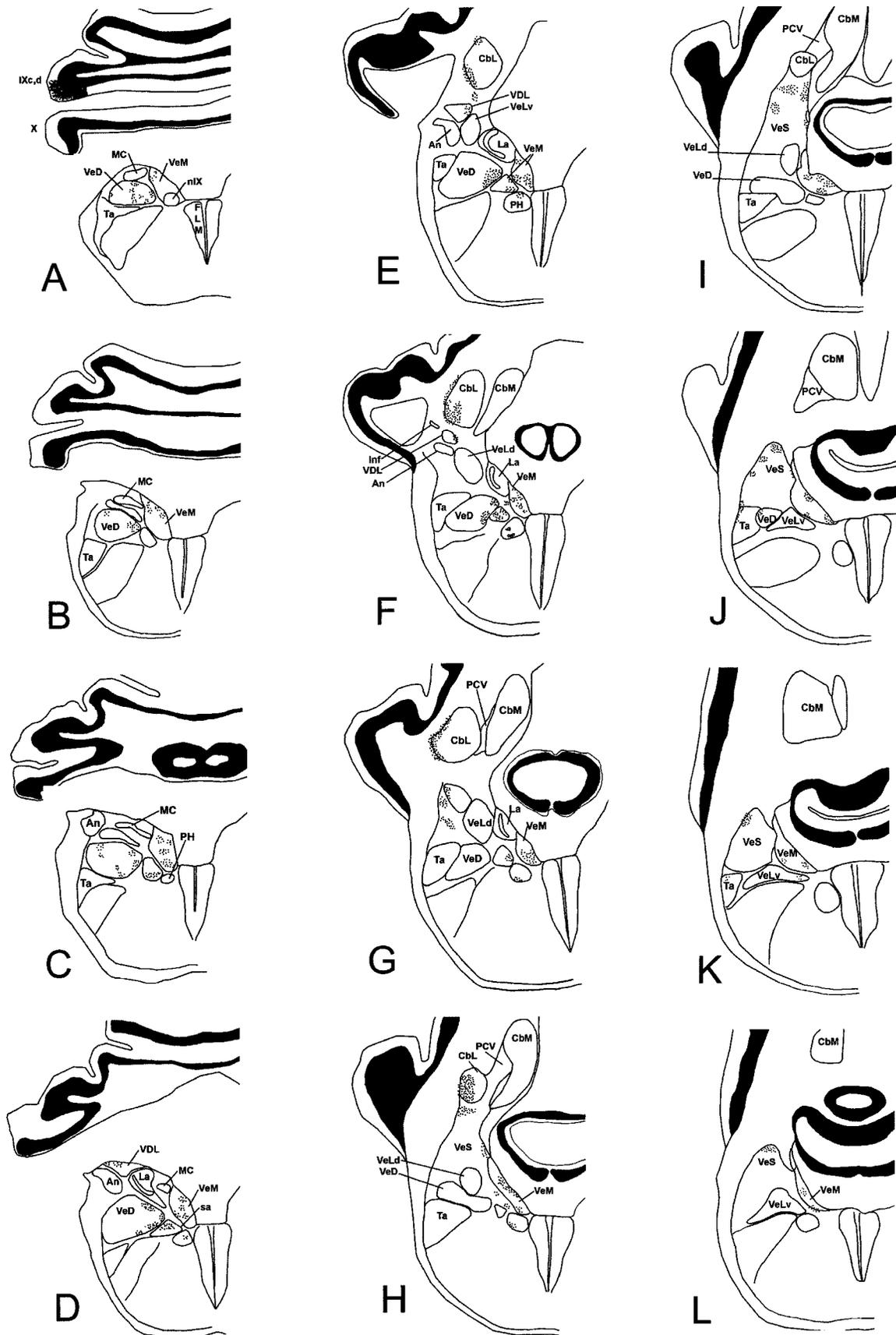


Fig. 4. Locations of the terminals of rotation zone Purkinje cells. A-L: This series of coronal sections through the vestibular nuclei complex is from case #R3 (A, most caudal; L, most rostral). The injection site is shown in A by the darkened area. It was centered in the

ventral IXc,d, but also labeled Purkinje cells in dorsal X, the lateral pole of IXc,d and dorsal IXc,d. The stippled regions indicate areas where terminal labeling was found, but not the density of terminal labeling. See text for a detailed description.

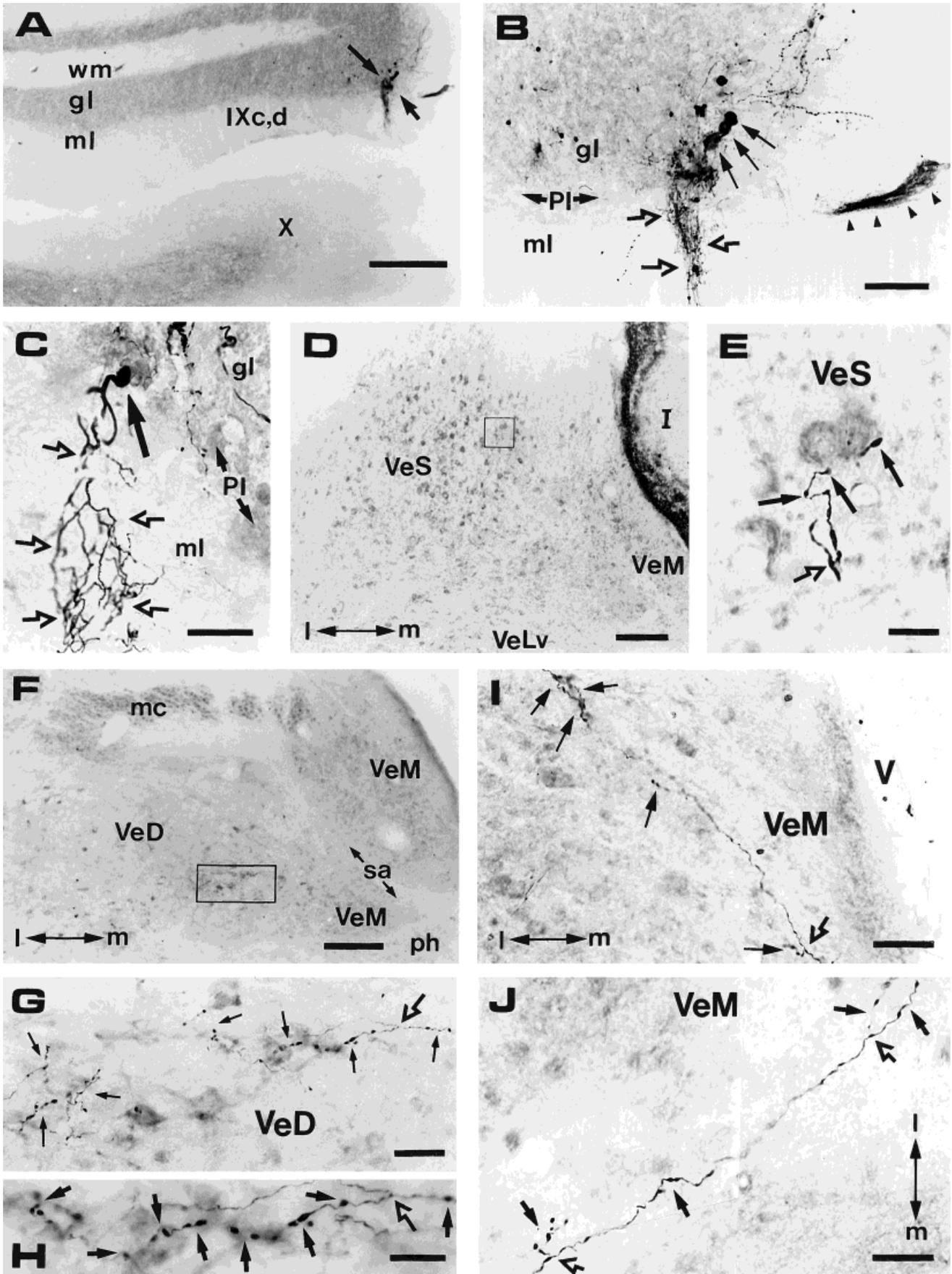


Figure 5

of VeS, some to the medial margin of VDL, and some to the pcv. We reiterate that this is the area in which group A resides in chickens (Wold, 1976; Dickman and Fang, 1996). Figure 5 shows photomicrographs of injection sites and terminal labeling in the cerebellar nuclei from the rotation zone cases.

Figure 6E summarizes our findings of the projections of Purkinje cells in the rotation (left side) and translation (right side) zones of the VbC. The size of the arrowheads qualitatively represents the magnitude of the projection.

## DISCUSSION

In this report, we have shown the projection sites of the VbC zones containing cells responsive to translational and rotational optic flow. After injections of BDA in the translation zone, extremely heavy terminal labeling was found in the pcv adjacent to CbM. A moderate amount of labeling was observed in CbM itself, VeS, and VeD, whereas a small amount of labeling was found in the Ta, VeM, VDL, and VeLv. After injections of BDA into the rotation zone, heavy terminal labeling was found in VeS, VeM, and VeD. A moderate amount of labeling was found in the VDL and the pcv adjacent to the CbL, whereas a small amount of terminal labeling was found in the CbL, Ta, ph, and VeLv. Thus, the projections of the rotation and translation zones to the cerebellar nuclei are quite distinct. The projections of the two zones to the vestibular nuclei are largely distinct, but there certainly is some overlap, particularly with respect to VeS and VeD. However, there were differential projections to these two nuclei. With respect to VeD, terminal labeling was found throughout VeD after injections into the rotation or translation zones, but the labeling was heavier in the medial half after injections into the rotation zones, and heavier in the lateral half after injections into the translation zone. With respect to the VeS, most of the labeling after injections in the rotation zone was located dorsally, medially, and centrally. Most of the terminals in the VeS from injections in the translation zone were also found in the dorsal and medial regions, but also in the lateral region. Compared with injections into the translation zone, we found more variability in the

distribution of the terminal labeling after injections into the rotation zone.

Together, these data emphasize that the neural systems subserving self-translation and self-rotation remain segregated in the vestibular and cerebellar nuclei. This is illustrated in Figure 6, where we show the rotation zones (D) and translation (D) of the mc of the IO (from Lau et al., 1998), and the projections of the translation and rotation zones of the VbC revealed from the present study (E). (In Fig. 6E we also note the input from the otolith organs [\*] and semicircular canals [#; see below]). Note that there is the possibility of integration of self-translation and self-rotation optic flow information, particularly in VeD and VeS. Since self-motion generally involves a combination of rotation and translation, one would expect this sort of integration. Previously we have emphasized that the processing of translational and rotational optic flow by the VbC is based on a common spatial frame of reference, which would permit easy integration (Wylie et al., 1998; Wylie and Frost, 1999).

## Comparison with previous studies of pigeons

Arends and Voogd (1989) divided the pigeon cerebellum into five parasagittal zones (zones A–C, E, and F) based on climbing fiber input from the IO. Zone F, which received input from the medial column (mc) of the IO, corresponds very well to the flocculus rotation zone, and we have recently confirmed that it receives projections from the dorsomedial mc (Lau et al., 1998). The determination of the other four zones was based on retrograde labeling from injections throughout the vermis. Arends and Voogd (1989) admitted that few of these injections were in the folia of the VbC, but nonetheless they had these four zones extend into the VbC. We (Lau et al., 1998) showed that the translation zone of the VbC received input from the ventrolateral mc and cautioned against applying the description offered by Arends and Voogd (1989) to the VbC. This is also the case in mammals, where the zones of the VbC (flocculus, uvula, and nodulus) do not correspond to the zones of other parts of the cerebellum (e.g., Groenewegen et al., 1979).

Arends and Zeigler (1991) provided a comprehensive description of the projections of pigeon cerebellum with the anterograde transport of WGA-HRP. They described the five zones, which they extended into the VbC, but again we would like to encourage caution. Arends and Zeigler (1991) provided a remarkably well-detailed description of the extent of their injection sites, which does permit a comparison with the data from the present study. In their study, they reported seven cases in which the injection included the uvula, nodulus, and/or auricle. It is important to note that the dorsal uvula, which we did not consider part of the translation zone (Fig 6E; Wylie et al., 1993), was included in some of these injections. Three of their "lateral" injections appear to be restricted to the rotation zone, and two "medial" injections appear to be restricted to the translation zone. On some accounts, our findings are in agreement with those of Arends and Zeigler (1991). Terminal labeling in the VeS was described as moderate to heavy in six of the seven cases and light in the last case. They did note that the medial injections resulted in more labeling in the dorsolateral VeS, and the lateral injections resulted in more labeling in the medial VeS. Labeling in VeD and VeM was moderate to heavy in four cases, light in two cases, and absent in one. Differential projections to the medial

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Fig. 5. Projections of Purkinje cells in the rotation zone of the vestibulocerebellum (VbC). **A:** Injection site from case #R2, indicated by the arrows. This section is from the caudal edge of the injection site: in other sections many more Purkinje cells were labeled. The bulk of the injection was centered 3.0 mm from the midline, in the extreme lateral edge of in the ventral layer of folium IXc,d. **B:** Higher power photomicrograph of the same injection site. The Purkinje cell dendrites in the molecular layer (ml) are indicated by the stylized arrows, and three clearly labeled somae of Purkinje cells are indicated by the small arrows. The arrowheads indicate the parallel fiber beam. **C:** A single Purkinje cell from the edge of the injection site from case #R1. The cell body and the dendritic tree are respectively indicated by the large arrow and stylized arrows. **E:** Terminal labeling in the medial margin of superior vestibular nucleus (VeS), from case #R1. The area shown in E is indicated by the rectangle in **D**. **G,H:** Different magnifications of the same terminal field in the descending vestibular nucleus (VeD) from case #R3. The area shown in G is indicated by the rectangle in **F**. **I,J:** Different magnifications of the same terminal field in the medial vestibular nucleus (VeM) from case #R4. In E and G–J, the stylized arrows indicate clear branch points whereas the other arrows indicate individual terminals (varicosities) or groups of terminals. I, lateral; m, medial. Scale bars = 500  $\mu$ m in A; 200  $\mu$ m in D,F; 100  $\mu$ m in B,I; 50  $\mu$ m in C,G,J; 20  $\mu$ m in E,H.

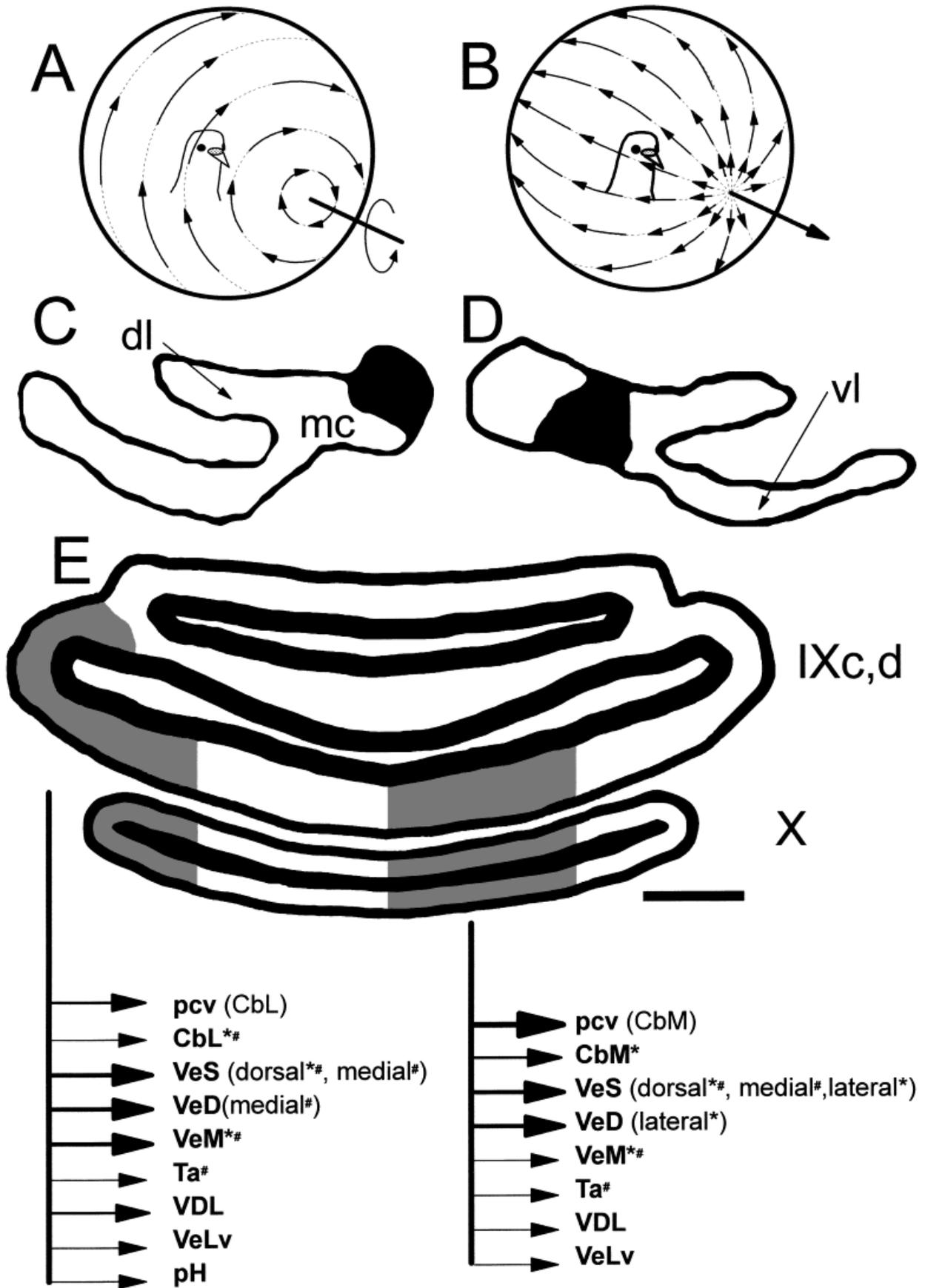


Figure 6

and lateral VeD were not noted. For those four cases in which there was moderate to heavy labeling in the VeM, two were cases in which the injection was located medially. In contrast, we found moderate to heavy labeling in the VeM after injections into the rotation zone, but sparse labeling after injections into the translation zone.

Arends and Zeigler (1991) found moderate to heavy labeling in the ph in two cases in which the injection included the lateral VbC, but not in the medial cases. Similarly we found labeling (light) in the ph only after rotation zone injections. However, for two of the "lateral" injections Arends and Zeigler (1991) noted no labeling in ph. They did note moderate to heavy labeling in VDL in three cases, and light labeling in the VDL in two cases, but there were no consistent differences with respect to the projections from the medial and lateral injections. They found moderate to heavy labeling in the Ta from the lateral but not the medial injections. This is at odds with our findings that injections into the rotation zone and translation zone resulted in light labeling in the Ta. Arends and Zeigler (1991) noted terminal labeling in the CbL only after lateral injections, which is consistent with our findings. Likewise, they found that the major projection of the medial VbC was to pcv as did we, but they did not ascribe any terminals to the CbM.

The major difference between our findings and those of Arends and Zeigler (1991) is with respect to the projection to Inf. They found heavy terminal labeling in Inf from the lateral injections, whereas we noted an absence of labeling in Inf from all our injections. This difference (as with any other differences), could be due to the fact that their injections were not restricted to the molecular layer; thus, some of the terminals that they observed could be collaterals of mossy fibers. It could also be due to the fact that their lateral injections may have been located more rostral than our rotation zone injections. However, it is more likely that they had different criteria for the defining the borders of Inf. We could only identify Inf in as many as five serial sections; however, the drawings of Arends and Zeigler (1991) suggest that Inf is quite extensive. Rostrally they showed Inf as medial to VDL, in an area that might include group A described by Dickman and Fang (1996). The termination that Arends and Zeigler (1991) ascribed to Inf we may have ascribed to CbL, pcv, VDL, and/or dorsal VeS. In the drawings of Arends et al. (1991), they showed some cells retrogradely labeled from the oculomotor complex that are quite dorsal to the VDL and on the lateral edge of CbL. They ascribed these to the Inf, whereas we would have ascribed them to the CbL or the

pcv adjacent to the CbL. In summary, Arends and Zeigler (1991) found a heavy projection to a restricted area dorsal to VDL from injections into the lateral VbC, which they ascribed to Inf. From the rotation zone, we found a heavy projection to the lateral edge of the CbL and the adjacent pcv but did not see terminals in Inf, in those few sections where Inf was clearly identifiable.

### Comparison with the primary vestibular projection

As with the systems analyzing optic flow, the vestibular system also has separate structures analyzing self-translation and self-rotation. The semicircular canals are sensitive to rotation of the head, whereas the otolith organs respond to linear acceleration that would result from self-translation (Wilson and Melvill Jones, 1979). (The otolith organs also respond to head tilt that occurs during rotation of the head about axes other than the vertical axis). Given this, one might hypothesize that the canals and otolith organs project to those areas of the vestibular and cerebellar nuclei receiving input from Purkinje cells in the rotation and translation zones, respectively. Detailed descriptions of the projections of the vestibular apparatus have been provided by Schwarz and Schwarz (1986) and Dickman and Fang (1996), and their findings do support the above stated hypothesis to some degree. The projection to the VeS is to the dorsal and medial aspects from the canals, and to the dorsal and lateral margins from the otoliths. Likewise, we found that the lateral margin of VeS received a heavier input from the translation zones, and the dorsal margin received input from both the rotation and translation zones. The projection to the VeD was largely to the medial half from the canals, and to the lateral half from the otolith organs. Similarly, we found that the medial and lateral halves of VeD received heaviest input from Purkinje cells in the rotation and translation zones, respectively.

Dickman and Fang (1996) reported that the canals, but not the otoliths organs, projected to Ta, but we found projections (light) from both the translation and rotation zones. Dickman and Fang (1996) did not distinguish the pcv from CbL and CbM, which makes a comparison in this regard a bit difficult. Nonetheless, they reported that the projection from the canals was largely to CbL, whereas the projection of the otoliths was to the lateral margin of CbM and CbL. The canals and otoliths projected to the medial and lateral margins of VeM, respectively (Schwarz and Schwarz, 1986; Dickman and Fang, 1996), but we found that the rotation zone projected throughout the VeM, and the projection from the translation zone was weak.

### Comparison with mammalian studies

It is difficult to compare the results of the present study with those of mammals for one simple reason: the olivocerebellar system responsive to translational optic flow has only been found in pigeons. However, physiologically, the rotational optic flow system in mammals is essentially identical to that in pigeons. Purkinje cells of the rotational optic flow system in both rabbits and pigeons prefer rotation about either the vertical axis or an horizontal axis that is perpendicular to the ipsilateral anterior canal (Simpson et al., 1981; Graf et al., 1988; Wylie and Frost, 1993).

In rabbits, rotation-sensitive Purkinje cells are found in four of five zones in the flocculus, and four of five zones in

Fig. 6. Brainstem-cerebellar systems analyzing translational and rotational self-motion. **A,B:** Rotational and translational, respectively, optic flowfields that result from rotation about, and translation along, the z axis. The arrows, as projected onto a sphere surrounding the pigeon, represent local image motion in the flowfield. **C,D:** Those areas of the medial column (mc) of the inferior olive that project to the rotation and translation zones, respectively, of the vestibulocerebellum (from Lau et al., 1998). **E:** Schematic of the results of the present study. The drawing is a camera lucida tracing of a coronal section through the VbC. The granule cell layers are filled black, and the areas containing optic flow responses are shaded gray. The projections of the rotation and translation zones are shown on the left and right, respectively. The size of the arrow represents the magnitude of the projection. # and \* indicate those structures that receive a projection from the semicircular canals and otolith organs, respectively (Schwarz and Schwarz, 1986; Dickman and Fang, 1996). Scale bar = 1 mm.

the ventral nodulus, as well as parts of the dorsal nodulus and ventral uvula (Graf et al., 1988; Kano et al., 1990; Kusunoki et al., 1990; DeZeeuw et al., 1994; Tan et al., 1995; Wylie et al., 1995). In rabbits, although there were some reported differences between the projection of the ventral nodulus and flocculus, the rotation zones projected to the group  $\gamma$  (which Arends et al. [1991] consider to be equivalent to the VDL in pigeons); the ventral dentate nucleus (which Arends et al. [1991] consider to be equivalent to Inf in pigeons); VeS; the parvocellular portion of the VeM; the magnocellular portion of the VeM (VeLv); and the white matter surrounding the posterior interposed nucleus (perhaps equivalent to the pcv/CbL; DeZeeuw et al., 1994; Wylie et al., 1994). In these studies, little labeling was found in VeD (DeZeeuw et al., 1994; Wylie et al., 1994). In rabbits, those zones responsive to rotation about the vertical axis project largely to the VeM, whereas those zones responsive to rotation about the horizontal axis perpendicular to the anterior canal project largely to the group  $\gamma$ , VeS, and the caudal parts of VeM. (e.g., DeZeeuw et al., 1994; Wylie et al., 1994; Tan et al., 1995). This work in rabbits emphasizes that the projections of the translation and rotation zones may be further subdivided. There are four subzones within the translation zone (Wylie and Frost, 1999). There are the two types of responsive cells in the rotation zone, essentially identical to the two types of rotation cells in mammals (Wylie and Frost, 1993), but the number of subzones remains undetermined.

Perhaps an appropriate comparison of the translation zone in pigeons is the most medial zone of the mammalian nodulus and ventral uvula. Purkinje cells in this zone receive climbing fiber input from the beta subnucleus of the IO and are responsive to head tilt: that is, these cells are responsive to signals originating in the otolith organs (Shojaku et al., 1991; Barmack and Shojaku, 1992, 1995). What this zone has in common with the translation zone in the pigeon VbC is that both would be active during self-translation. In rabbits, this zone projects to the white matter abutting on the fastigial nucleus (Wylie et al., 1994), which might be an analogous to the pcv/CbM in pigeons. However, this zone in mammals also projects heavily to the caudal VeM and the white matter surrounding the interposed nuclei (Wylie et al., 1994), but in the present study, the projection from the translation zone to the VeM was usually sparse.

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