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# CONGRUENCE OF ZEBRIN II EXPRESSION AND FUNCTIONAL ZONES DEFINED BY CLIMBING FIBER TOPOGRAPHY IN THE FLOCCULUS

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**Abstract—The cerebellum is organized into parasagittal zones with respect to the topography of climbing fiber (CF) afferents and the expression of molecular markers such as zebrin II. Zebrin is expressed by a subset of Purkinje cells that are distributed as a parasagittal array of immunopositive and immunonegative stripes. Several studies in rodents suggest that, in general, CFs to the zebrin negative stripes convey somatosensory information, whereas CFs to the zebrin positive stripes convey information from visual and other sensory systems. The pigeon flocculus consists of four pairs of zebrin+/− stripes (P4 +/− through P7 +/−), however the CF input consists entirely of visual inputs. Thus, because the correspondence of zebrin expression and CF information must be different from that proposed for rodents, we investigated this relationship in the pigeon flocculus. Floccular Purkinje cells respond to patterns of optic flow resulting from self-rotation about one of two axes: either the vertical axis (zones 0 and 2), or a horizontal axis (zones 1 and 3). Visual CF afferents projecting to the flocculus arise from the medial column of the inferior olive (mcIO). Zones 0 and 2 receive input from the caudal mcIO, whereas zones 1 and 3 receive input from the rostral mcIO. We injected a fluorescent anterograde tracer into the rostral and/or caudal mcIO and visualized zebrin expression. There was a strict concordance between CF organization and zebrin labeling: caudal mcIO injections resulted in CFs in zebrin bands P4 +/− and P6 +/−, whereas rostral mcIO injections resulted in CFs in zebrin bands P5 +/− and P7 +/−. Thus, zebrin stripes P4 +/− and P6 +/− correspond to the vertical axis zones 0 and 2, whereas P5 +/− and P7 +/− correspond to the horizontal axis zones 1 and 3. This is the first explicit demonstration that a series of zebrin stripes corresponds with functional zones in the cerebellum.** 

**Key words: cerebellum, optokinetic, vestibulocerebellum, aldolase C, optic flow, compartmentation.**

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*Abbreviations:* BDA, biotinylated dextran amine (anterograde tracer); CF, climbing fiber; CSA, complex spike activity; Hsp 25, heat-shock protein 25; IO, inferior olive; IXcd, folium IXcd of the cerebellum; mcIO or mc, medial column of the inferior olive; PBS, phosphate-buffered saline; rH45, rotation about the horizontal axis, 45° azimuth; rVA, rotation about the vertical axis; X, folium X of the cerebellum; zebrin+, zebrin immunopositive; zebrin−, zebrin immunonegative.

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The fundamental architecture of the cerebellum consists of parasagittal zones that are oriented perpendicular to the length of the folia (e.g. Voogd and Bigaré, 1980). These parasagittal zones can be defined by climbing fiber (CF) and mossy fiber input, Purkinje cell projection patterns, and Purkinje cell response properties (Llinas and Sasaki, 1989; de Zeeuw et al., 1994; Voogd and Glickstein, 1998; Wu et al., 1999; Ruigrok, 2003; Apps and Garwicz, 2005). A parasagittal organization has also been revealed with several molecular markers (for review see Herrup and Kuemerle, 1997), but the most thoroughly studied of these is zebrin II (aldolase C; Brochu et al., 1990; Ahn et al., 1994; Hawkes and Herrup, 1995), which is expressed by Purkinje cells. Zebrin immunopositive (zebrin+) Purkinje cells are distributed as a parasagittal array of stripes, separated by zebrin immunonegative (zebrin−) stripes (e.g. Larouche and Hawkes, 2006). Zebrin stripes have been shown in several mammalian species, and recently we have shown that zebrin is also expressed in the pigeon cerebellum with a pattern that is strikingly similar to that found in mammals (Fig. 1F, G; Pakan et al., 2007). Thus, the pattern of zebrin stripes is highly conserved, and is likely critical for fundamental cerebellar function. One difference between mammals and the pigeon is in the vestibulocerebellum (uvula, nodulus and flocculus; see Fig. 1C, E). In both pigeons and mammals, the nodulus (folium X) is uniformly zebrin+ (see Fig. 1G; Hawkes and Herrup, 1995; Pakan et al., 2007). Folium IXcd of the cerebellum (IXcd) in the pigeon, which includes the uvula (medial half) and the flocculus (lateral half; Wylie and Frost, 1993; Wylie et al., 1993), consists of an array of seven striking zebrin+/− stripes (P1 +/− through P7 +/−; see Fig. 1F, G; Pakan et al., 2007). In mammals, the uvula also consists of a series of zebrin+/− stripes, however, the flocculus is uniformly zebrin+ (Brochu et al., 1990; Hawkes and Gravel, 1991; Hawkes et al., 1993; Hawkes and Herrup, 1995; Ozol et al., 1999; Armstrong and Hawkes, 2000; Sanchez et al., 2002; Marzban et al., 2003; Sillitoe et al., 2003). Although the specific function of zebrin II expression in the cerebellum is largely unknown, zebrin II expression is useful for, and often used as, a positional landmark in the cerebellar cortex (Hawkes and Gravel, 1991; Hawkes, 1992; Hawkes et al., 1993; Ozol et al., 1999).

The purpose of the present study was to examine the relationship between CF input and zebrin stripes in the flocculus of pigeons, for two main reasons. First, based on studies in rodents, it has been suggested that the CFs to the zebrin− stripes carry somatosensory information, whereas the CFs that project to the zebrin+ stripes carry information from the visual and perhaps other sensory

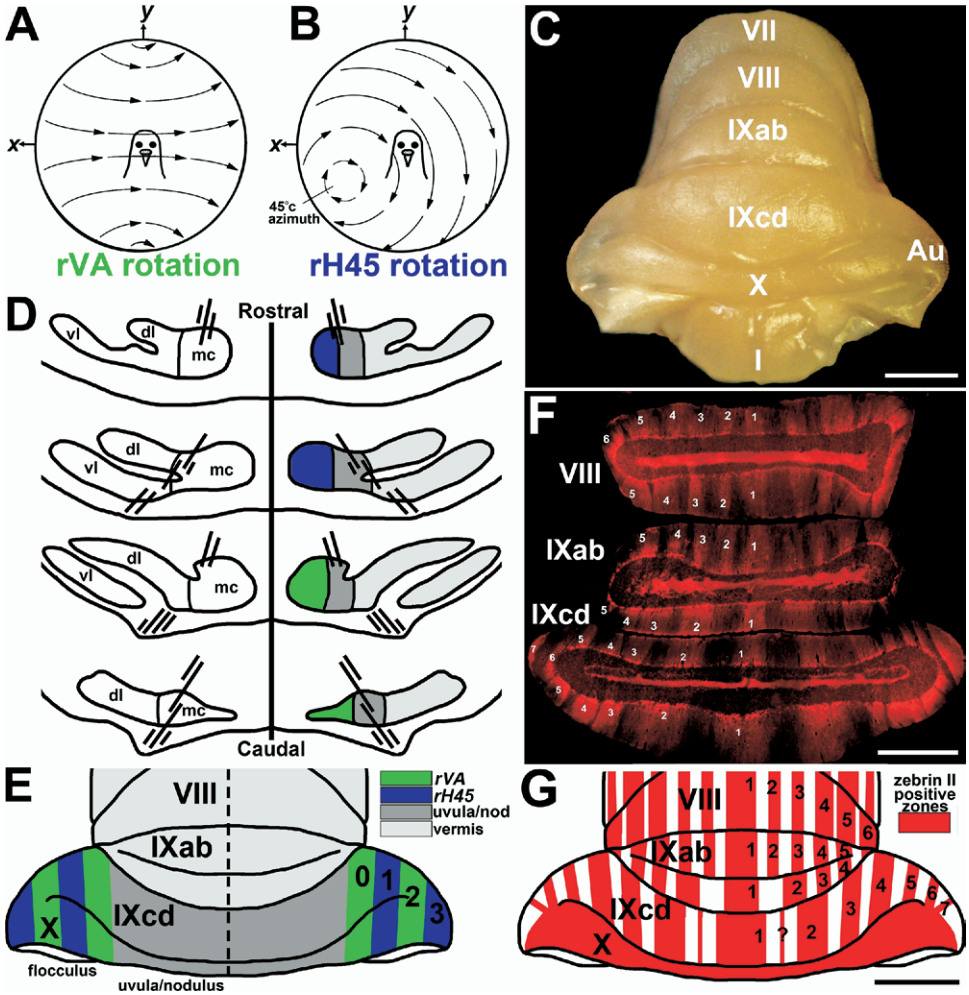


**Fig. 1.** Parasagittal organization of the pigeon flocculus. Purkinje cell CSA in the flocculus, the lateral portion of folium IXcd and X, responds best to rotational optic flow about either the vertical axis (rVA; green; A) or an horizontal axis oriented 45° to midline (rH45; blue; B; Graf et al., 1988; Wylie and Frost, 1993). (C) A photograph of the posterior pigeon cerebellum, indicating folia IXcd and X, as well as their lateral extension forming the auricle (Au). (E) The rVA and rH45 cells are organized into four zones in the flocculus: two rVA zones (0 and 2, green) interdigitated with two rH45 zones (1 and 3, blue; Winship and Wylie, 2003). The rVA and rH45 zones receive climbing fibre input from the caudal and rostral medial column (mc) of the inferior olive, respectively (D; Wylie et al., 1999). (F, G) The pattern of zebrin II expression in the pigeon posterior cerebellum (adapted from Pakan et al., 2007), shown with a coronal section through the posterior cerebellum (F) and a schematic of the pattern of zebrin II positive stripes (G). The zebrin stripes are numbered from 1 to 7 through folium IXcd (F,G). dl, vl=dorsal and ventral lamellae of the IO. All scale bars=1 mm.

systems (Voogd et al., 2003; Sugihara and Shinoda, 2004, 2007; Sugihara and Quy, 2007). This scheme cannot apply to the pigeon flocculus. Folium IXcd of the pigeon flocculus clearly consists of zebrin+ and − stripes (see Fig. 1F; Pakan et al., 2007), yet there is no somatosensory CF input. The pigeon inferior olive (IO) consists of three regions, the ventral and dorsal lamella, and the medial column (mcIO; Arends and Voogd, 1989). The flocculus receives CFs from medial subnuclei of the mcIO, and it appears that this input conveys only visual optic flow information. This is supported by anatomical studies demonstrating that the mcIO receives visual input from retino-recipient nuclei in the pretectum and accessory optic system (Clarke, 1977; Brecha et al., 1980; Gamlin and Cohen, 1988; Wylie et al., 1997; Wylie, 2001), and by electrophysiological studies detailing the responses of neurons in the mcIO and flocculus to optic flow stimuli (Wylie and Frost, 1993; Winship and Wylie, 2001). Also, somatosensory information, from both ascending (Wild, 1989) and descending systems (Wild and Williams, 2000) reaches the ventral lamella of the IO and not the mcIO. Second, there have been recent attempts to determine if the zebrin stripes correspond with functional zones of the cerebellum (Chockkan and Hawkes, 1994; Hallem et al., 1999; Voogd et al., 2003; Sugihara and Shinoda, 2004, 2007; Voogd and Ruigrok, 2004; Pijpers et al., 2006; Sugihara et al., 2007) and the pigeon flocculus offers a remarkable opportunity in this regard. The functional zonal organization of the flocculus has been extensively documented, and is essentially identical in mammals and birds (Voogd and Wylie, 2004). The complex spike activity (CSA) of floccular Purkinje cells, which represents CF activity (Eccles et al., 1966), responds best to patterns of optic flow that result from self-rotation about one of two axes: either the vertical axis (rVA, Fig. 1A), or a horizontal axis oriented at 45° azimuth (rH45; Fig. 1B; rabbits, Simpson et

al., 1981; Graf et al., 1988; pigeon, Wylie and Frost, 1993). In several species, it has been shown that the rVA and rH45 cells are organized into parasagittal zones (Voogd and Wylie, 2004). The pattern of the zones in the pigeon flocculus is shown in Fig. 1E. There are two rVA zones (0 and 2) interdigitated with two rH45 zones (1 and 3). In caudal sections, the four zones can be seen in the coronal plane, but much of zone three resides rostrolaterally in the auricle, a distinct lateral protrusion of the avian cerebellum (Larsell, 1967). The CF inputs to the rVA and rH45 zones originate in the caudal and rostral regions of the mcIO, respectively (see Fig. 1D; Wylie et al., 1999; Winship and Wylie, 2003). These subregions of the mcIO can be considered homologous with the mammalian dorsal cap of Kooy as the connectivity is strikingly similar. It has been shown in several mammalian species that the caudal and rostral regions of the dorsal cap project to the rVA and rH45 zones, respectively (for review see Voogd and Wylie, 2004).

In order to determine the relationship of the zebrin stripes to the CF projections in the pigeon flocculus, we made small injections of the anterograde tracer biotinylated dextran amine (BDA) into either the caudal (rVA) or rostral (rH45) regions of the mcIO in pigeons. We then examined the resulting olivocerebellar CF labeling in relation to the zebrin expression pattern in the flocculus.

## EXPERIMENTAL PROCEDURES

### 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. Procedures were optimized for minimizing the number of animals used and their suffering. Silver King and Homing pigeons (*Columba livia*), obtained from a local supplier, were anesthetized by an i.m. injection of a ketamine (65 mg/kg)/xylazine (8 mg/kg) cocktail. Supplemental doses were administered as necessary. Animals were placed in a stereotaxic device with pigeon ear bars and a beak bar adapter so that the orientation of the skull conformed to the atlas of Karten and Hodos (1967). To access the IO, bone and dura were removed from the dorsomedial surface of the cerebellum, lateral to the mid-sagittal sinus.

The intent was to make localized injections into the subnuclei of the IO that provide CF input to the flocculus. The pigeon IO is divided into ventral and dorsal lamella, which are conjoined medially by the mcIO (Arends and Voogd, 1989). Throughout the text we refer to the olivary input to the rVA and rH45 zones as originating in the caudal and rostral mcIO, respectively, as illustrated in Fig. 1D. The rostro-caudal extent of the mcIO ranges from about 1.5–1.8 mm in length. From our previous work (Wylie et al., 1999) we showed that retrograde labeling from injections into the rVA zones is concentrated in the caudal 700–800 $\mu$m, whereas labeling from rH45 zones is concentrated in the rostral 700–800 $\mu$m. The border between those areas projecting to the rVA and rH45 zones could be quite sharp (see Fig. 1 of Wylie et al., 1999), and double-labeling has shown that there is no overlap (Pakan et al., 2005). There is an essentially identical distinction between caudal and rostral dorsal cap in rabbits and rats, which project to the rVA and rH45 zones in these species (Voogd and Wylie, 2004).

To ensure that we were in the desired olivary subnuclei, single-unit extracellular recordings were used to confirm the location of the injection sites. To record the activity of optic flow units in the IO, glass micropipettes filled with 2 M NaCl, with tip diameters of 4–5 $\mu$m, were advanced through the cerebellum and into the brainstem using an hydraulic microdrive (Frederick Haer & Co., Millville, NJ, USA). Extracellular signals were amplified, filtered, and fed to a window discriminator. Inferior olivary units are easily identified based on their characteristically low firing rate (approximately one spike/s) and proximity to the base of the brain. Upon isolation of a unit in the IO, the optic flow preference of the unit was qualitatively determined. The direction-selectivity of the olivary neuron was determined by moving a large (90×90°) hand-held visual stimulus, consisting of black bars, wavy lines and dots on a white background, in the receptive field of the unit. With such stimuli, rVA and rH45 units are easily identified (Winship and Wylie, 2001, 2003). Once the desired area was isolated, the recording electrode was replaced with a micropipette (tip diameter 20–30 $\mu$m) containing fluorescent BDA; either mini-ruby (red; D-3312) or mini-emerald (green; D-7178; 10,000 molecular weight; Invitrogen, Carlsbad, CA, USA). The tracers (0.01–0.05 $\mu$l of 10% solution in 0.1 M phosphate buffer) were pressure injected using a Picospritzer II (General Valve Corporation, Fairfield, NJ, USA). After surgery the craniotomy was filled with bone wax and the wound was sutured. Birds were given an i.m. injection of buprenorphine (0.012 mg/kg) as an analgesic.

After a recovery period of 3–5 days, the animals were deeply anesthetized with sodium pentobarbital (100 mg/kg) and immediately transcardially perfused with phosphate buffered saline (PBS; 0.9% NaCl, 0.1 M phosphate buffer) followed by 4% paraformaldehyde in 0.1 M PBS (pH 7.4). The brain was extracted from the skull and immersed in paraformaldehyde for 7 days at 4 °C. The brain was then embedded in gelatin and cryoprotected in 30% sucrose in 0.1 M PBS overnight. Using a microtome, frozen serial sections in the coronal plane (40 $\mu$m thick) were collected throughout the rostro-caudal extent of the cerebellum.

### Immunohistochemistry

Tissue sections were rinsed thoroughly in 0.1 M PBS and blocked with 10% normal donkey serum (Jackson Immunoresearch Laboratories, West Grove, PA, USA) and 0.4% Triton X-100 in PBS for 1 h. Tissue was then incubated in PBS containing 0.1% Triton X-100 and the primary antibody, mouse monoclonal anti-zebrin II (kindly provided by Richard Hawkes, University of Calgary, AB, Canada; Brochu et al., 1990) for 60–75 h at room temperature. Anti–zebrin II is a monoclonal antibody grown in mouse, produced by immunization with a crude cerebellar homogenate from the weakly electric fish *Apteronotus* (Brochu et al., 1990) and recognizes in mouse a single polypeptide band with an apparent molecular weight 36 kDa, which cloning studies have shown to be the metabolic isoenzyme aldolase C (Ahn et al., 1994; Hawkes and Herrup, 1995). It was used directly from spent hybridoma culture medium diluted 1:200. Anti–zebrin II Western blot analysis of homogenate of pigeon cerebellum also detects a single immunoreactive polypeptide band, identical in size to the band detected in extracts from the adult mouse cerebellum (Pakan et al., 2007). Tissue was then rinsed in PBS and sections were incubated in either Cy3, Cy2 or AMCA conjugated donkey anti-mouse antibody (Jackson Immunoresearch Laboratories, West Grove, PA, USA: diluted 1:100 in PBS, 2.5% normal donkey serum, and 0.4% Triton X-100) for 2 h at room temperature. The tissue was then rinsed in PBS and mounted onto gelatinized slides for viewing.

### Microscopy and image analysis

Sections were viewed with a compound light microscope (Leica DMRE, Richmond Hill, ON, Canada) equipped with the appropriate fluorescence filters (rhodamine and FITC). Images were acquired using a Retiga EXi *FAST* Cooled mono 12-bit camera (Qimaging, Burnaby, BC, Canada) and analyzed with OPENLAB imaging software (Improvision, Lexington, MA, USA). Adobe Photoshop was used to compensate for brightness and contrast.

### Nomenclature of the pigeon flocculus

As in mammals, the cerebellum in birds is highly foliated, but is restricted to a vermis without hemispheres. Folia IXcd (uvula) and X (nodulus) comprise the vestibulocerebellum and merge rostrolaterally to form the auricle. Larsell (1967) considered the lateral extensions of folium IXcd and X as the paraflocculus and flocculus, respectively. In recent years we (Wylie and Frost, 1999; Winship and Wylie, 2003; Wylie et al., 2003a,b) divided the vestibulocerebellum into flocculus, nodulus and ventral uvula based on function and homology with mammals. Purkinje cells throughout the vestibulocerebellum respond to optokinetic stimulation (e.g. Wylie et al., 1993). In the medial half, the CSA of Purkinje cells responds best to patterns of optic flow resulting from self-translation (Wylie et al., 1993, 1998a). In the lateral half of IXcd and X, CSA responds best to optic flow resulting from self-rotation about the vertical axis (Fig. 1A, rVA neurons) or an horizontal axis oriented 45° to the midline (Fig. 1B, rH45 neurons; Wylie and Frost, 1993). These responses are essentially identical to those observed in the mammalian flocculus (Graf et al., 1988; Wylie and Frost, 1993). Thus, we consider these zones in the lateral half of both IXcd and X, including the auricle, as the flocculus. The numbering of the floccular zones, 0–3 as shown in Fig. 1A, follows that used for rats and rabbits (Voogd and Wylie, 2004). In mammals, a similar phenomenon has occurred: parts of the cerebellum traditionally included in the ventral paraflocculus are now considered part of the "floccular region," "lobe" or "complex" (see Voogd and Barmack, 2006). For example, the four optokinetic zones of the flocculus, as well as the C2 zone extend significantly into folium p, traditionally considered part of the paraflocculus (de Zeeuw et al., 1994; Tan et al., 1995).

## RESULTS

The results are based on observations in nine animals, where injections of red and/or green fluorescent BDA were made into the mcIO. In seven animals, a single injection of red BDA was made in the mcIO. In three of these cases, the injections were aimed at the caudal mcIO and rVA cells were recorded at the injection sites (cases VA#1–3). In the other four cases the injections were aimed at the rostral mcIO and rH45 cells were recorded at the injection sites (cases H45#1–4). The remaining two animals received two injections each: a red injection was made into the caudal mcIO (rVA) and a green injection was made into the rostral mcIO (rH45) (cases VA/H45#1 and 2). The extent of the injections is shown in Fig. 2. The injections were small, covering from 200 to 360 $\mu$m of the rostro-caudal extent of the mcIO (average=270 $\mu$m). All injections at sites where H45 cells were recorded were confined to the rostral mcIO. All but one of the injections at sites where rVA cells were recorded, were confined to the caudal half of the mcIO. In the exception (case VA#3), the injection was largely in the caudal mcIO, but spread to the rostral half. Critically, in the cases involving two injections (VA/H45 #1 and 2), the red and green injections were non-overlapping. Fig. 3A, B shows photomicrographs of representative injection sites (case VA/H45#2), illustrating a red BDA injection in the caudal (rVA) mcIO (A) and a green BDA injection in the rostral (H45) mcIO (B).

The resulting CF labeling from both the red and green BDA was entirely contralateral in all cases, robust, and easily distinguishable in the molecular layer (Fig. 3C, D, F and H). From our injections in the caudal (rVA) mcIO resulting CF labeling consisted of a caudomedially located zone (zone 0) and a more rostrolaterally located zone (zone 2) in both folia IXcd and X. From our injections in the rostral (H45) mcIO we observed CF labeling clearly organized into two zones (zones 1 and 3). In cases VA/H45#1 and 2, the CF labeling from the caudal (red) and rostral (green) mcIO were clearly interdigitated and non-overlapping (see Figs. 3D, 4–6).

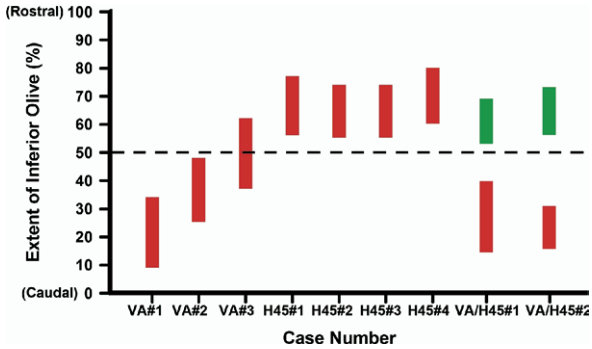


**Fig. 2.** Location and extent of the injection sites in the IO. A bar graph shows the location and size of each injection, expressed as a proportion of the rostro-caudal extent of the mcIO. The caudal and rostral borders of mcIO olive are designated 0% and 100%, respectively, and the dashed line represents the midpoint. It is known from previous research that the caudal half of the mcIO contains rVA cells and projects to zones 0 and 2 in the flocculus. Likewise, the rostral half of the mcIO contains rH45 cells and projects to floccular zones 1 and 3 (Wylie et al., 1999; Pakan et al., 2005). Single injections of red-BDA were made in cases VA#1–3 and H45#1–4. In cases VA/H45#1 and 2, red- and green-BDA were injected in the caudal and rostral mcIO, respectively.

In all animals we also observed the expected pattern of zebrin immunoreactivity in folium IXcd (Figs. 1F, G, 4–6) consisting of seven zebrin+/− stripes. The stripes themselves are numbered following the nomenclature used in Pakan et al. (2007) which is the same as that in mammals, whereby the most medial positive stripe is designated P1+ and the number increases as the stripes move laterally to P7+ (see Fig. 1F, G; Brochu et al., 1990; Eisenman and Hawkes, 1993; Ozol et al., 1999; Sillitoe and Hawkes, 2002; reviewed in Sillitoe et al., 2005). The seven stripe pairs were consistently seen in all specimens, however, the width of individual stripes can vary both between animals, as well as along the rostro-caudal dimension within animals. Therefore, in designating the band numbers it is important to complete an examination of all sections throughout the rostro-caudal extent of the vestibulocerebellum (see Fig. 6 for a complete reconstruction in this regard). Folium X is generally immunopositive, with the exception of the dorsal lamella at rostral levels, where IXcd and X merge to form the auricle (see below, Figs. 5, 6).

From the injections in the caudal and rostral mcIO (rVA, rH45), there was a clear correspondence with the zebrin stripes, which is illustrated in Figs. 4–6. The injections in the caudal mcIO resulted in CF labeling in zebrin zones P4 +/−, and P6 +/−, whereas the injections in the rostral mcIO resulted in CF labeling in zebrin zones P5 +/−, and P7 +/− (see Table 1). Figs. 4 and 5A–C show data from case VA/H45#1, where red- and green-BDA was injected in

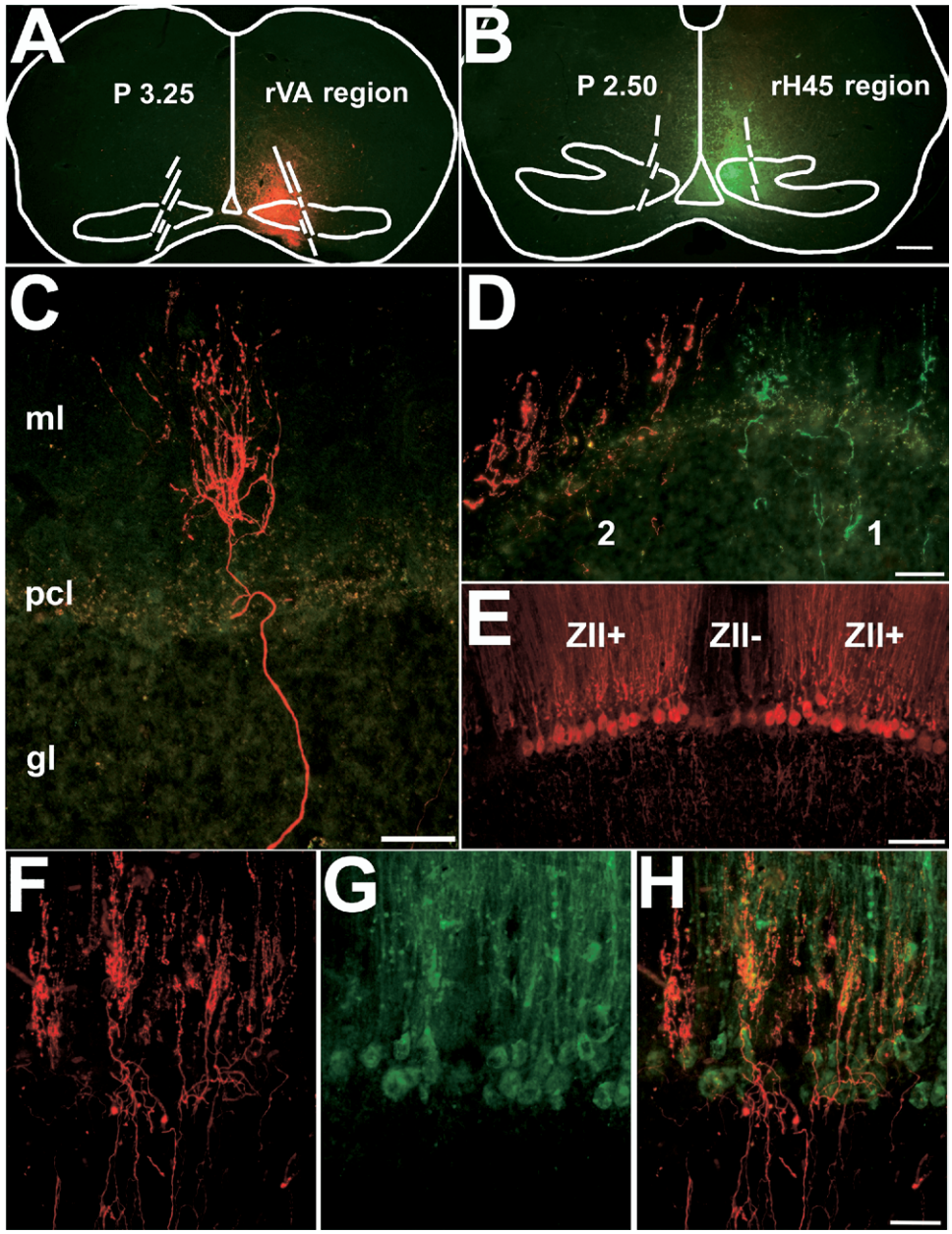


**Fig. 3.** Olivary injections sites, CF labeling, and zebrin immunohistochemistry in the flocculus. (A, B) Photomicrographs of red- and green-BDA injections in the caudal (rVA) and rostral (rH45) regions of the mcIO from case VA/H45#2. P3.25 and P2.50 refer to the rostrocaudal location of the coronal section in the pigeon atlas of Karten and Hodos (1967). (C–H) The molecular layer (ml) is represented dorsally, followed by the Purkinje cell layer (pcl), and the granule layer (gl) ventrally. (C, D) Photomicrographs of typical BDA labeled CFs from case H45#2 and VA/H45#1, respectively. (D) The red and green labeling are easily distinguishable, as illustrated with the labeling in zones 1 (rH45) and 2 (rVA). (E) An example of zebrin II immunohistochemistry from case H45#1, illustrating zebrin II positive (zebrin+) and negative (zebrin−) stripes. The zebrin II expression is apparent in the Purkinje cell bodies, axons and dendrites. (F–H) Red BDA labeled CFs (F) in a zebrin+ stripe (green, G) from case VA#1. The overlay is shown in H. Scale bars=250 $\mu$m (B, also applies to A); 100 $\mu$m (C–E); 50 $\mu$m (H, also applies to F and G).

the caudal and rostral mcIO, respectively. From the green-BDA injection labeling was seen in two zones (1 and 3; Figs. 4A, 5A) interdigitated with labeling in two zones (0 and 2; Figs. 4B, 5A) from the red-BDA injection. This labeling spanned the P4 +/− to P7 +/− zebrin stripes in IXcd (Figs. 4C, 5B). From the overlay of the CF and zebrin labeling (Figs. 4D and 5B), the correspondence was clear: CF zones 0 and 2 (rVA) correspond to zebrin stripes P4 +/−, and P6 +/−, whereas CF zones 1 and 3 (rH45) correspond to zebrin stripes P5 +/−, and P7 +/− (see also Fig. 6). As indicated in Table 1, there was strong support for this scheme from all cases. The only exceptions were that from the injections in the caudal mcIO in cases VA/H45#1 and 2, a very small amount of CF labeling was observed in the P7− stripe (see Fig. 6). In both cases, this labeling was found on the ventral–lateral border of the auricle, where X joins IXcd. We are

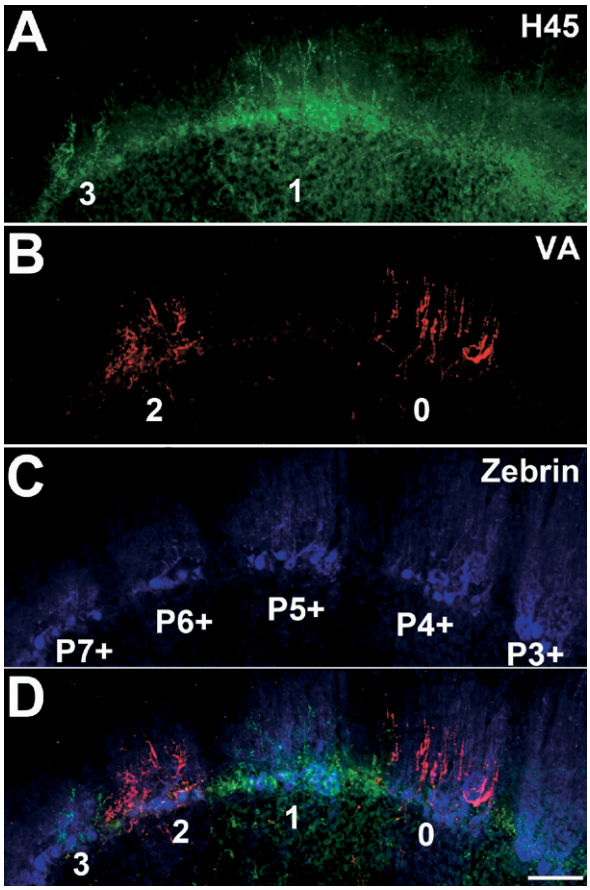


**Fig. 4.** Correspondence of CF zones and zebrin II stripes in folium IXcd of the flocculus. (A, B) CF labeling in IXcd after an injection of green BDA into the rostral mcIO (rH45 region) and a red BDA injection into the caudal mcIO (rVA region), respectively. The two rH45 zones (1 and 3), and two rVA zones (0 and 2) were clearly labeled. (C) Zebrin II labeling in blue, with the positive zebrin II stripes labeled P3+ to P7+. (D) Overlay of all three colors, which illustrates the concordance between zone 0 and zebrin stripe P4, zone 1 and zebrin stripe P5, zone 2 and zebrin stripe P6 and zone 3 and zebrin stripe P7. Scale bar=100 $\mu$m.

unsure if this area actually represents ventral lamella of X as opposed to IXcd. The injection from case VA#3, which was centered in the caudal rVA region but spread to the rostral half of the mcIO, resulted in CF labeling which was largely in P4 +/− and P6 +/−, but also included moderate labeling in P7− and a small amount in P7+.

We wish to emphasize, that a CF zone (i.e. zone 0, 1, 2, or 3) spanned a single zebrin stripe including the positive and negative portion. This is illustrated in Fig. 5D where a red BDA injection was made in the rH45 region of the mcIO (from case H45#1) and zebrin expression was visualized in green. This photomicrograph shows CFs terminating in zone 1 which is concentrated in P5−, but clearly spans into the adjacent P5+ as well. The presence of CFs in both positive and negative portions of a single zebrin stripe can also be seen in Figs. 4D, 5B, C, G, and 6.

From our injections in the caudal and rostral mcIO, the four CF zones (0–3) were clearly visible in folium X, and contiguous with the zones in IXcd (Figs. 5A, E, F and 6A). Folium X is generally uniformly immunopositive for zebrin, especially in caudal regions of the folium (Fig. 5B). In the rostral part of the dorsal lamella of X, where it joins with IXcd, there appears to be a region of transition, where the zebrin stripes in IXcd persist into the lateral margin of X. This is shown in the zebrin expression pattern in Fig. 5F (green); arrowheads indicate where the immunonegative regions of P3−, P4− and P5− of IXcd are extending into dorsal X (see also Fig. 6). With the exception of this transition region in the lateral portion, folium X is uniformly zebrin positive, without stripes. The CF zones however persist, with the same pattern observed in IXcd.

Fig. 6 shows a reconstruction of the pattern of CF labeling as related to the zebrin stripes from case VA/H45#2. The Purkinje cell layer from 27 serial sections has been unfolded and flattened onto the surface of the page, illustrating the CF labeling from injections in the rostral (rH45; green) and caudal (rVA; red) mcIO along with the zebrin+ (blue) and zebrin− (gray) stripes. The congruence of zones 0 and 2 with the P4 +/− and P6 +/− stripes and zones 1 and 3 with the P5 +/− and P7 +/− stripes is evident.

## DISCUSSION

For several decades it has been known that the CF inputs to the cerebellum are organized into parasagittal zones (Voogd and Bigaré, 1980). This is especially apparent in the flocculus, where there are several rVA zones interdigitated with rH45 zones. Although the absolute number of zones varies between species, a similar pattern is observed in both mammals and aves, indicating that the system is highly conserved. Current research suggests that there are five visual zones in rats, three rVA zones (zones 0, 2 and 4) interdigitated with two H45 zones (zones 1 and 3; Sugihara et al., 2004), four zones in pigeons (zones 0–3; Winship and Wylie, 2003) and four zones (zones 1–4) in rabbits (de Zeeuw et al., 1994; Tan et al., 1995a) and mice (for review see Voogd and Wylie, 2004; Schonewille et al., 2006).

Zebrin II and other molecular markers are also expressed as a series of parasagittal stripes in the cerebellum (Hawkes and Gravel, 1991; Herrup and Kuemerle, 1997). Again, this principle is highly conserved as a similar pattern of zebrin stripes is apparent in avian and mammalian species, although there are some differences (Pakan et al., 2007). Perhaps the most striking difference is, whereas zebrin positive and negative stripes are quite distinct in IXcd of the avian flocculus, the mammalian flocculus is uniformly zebrin positive (Ozol et al., 1999; Sanchez et al., 2002; Sillitoe and Hawkes, 2002; Marzban et al., 2003). This is quite surprising in view of physiological and anatomical studies that have underscored that in terms of function, response properties and connectivity, the flocculus is virtually identical in birds and mammals (Wylie, 2001; Voogd and Wylie, 2004). Nonetheless, the appearance of zebrin stripes in the avian flocculus, combined with an extensive literature that has examined the physiology and CF afferents to the flocculus in birds (Wylie and Frost, 1991, 1993, 1999; Lau et al., 1998; Wylie et al., 1998b, 1999; Crowder et al., 2000; Winship and Wylie, 2001, 2003; Wylie, 2001), affords a unique opportunity to exam-

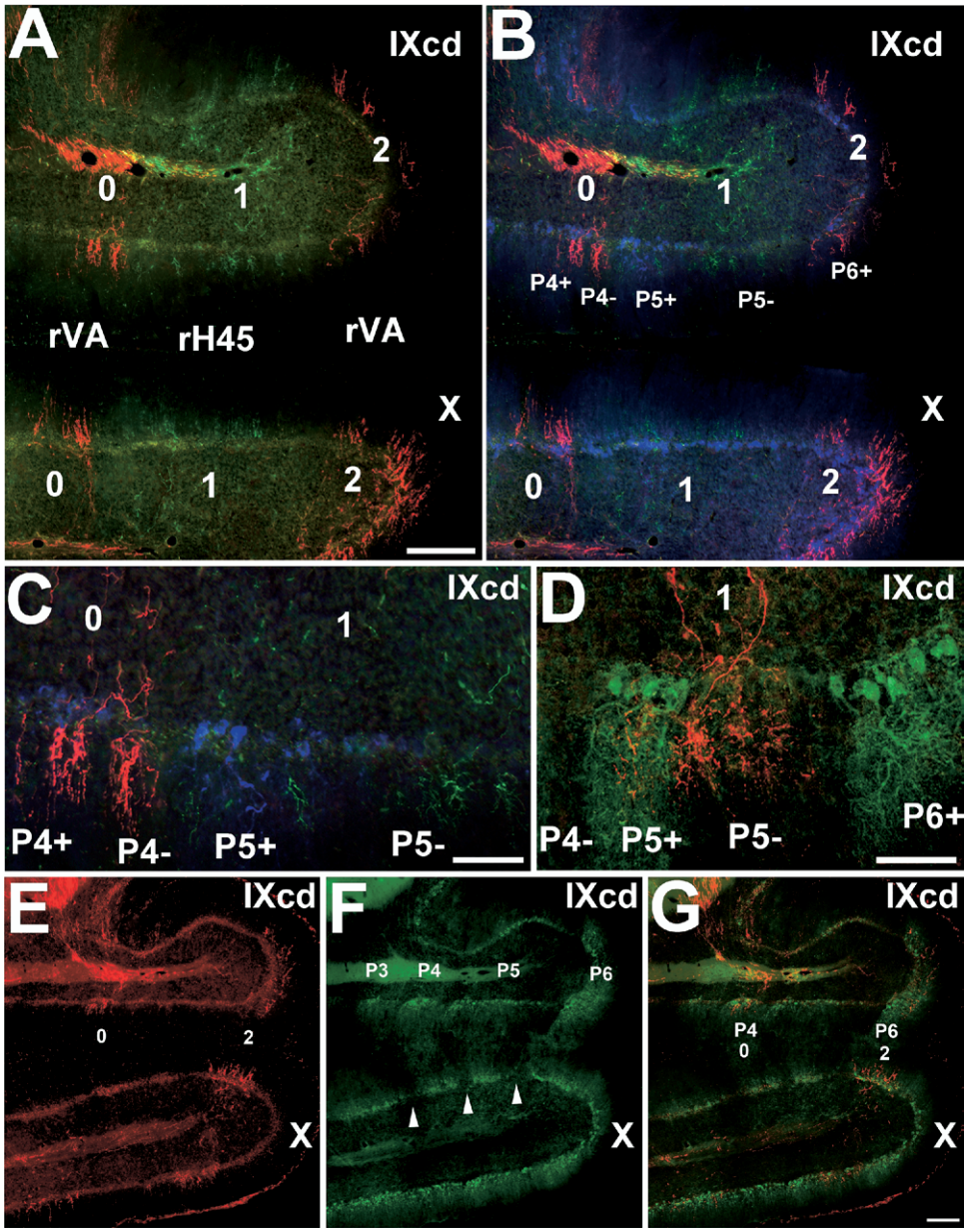


**Fig. 5.** CF zones and zebrin II stripes in the flocculus of folia IXcd and X. (A, B) Photomicrographs of a coronal section of IXcd and X through the flocculus from case VA/H45#1. (A) Red CFs can be seen from a BDA injection in the caudal mcIO (rVA region), in two parasagittal clusters: a medial zone 0 and a lateral zone 2. Green CFs can be seen in zone 1 from a BDA injection in the rostral mcIO (rH45 region). This injection also resulted in CF labeling in zone 3 (not shown). The CF zones clearly extend through folium X as well. (B) Zebrin II expression (blue) is superimposed with the CF zones to reveal the concordance in IXcd of the P4, P5 and P6 zebrin stripes with CF zones 0, 1 and 2 respectively. In folium X, the zebrin expression pattern is uniformly positive throughout the mediolateral extent of the flocculus. (C) A magnified version of zones 0 and 1 in the ventral lamella of folium IXcd. This panel illustrates the concordance of the boundaries of the zebrin and CF zones, as well as the presence of CFs in both the positive and negative regions of a particular zebrin stripe (e.g. zone 0 CFs (red) in both P4+ and P4− zebrin regions, and zone 1 CFs (green) in both P5+ and P5− zebrin regions, with no spread from either color into the adjacent zebrin stripe). (D) A photomicrograph of IXcd from case H45#1 where red-labeled CFs resulting from an injection in the rostral mcIO (rH45 region) extend into both the P5+ and P5− zebrin regions but not into the P4 or P6 stripes. (E–G) A photomicrograph of a coronal section of the flocculus through folia IXcd and X, at the region where these two folia are joining to eventually form the auricle (case VA#2). (E) CF labeling in zones 0 and 2 from an injection of red-BDA in the caudal region of the mcIO (rVA region). (F) Zebrin II expression in green, and G shows the overlay, illustrating the concordance of the CF zones 0 and 2 in IXcd with zebrin II stripes P4 and P6, respectively. In folium X, the CFs are contiguous with those in IXcd. Although the majority of folium X shows uniform positive zebrin II expression, three areas of weak/negative zebrin II expression can be seen in the dorsal lamella (indicated by arrowheads; see also reconstruction in Fig. 6A). These zebrin II negative regions in folium X can only be seen in rostral sections, immediately before folia IXcd and X join to become the auricle, and are prominent only in the dorsal lamella and the lateral regions. These three zebrin negative stripes in folium X correspond to P3−, P4− and P5− from medial to lateral. Scale bars=250 $\mu$m (A, also applies to B, E–G); 100 $\mu$m (C, D).

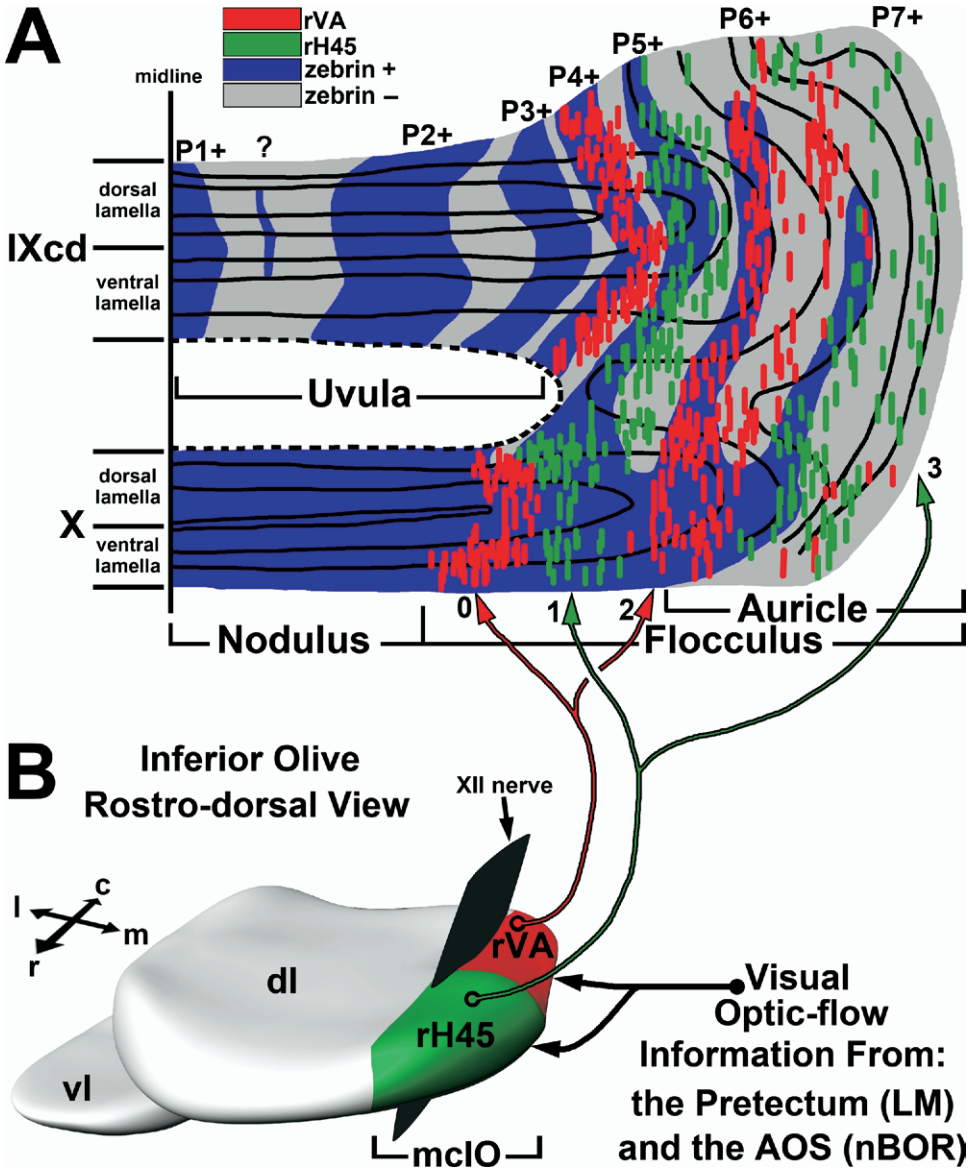


**Fig. 6.** A reconstruction of CF projections and zebrin II expression in the flocculus and the origins of the CF projections from the IO. (A) An "unfolded" reconstruction of labeled CFs as well as zebrin II expression in the right half of folia IXcd and X, including the lateral extension that forms the auricle, from case VA/H45#2. In this case, an injection of red BDA was made in the caudal mcIO (rVA region) and an injection of green BDA was made in the rostral mcIO (rH45 region). The reconstruction was from 27 coronal sections (40 $\mu$m thick and separated by 40 $\mu$m) through the extent of the flocculus. The black lines represent the outline of the Purkinje cell layer of selected sections (approximately every fourth) and indicate the shape of the folia at caudal (inside lines) and progressively more rostral (outside lines) extents. The dashed line indicates that IXcd has been cut away from X in the unfolding process, but in fact they are contiguous at this point. Each CF was marked with a line of the corresponding color and the positive zebrin II expression marked with blue (the zebrin II negative stripes are gray). The CF projection zones are labeled 0–3 and the zebrin II stripes are labeled P1+ to P7+. (B) A 3-D schematic of the left IO viewed from the rostrodorsal aspect; the regions of the IO which are responsive to rotational optic flow (the mcIO) are indicated in red (caudal rVA region) and green (rostral rH45 region). The CF projections from these two regions of the IO to folium IXcd and X are indicated by arrows. The afferent information that the mcIO receives is also indicated and includes visual optic-flow information from two retinal-recipient nuclei, lentiformis mesencephali (LM) and the nucleus of the basal optic root (nBOR; Wylie, 2001). dl, vl=dorsal and ventral lamellae of the IO, respectively. Scale bar=1 mm in A.

ine the relationship between zebrin expression, CF zones, and physiology in the flocculus.

### The relationship between CF zones and zebrin stripes

In this study, by injecting anterograde tracers into the mcIO and observing CF labeling as well as zebrin II expression, we have shown that there is a strict concordance between CF zones and zebrin stripes in folium IXcd of the flocculus in pigeons. Injection of anterograde tracer in the caudal mcIO resulted in CFs in zebrin bands P4 +/− and P6 +/−, whereas rostral mcIO injections resulted in CFs in zebrin bands P5 +/− and P7 +/−. Thus, zebrin stripes P4 +/− and P6 +/− correspond to the rVA zones 0 and 2, whereas

**Table 1.** Summary of zebrin II and climbing fiber correlation from each case

| Caudal mcIO injections | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Case | P4+ | P4− | P5+ | P5− | P6+ | P6− | P7+ | P7− |
| VA#1 | +++ | + | | | + | ++ | | |
| VA/H45#1 | +++ | + | | | +++ | + | | +[a] |
| VA/H45#2 | +++ | + | | | +++ | ++ | | +[a] |
| VA#2 | ++ | + | | | +++ | ++ | + | |
| VA#3 | ++ | + | | | +++ | + | + | + |
| Rostral mcIO injections | | | | | | | | |
| Case | P4+ | P4− | P5+ | P5− | P6+ | P6− | P7+ | P7− |
| VA/H45#1 | | | ++ | + | | | ++ | +++ |
| VA/H45#2 | | | ++ | ++ | | | + | ++ |
| H45#1 | | | +++ | ++ | | | + | +++ |
| H45#2 | | | ++ | + | | | ++ | +++ |
| H45#3 | | | ++ | + | | | + | +++ |
| H45#4 | | | ++ | + | | | | ++ |

Single, double and triple "+" signs indicate the relative amount of climbing fiber labeling in each of the zebrin stripes. Note that this is not an indication of the density of the labeling in the zones. The P4− stripe is thinner than the P4+ stripe (see Fig. 1F, G). That is, there is generally less labeling in the P4− stripe compared to P4+ stripe simply because it is thinner. In contrast, the P7− stripe includes the majority of the auricle, and is much wider than the P7+ stripe (see Fig. 1G).

[a] Very few climbing fibers were found ventrally in the auricle.

P5 +/− and P7 +/− correspond to the rH45 zones 1 and 3, respectively. Fig. 6 summarizes these findings with a schematic of the pigeon IO and a reconstruction of the CF projections to the flocculus.

Note that the concordance between the zebrin stripes and the CF zones only applies to folium IXcd and the rostral-most parts of the dorsal lamella of X. The bulk of folium X does not contain zebrin stripes, but is uniformly zebrin positive (Figs. 5 and 6; Pakan et al., 2007), as is the case in mammals (Hawkes et al., 1993; Hawkes and Herrup, 1995; Ozol et al., 1999; Sanchez et al., 2002; Marzban et al., 2003). However, the CF zones clearly extend through IXcd and X (Figs. 5 and 6). Perhaps the CF zones in X are related to the expression of some other molecular marker. For example, in the mouse cerebellum, heat-shock protein-25 (Hsp 25) is expressed as parasagittal stripes of high and low immunoreactivity, but only in regions where zebrin immunoreactivity is uniformly positive, including the nodulus and flocculus (Armstrong et al., 2000). However, the equivalent to Hsp 25 in rats (Hsp 27) was not detected in the adult rat cerebellum by using immunocytochemistry (Wilkinson and Pollard, 1993; Plumier et al., 1997). Whether Hsp 25 is expressed as stripes anywhere in the pigeon cerebellum has yet to be investigated.

The finding that a CF zone in the pigeon flocculus corresponds to a pair of positive/negative zebrin stripes, (i.e. zone 0 corresponds to zebrin stripes P4+ and P4−, etc.) is unique and perhaps contrary to previous investigations of the correspondence of CF afferents and zebrin stripes in mammals. Previous studies emphasized that an olivary subnucleus projects to either a positive or negative zebrin stripe, but not both (Gravel et al., 1987; Sugihara and Shinoda, 2004; Apps and Garwicz, 2005; Pijpers et al., 2006; Sugihara and Quy, 2007). Voogd and colleagues (2003) investigated the distribution of CFs to the copula pyramidis and the paramedian lobule in relation to the pattern of zebrin II expression in the rat. They found that, with few exceptions, olivocerebellar fibers originating from the rostral dorsal accessory olive innervate the zebrin negative stripes of the C1 and C3 zones, the rostral medial accessory olive and principal olive innervate respectively the zebrin positive stripes of the C2 and D zones, and that the A2 zone corresponds to the region of the P4b+ and P5a+ bands in the medial paramedian lobule and lobulus simplex. Voogd and Ruigrok (2004) investigated the zonal organization of the corticonuclear and the olivocerebellar CF projections to the vermis of the cerebellum in relation to zebrin II stripes in rats. They found that small injections in various subnuclei of the IO produced CF bands which were generally confined to either a positive or a negative zebrin II stripe, but not both. In a comprehensive study of the entire cerebellum, Sugihara and Shinoda (2004) identified olivocerebellar projections to zebrin II compartments by labeling CFs with BDA injected into various small areas within the IO in rats. They found that the principal olive (as well as neighboring areas) and several medial subnuclei innervated zebrin positive stripes, whereas the centrocaudal portion of the medial accessory olive innervated zebrin negative stripes in the vermis. The dorsal accessory olive and neighboring regions innervated zebrin negative and lightly positive stripes in the hemisphere and the rostral and caudal pars intermedia. To reiterate, the correspondence between a given olivary region and zebrin stripes of a particular sign (positive or negative) was not found in the present study of the pigeon flocculus. Rather, an olivary region was associated with a particular positive/negative zebrin pair. Whether the type of zebrin-CF correspondence that we observed is peculiar to pigeons, or even just the flocculus of pigeons, remains to be seen. It is possible that within the cerebellum the nature of the concordance between zebrin stripes and CF zones is different for different olivocerebellar systems. This idea is supported by the fact that the zebrin-CF concordance that we observed in the flocculus applies to folium IXcd but not X, despite the fact that the CF zones are identical in IXcd and X.

### Linking functional cerebellar zones with zebrin stripes

In recent years there has been an attempt to reveal an underlying unit of function associated with zebrin stripes. Sugihara et al. (2004) concluded that the zebrin stripes are related to function insofar as IO subnuclei project to either zebrin+ or zebrin− bands, and the subnuclei of the IO receive input from particular sensory systems. Furthermore, Sugihara et al. (2004) concluded that the zebrin− stripes receive input from CFs conveying somatosensory information whereas zebrin+ stripes receive input from CFs conveying information from visual, auditory and other sensory systems (see also Voogd et al., 2003; Voogd and Ruigrok, 2004; Sugihara and Quy, 2007; Sugihara and Shinoda, 2007). A clear example of this is the ventral lateral outgrowth, which processes visual-optokinetic information, projection to zebrin+ stripes in lobule X and ventral IXcd. In the dorsal margin of IXcd, thin zebrin− stripes are innervated by somatosensensory olivary subnuclei (Voogd et al., 1996). Clearly, this scheme does not apply to the pigeon flocculus as the CF inputs convey only visual information (see introduction). Furthermore, there are several exceptions to this scheme in mammalian studies. For example, vestibular information appears to reach zebrin+ stripes in lobules VIII–X via the group beta and the dorsomedial cell column, but also a zebrin− stripe in the lateral A subzone of the anterior vermis via the subnucleus B of the caudal medial accessory olive (Gerrits et al., 1985; Voogd and Ruigrok, 2004; Voogd and Barmack, 2006). Also, the majority of the CF afferents to zebrin+ stripes arise from nuclei at the midline of the mesodiencephalic junction from structures regarded as motor, rather than sensory (including the red nucleus and accessory oculomotor nuclei; Swenson and Castro, 1983; Onodera, 1984; Holstege and Tan, 1988; de Zeeuw et al., 1989), as well as the subnucleus a, which receives input from the spinal cord (Matsushita et al., 1991).

In the present study, because there is a great deal of research detailing the optic flow information conveyed by CFs to the flocculus, we suggest a functional link between the four optokinetic zones and the zebrin stripes. The caudal and rostral mcIO provide CF afferents to floccular zones 0 and 2, and 1 and 3, respectively. In the present study, we showed that each zone corresponds to a particular zebrin positive/negative stripe pair (P4 +/− to P7 +/−; see Fig. 6). Previous studies from our laboratory have shown that the caudal mcIO and zones 0 and 2 respond best to rVA optic flow, whereas the rostral mcIO and zones 1 and 3 respond best to rH45 optic flow (Winship and Wylie, 2001; Pakan et al., 2005). Thus the P4 +/− and P6 +/− stripes are rVA zones, and the P5 +/− and P7 +/− are rH45 zones. Whether the zebrin+ and zebrin− regions of each of the rVA and rH45 zones have different roles in oculomotor function is unknown. Because zebrin+ and zebrin− cells may differ with respect to plasticity and excitability (Welsh et al., 2002; Wadiche and Jahr, 2005) this may be the case. We must acknowledge that it is possible that the zebrin− and zebrin+ stripes receive differential sensory information. Although our research over the past two decades has shown that the CSA of all Purkinje cells in the flocculus responds to optokinetic stimulation (e.g. Wylie et al., 1991, 1993, 1998; e.g. Wylie et al., 1991, 1993, 1998; Wylie and Frost, 1993), as do the olivary cells that provide CF input to the flocculus (e.g. Winship and Wylie, 2001), whether some cells also respond to vestibular stimulation has not been tested. This is the case in the rabbit uvula and nodulus (Shojaku et al., 1991) as well as the flocculus (Simpson et al., 2002). Thus, it is possible that either the zebrin+ or zebrin− stripes (or both) also receive vestibular CF information. If this were the case, it would likely arise from a secondary vestibular input, as a primary vestibular input to the mcIO has not been reported in pigeons (Schwarz and Schwarz, 1986; Dickman, 1996; Dickman and Fang, 1996).

The direct congruence between several functional CF zones with clearly defined response properties, and a series of zebrin stripes that we show in the pigeon flocculus in the present paper, has not been described in other cerebellar systems in any species (cf. Chockkan and Hawkes, 1994; Hallem et al., 1999). However, a few recent papers have suggested a direct relationship between zebrin stripes and other aspects of cerebellar physiology. Sugihara et al. (2007) showed that synchronous activity was higher among Purkinje cells within a zebrin stripe. Gao et al. (2006) demonstrated that the inhibition evoked by parallel fiber stimulation results in parasagittal bands of decreases in activity along a folium that correspond to the location of zebrin stripes. Thus, with the present study and those of Sugihara et al. (2007) and Gao et al. (2006), the correspondence of zebrin immunoreactivity with cerebellar function is beginning to emerge.

Finally, although the Purkinje cells in the flocculus are uniformly zebrin II immunopositive in mammals (Leclerc et al., 1992; Eisenman and Hawkes, 1993), stripes in either the Purkinje cell axons, the afferent input, or the Purkinje cells themselves are revealed with several other molecular markers, such as acetylcholine esterase (de Zeeuw et al., 1994; Tan et al., 1995b), corticotropin-releasing factor (Sawada et al., 2008) and Hsp25 (Armstrong et al., 2000), respectively. Schonewille et al. (2006) investigated the correspondence of the floccular zones, defined by recording responses to optic flow stimuli, with Hsp25. The relationship they found between this molecular marker (Hsp25) and the physiological zones in the mouse flocculus was completely different to what we found in the present study using zebrin as a molecular marker. An Hsp25 positive stripe encompassed zones 1 (rH45) and 2 (rVA), and an Hsp25 negative stripe encompassed zones 3 (rH45) and 4 (rVA). Therefore, it is evident that the relationship between various molecular markers and the physiological zones in the cerebellum is complex. Even though the function of zebrin II is unclear (cf. Welsh et al., 2002), the unique parasagittal organization of both zebrin II and other molecular markers would suggest that examining the relationship between these markers and other well-known aspects of cerebellar organization, such as anatomy and electrophysiology, will lead to insights on the

fundamental functional organization of the cerebellar cortex.

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## REFERENCES

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

Apps R, Garwicz M (2005) Anatomical and physiological foundations of cerebellar information processing. Nat Rev Neurosci 6:297–311.

Arends J, Voogd J (1989) Topographic aspects of the olivocerebellar system in the pigeon. Exp Brain Res 17:52–57.

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

Armstrong CL, Krueger-Naug AM, Currie RW, Hawkes R (2000) Constitutive expression of the 25-kDa heat shock protein Hsp25 reveals novel parasagittal bands of Purkinje cells in the adult mouse cerebellar cortex. J Comp Neurol 416:383–397.

Brecha N, Karten HJ, Hunt SP (1980) Projections of the nucleus of the basal optic root in the pigeon: an autoradiographic and horseradish peroxidase study. J Comp Neurol 189:615–670.

Brochu G, Maler L, Hawkes R (1990) Zebrin II: a polypeptide antigen expressed selectively by Purkinje cells reveals compartments in rat and fish cerebellum. J Comp Neurol 291:538–552.

Chockkan V, Hawkes R (1994) Functional and antigenic maps in the rat cerebellum: zebrin compartmentation and vibrissal receptive fields in lobule IXa. J Comp Neurol 345:33–45.

Clarke PG (1977) Some visual and other connections to the cerebellum of the pigeon. J Comp Neurol 174:535–552.

Crowder NA, Winship IR, Wylie DR (2000) Topographic organization of inferior olive cells projecting to translational zones in the vestibulocerebellum of pigeons. J Comp Neurol 419:87–95.

de Zeeuw CI, Holstege JC, Ruigrok TJ, Voogd J (1989) Ultrastructural study of the GABAergic, cerebellar, and mesodiencephalic innervation of the cat medial accessory olive: anterograde tracing combined with immunocytochemistry. J Comp Neurol 284:12–35.

de Zeeuw CI, Wylie DR, DiGiorgi PL, Simpson JI (1994) Projections of individual Purkinje cells of identified zones in the flocculus to the vestibular and cerebellar nuclei in the rabbit. J Comp Neurol 349:428–447.

Dickman JD (1996) Vestibular afferent projections to the brain stem in pigeons. Ann N Y Acad Sci 781:611–613.

Dickman JD, Fang Q (1996) Differential central projections of vestibular afferents in pigeons. J Comp Neurol 367:110–131.

Eccles JC, Llinas R, Sasaki K, Voorhoeve PE (1966) Interaction experiments on the responses evoked in Purkinje cells by climbing fibres. J Physiol 182:297–315.

Eisenman LM, Hawkes R (1993) Antigenic compartmentation in the mouse cerebellar cortex: zebrin and HNK-1 reveal a complex, overlapping molecular topography. J Comp Neurol 335:586–605.

Gamlin PD, Cohen DH (1988) Projections of the retinorecipient pretectal nuclei in the pigeon (Columba livia). J Comp Neurol 269: 18–46.

Gao W, Chen G, Reinert KC, Ebner TJ (2006) Cerebellar cortical molecular layer inhibition is organized in parasagittal zones. J Neurosci 26:8377–8387.

Gerrits NM, Voogd J, Magras IN (1985) Vestibular afferents of the inferior olive and the vestibulo-olivo-cerebellar climbing fiber pathway to the flocculus in the cat. Brain Res 332:325–336.

Graf W, Simpson JI, Leonard CS (1988) Spatial organization of visual messages of the rabbit's cerebellar flocculus. II. Complex and simple spike responses of Purkinje cells. J Neurophysiol 60: 2091–2121.

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.

Hallem JS, Thompson JH, Gundappa-Sulur G, Hawkes R, Bjaalie JG, Bower JM (1999) Spatial correspondence between tactile projection patterns and the distribution of the antigenic Purkinje cell markers anti-zebrin I and anti-zebrin II in the cerebellar folium crus IIA of the rat. Neuroscience 93:1083–1094.

Hawkes R (1992) Antigenic markers of cerebellar modules in the adult mouse. Biochem Soc Trans 20:391–395.

Hawkes R, Blyth S, Chockkan V, Tano D, Ji Z, Mascher C (1993) Structural and molecular compartmentation in the cerebellum. Can J Neurol Sci 20 (Suppl 3):S29–S35.

Hawkes R, Gravel C (1991) The modular cerebellum. Prog Neurobiol 36:309–327.

Hawkes R, Herrup K (1995) Aldolase C/zebrin II and the regionalization of the cerebellum. J Mol Neurosci 6:147–158.

Herrup K, Kuemerle B (1997) The compartmentalization of the cerebellum. Annu Rev Neurosci 20:61–90.

Holstege G, Tan J (1988) Projections from the red nucleus and surrounding areas to the brainstem and spinal cord in the cat. An HRP and autoradiographical tracing study. Behav Brain Res 28:33–57.

Karten H, Hodos W (1967) A stereotaxic atlas of the brain of the pigeon (Columba livia). Baltimore: Johns Hopkins Press.

Larouche M, Hawkes R (2006) From clusters to stripes: the developmental origins of adult cerebellar compartmentation. Cerebellum 5:77–88.

Larsell O (1967) The cerebellum: from myxinoids through birds. Minneapolis: University of Minnesota Press.

Lau KL, Glover RG, Linkenhoker B, Wylie DR (1998) Topographical organization of inferior olive cells projecting to translation and rotation zones in the vestibulocerebellum of pigeons. Neuroscience 85:605–614.

Leclerc N, Schwarting GA, Herrup K, Hawkes R, Yamamoto M (1992) Compartmentation in mammalian cerebellum: Zebrin II and P-path antibodies define three classes of sagittally organized bands of Purkinje cells. Proc Natl Acad Sci U S A 89:5006–5010.

Llinas R, Sasaki K (1989) The functional organization of the olivocerebellar system as examined by multiple Purkinje cell recordings. Eur J Neurosci 1:587–602.

Marzban H, Zahedi S, Sanchez M, Hawkes R (2003) Antigenic compartmentation of the cerebellar cortex in the Syrian hamster Mesocricetus auratus. Brain Res 974:176–183.

Matsushita M, Ragnarson B, Grant G (1991) Topographic relationship between sagittal Purkinje cell bands revealed by a monoclonal antibody to zebrin I and spinocerebellar projections arising from the central cervical nucleus in the rat. Exp Brain Res 84:133–141.

Onodera S (1984) Olivary projections from the mesodiencephalic structures in the cat studied by means of axonal transport of horseradish peroxidase and tritiated amino acids. J Comp Neurol 227:37–49.

Ozol K, Hayden JM, Oberdick J, Hawkes R (1999) Transverse zones in the vermis of the mouse cerebellum. J Comp Neurol 412: 95–111.

Pakan JM, Iwaniuk AN, Wylie DR, Hawkes R, Marzban H (2007) Purkinje cell compartmentation as revealed by zebrin II expression in the cerebellar cortex of pigeons (Columba livia). J Comp Neurol 501:619–630.

Pakan JM, Todd KG, Nguyen AP, Winship IR, Hurd PL, Jantzie LL, Wylie DR (2005) Inferior olivary neurons innervate multiple zones of the flocculus in pigeons (Columba livia). J Comp Neurol 486: 159–168.

Pijpers A, Apps R, Pardoe J, Voogd J, Ruigrok TJ (2006) Precise spatial relationships between mossy fibers and climbing fibers in rat cerebellar cortical zones. J Neurosci 26:12067–12080.

Plumier JC, Hopkins DA, Robertson HA, Currie RW (1997) Constitutive expression of the 27-kDa heat shock protein (Hsp27) in sensory and motor neurons of the rat nervous system. J Comp Neurol 384:409–428.

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.

Sanchez M, Sillitoe RV, Attwell PJ, Ivarsson M, Rahman S, Yeo CH, Hawkes R (2002) Compartmentation of the rabbit cerebellar cortex. J Comp Neurol 444:159–173.

Sawada K, Fukui Y, Hawkes R (2008) Spatial distribution of corticotropin-releasing factor immunopositive climbing fibers in the mouse cerebellum: Analysis by whole mount immunohistochemistry. Brain Res 1222C:106–117.

Schonewille M, Luo C, Ruigrok TJ, Voogd J, Schmolesky MT, Rutteman M, Hoebeek FE, De Jeu MT, de Zeeuw CI (2006) Zonal organization of the mouse flocculus: physiology, input, and output. J Comp Neurol 497:670–682.

Schwarz DW, Schwarz IE (1986) Projection of afferents from individual vestibular sense organs to the vestibular nuclei in the pigeon. Acta Otolaryngol 102:463–473.

Shojaku H, Barmack NH, Mizukoshi K (1991) Influence of vestibular and visual climbing fiber signals on Purkinje cell discharge in the cerebellar nodulus of the rabbit. Acta Otolaryngol Suppl 481: 242–246.

Sillitoe RV, Hawkes R (2002) Whole-mount immunohistochemistry: a high-throughput screen for patterning defects in the mouse cerebellum. J Histochem Cytochem 50:235–244.

Sillitoe RV, Hulliger M, Dyck R, Hawkes R (2003) Antigenic compartmentation of the cat cerebellar cortex. Brain Res 977:1–15.

Sillitoe RV, Marzban H, Larouche M, Zahedi S, Affanni J, Hawkes R (2005) Conservation of the architecture of the anterior lobe vermis of the cerebellum across mammalian species. Prog Brain Res 148:283–297.

Simpson J, Graf W, Leonard C (1981) The coordinate system of visual climbing fibres to the flocculus. In: Progress in oculomotor research (Fuchs AF, Becker KA, eds), pp 475–485. Oxford: Elsevier.

Simpson JI, Belton T, Suh M, Winkelman B (2002) Complex spike activity in the flocculus signals more than the eye can see. Ann N Y Acad Sci 978:232–236.

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.

Sugihara I, Marshall SP, Lang EJ (2007) Relationship of complex spike synchrony bands and climbing fiber projection determined by reference to aldolase C compartments in crus IIa of the rat cerebellar cortex. J Comp Neurol 501:13–29.

Sugihara I, Quy PN (2007) Identification of aldolase C compartments in the mouse cerebellar cortex by olivocerebellar labeling. J Comp Neurol 500:1076–1092.

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, Shinoda Y (2007) Molecular, topographic, and functional organization of the cerebellar nuclei: analysis by three-dimensional mapping of the olivonuclear projection and aldolase C labeling. J Neurosci 27:9696–9710.

Swenson RS, Castro AJ (1983) The afferent connections of the inferior olivary complex in rats. An anterograde study using autoradiographic and axonal degeneration techniques. Neuroscience 8: 259–275.

Tan J, Gerrits NM, Nanhoe R, Simpson JI, Voogd J (1995a) Zonal organization of the climbing fiber projection to the flocculus and nodulus of the rabbit: a combined axonal tracing and acetylcholinesterase histochemical study. J Comp Neurol 356: 23–50.

Tan J, Simpson JI, Voogd J (1995b) Anatomical compartments in the white matter of the rabbit flocculus. J Comp Neurol 356:1–22.

Voogd J, Barmack NH (2006) Oculomotor cerebellum. Prog Brain Res 151:231–268.

Voogd J, Bigaré F (1980) Topographical distribution of olivary and cortico nuclear fibers in the cerebellum: a review. New York: Raven.

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

Voogd J, Glickstein M (1998) The anatomy of the cerebellum. Trends Neurosci 21:370–375.

Voogd J, Pardoe J, Ruigrok TJ, 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.

Voogd J, Ruigrok TJ (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, Wylie DR (2004) Functional and anatomical organization of floccular zones: a preserved feature in vertebrates. J Comp Neurol 470:107–112.

Wadiche JI, Jahr CE (2005) Patterned expression of Purkinje cell glutamate transporters controls synaptic plasticity. Nat Neurosci 8:1329–1334.

Welsh JP, Yuen G, Placantonakis DG, Vu TQ, Haiss F, O'Hearn E, Molliver ME, Aicher SA (2002) Why do Purkinje cells die so easily after global brain ischemia? Aldolase C, EAAT4, and the cerebellar contribution to posthypoxic myoclonus. Adv Neurol 89:331–359.

Wild JM (1989) Avian somatosensory system: II. Ascending projections of the dorsal column and external cuneate nuclei in the pigeon. J Comp Neurol 287:1–18.

Wild JM, Williams MN (2000) Rostral wulst in passerine birds. I. Origin, course, and terminations of an avian pyramidal tract. J Comp Neurol 416:429–450.

Wilkinson JM, Pollard I (1993) Immunohistochemical localisation of the 25 kDa heat shock protein in unstressed rats: possible functional implications. Anat Rec 237:453–457.

Winship IR, Wylie DR (2001) Responses of neurons in the medial column of the inferior olive in pigeons to translational and rotational optic flowfields. Exp Brain Res 141:63–78.

Winship IR, Wylie DR (2003) Zonal organization of the vestibulocerebellum in pigeons (Columba livia): I. Climbing fiber input to the flocculus. J Comp Neurol 456:127–139.

Wu HS, Sugihara I, Shinoda Y (1999) Projection patterns of single mossy fibers originating from the lateral reticular nucleus in the rat cerebellar cortex and nuclei. J Comp Neurol 411:97–118.

Wylie DR (2001) Projections from the nucleus of the basal optic root and nucleus lentiformis mesencephali to the inferior olive in pigeons (Columba livia). J Comp Neurol 429:502–513.

Wylie DR, Bischof WF, Frost BJ (1998a) Common reference frame for neural coding of translational and rotational optic flow. Nature 392:278–282.

Wylie DR, Glover RG, Lau KL (1998b) Projections from the accessory optic system and pretectum to the dorsolateral thalamus in the pigeon (Columbia livia): a study using both anteretrograde and retrograde tracers. J Comp Neurol 391:456–469.

Wylie DR, Brown MR, Barkley RR, Winship IR, Crowder NA, Todd KG (2003a) Zonal organization of the vestibulocerebellum in pigeons (Columba livia): II. Projections of the rotation zones of the flocculus. J Comp Neurol 456:140–153.

Wylie DR, Brown MR, Winship IR, Crowder NA, Todd KG (2003b) Zonal organization of the vestibulocerebellum in pigeons (Columba

livia): III. Projections of the translation zones of the ventral uvula and nodulus. J Comp Neurol 465:179–194.
Wylie DR, Frost BJ (1991) Purkinje cells in the vestibulocerebellum of the pigeon respond best to either translational or rotational whole-field visual motion. Exp Brain Res 86:229–232.
Wylie DR, Frost BJ (1993) Responses of pigeon vestibulocerebellar neurons to optokinetic stimulation. II. The 3-dimensional reference frame of rotation neurons in the flocculus. J Neurophysiol 70:2647–2659.
Wylie DR, Frost BJ (1999) Complex spike activity of Purkinje cells in the ventral uvula and nodulus of pigeons in response to translational optic flow. J Neurophysiol 81:256–266.
Wylie DR, Kripalani T, Frost BJ (1993) Responses of pigeon vestibulocerebellar neurons to optokinetic stimulation. I. Functional organization of neurons discriminating between translational and rotational visual flow. J Neurophysiol 70:2632–2646.
Wylie DR, Linkenhoker B, Lau KL (1997) Projections of the nucleus of the basal optic root in pigeons (Columba livia) revealed with biotinylated dextran amine. J Comp Neurol 384:517–536.
Wylie DR, Winship IR, Glover RG (1999) Projections from the medial column of the inferior olive to different classes of rotation-sensitive Purkinje cells in the flocculus of pigeons. Neurosci Lett 268: 97–100.

## APPENDIX

### Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neuroscience.2008.08.062.

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