Neuroscience 185 (2011) 73–84

HETEROGENEITY OF PARVALBUMIN EXPRESSION IN THE AVIAN CEREBELLAR CORTEX AND COMPARISONS WITH ZEBRIN II

D. R. WYLIE,[a,b]* C. GUTIERREZ-IBANEZ,[a] D. J. GRAHAM,[a] M. B. KREUZER,[a] J. M. P. PAKAN[a] AND A. N. IWANIUK[c]

[a]*University Centre for Neuroscience, University of Alberta, Edmonton, AB, T6G 2E9, Canada*

[b]*Department of Psychology, University of Alberta, Edmonton, AB, T6G 2E9, Canada*

[c]*Department of Neuroscience, Canadian Centre for Behavioural Neuroscience, University of Lethbridge, Lethbridge, AB, T1K 3M4, Canada*

Abstract—The cerebellar cortex has a fundamental parasagittal organization that is reflected in the physiological responses of Purkinje cells, afferent and efferent connections, and the expression of several molecular markers. The most thoroughly studied of these molecular markers is zebrin II (ZII; a.k.a. aldolase C). ZII is differentially expressed in Purkinje cells, resulting in a pattern of sagittal stripes of high expression interdigitated with stripes of little or no expression. In this study, we examined the expression of the calcium binding protein parvalbumin (PV) in the cerebellum of several avian species (pigeons, hummingbirds, zebra finches) and compared it to the expression of ZII. We found that PV immunoreactivity was distributed across the cerebellar cortex such that there were sagittal stripes of PV immunopostive (PV+) Purkinje cells alternating with PV immunonegative (PV−) Purkinje cells. Although most Purkinje cells in the anterior lobe were PV+, there were several thin (i.e. only a few Purkinje cells wide) PV− stripes spanning the folia. In the posterior lobe, PV+ and PV− stripes were also apparent, but the PV− stripes were much wider than in the anterior lobe. In sections processed for both ZII and PV, the expression was generally complementary: PV+ stripes were ZII−, and vice-versa. This complementary expression was most apparent in folia II–IV and VIII–IXcd. The complementary expression was not, however, absolute; some Purkinje cells co-expressed PV and ZII whereas others lacked both. These novel findings relate to the complex neurochemical organization of the cerebellum, and are likely important to issues regarding cerebellar plasticity.

Key words: calcium binding proteins, aldolase C, zebrin, parvalbumin, cerebellum, Purkinje cells.

*Correspondence to: D. R. Wylie, Department of Psychology, University Centre for Neuroscience, University of Alberta, Edmonton, AB, T6G 2E9, Canada. Tel: +1-780-492-5274; fax: +1-780-492-1768.
E-mail address: dwylie@ualberta.ca (D. R. Wylie).

Abbreviations: CF, climbing fibre; HA, horizontal axis floccular Purkinje cells; LM, nucleus lentiformis mesencephali; mcIO, medial column of the inferior olive; nBOR, nucleus of the basal optic root; PBS, phosphate buffered saline; PCβ4, phospholipase cβ4; PFA, para-formaldehyde; PV(±), parvalbumin immunopositive/immunonegative); VA, vertical axis floccular Purkinje cells; VbC, vestibulocerebelluml; ZII(±), zebrin II immunopositive/immunonegative.

Evidence accumulated over the past 20 years has shown that Purkinje cells, despite their homogeneous appearance in morphology and connectivity, are heterogeneous with respect to the expression of a number of molecular markers (for reviews see, Hawkes and Gravel, 1991; Herrup and Kuemerle, 1997; Armstrong and Hawkes, 2000; Apps and Hawkes, 2009). The most extensively studied in this regard, is zebrin II (ZII; a.k.a. aldolase C) (Brochu et al., 1990; Ahn et al., 1994; Hawkes and Herrup, 1995). ZII is distributed such that there are sagittally oriented stripes of ZII immunopositive (ZII+) Purkinje cells alternating with stripes of ZII immunonegative (ZII−) Purkinje cells (Brochu et al., 1990; Eisenman and Hawkes, 1993; Akintunde and Eisenman, 1994; Chockkan and Hawkes, 1994; Sillitoe and Hawkes, 2002; Voogd and Ruigrok, 2004; Sugihara et al., 2004; Sugihara and Shinoda, 2007; Sugihara and Quy, 2007). The parasagittal distribution of ZII reinforces the fundamental organization of the cerebellar cortex based on sagittal "zones" (Voogd and Bigaré, 1980), which are known from the distribution of climbing fiber and mossy fiber afferents, Purkinje cell projection patterns, and Purkinje cell response properties (Voogd et al., 1969; Andersson and Oscarsson, 1978; Llinas and Sasaki, 1989; Voogd and Glickstein, 1998; Wu et al., 1999; Ruigrok, 2003; Apps and Garwicz, 2005). The pattern of ZII+ and ZII− stripes in the cerebellar cortex is remarkably similar across all mammalian species studied (e.g. Sillitoe et al., 2005; Fujita et al., 2010). Furthermore, ZII± stripes occur in avian species with a pattern highly similar to that observed in mammals (Pakan et al., 2007; Iwaniuk et al., 2009b; Marzban et al., 2010). Thus, the spatial pattern of ZII expression is highly conserved across species and likely critical for cerebellar function.

ZII is not, however, the only molecular marker that reveals this fundamental parasagittal organization of the cerebellar cortex. For example, motilin (Chan-Palay et al., 1981), acetylcholinesterase (Jaarsma et al., 1995), corticotropin-releasing factor (van den Dungen et al., 1988; Cummings, 1989; Cummings et al., 1989; King et al., 1997; Sawada et al., 2008), heat shock protein 25 (Armstrong et al., 2000), human natural killer cell antigen (Eisenman and Hawkes, 1993; Marzban et al., 2004), and phospholipases (Sarna et al., 2006; Marzban et al., 2007; Iwaniuk et al., 2009b) all reveal parasagittal stripes in the cerebellar cortex. Because ZII has received so much research effort, it has become the benchmark to which other molecular markers are compared (Dehnes et al., 1998; Armstrong et al., 2000; Sarna et al., 2006; Iwaniuk et al., 2009b; Marzban et al., 2010; Sawada et al., 2010; Sillitoe et al., 2010). For example, the expression of phospholipase Cβ3 is con-

0306-4522/11 $ - see front matter
doi:10.1016/j.neuroscience.2011.04.014

cordant with that of ZII, whereas phospholipase Cβ4 shows a complementary expression pattern to that of ZII (Sarna et al., 2006; Iwaniuk et al., 2009b; Marzban et al., 2010). The expression of corticotrophin releasing factor is more complex, and neither concordant nor complementary to ZII (Sawada et al., 2008).

Several studies suggest that ZII+ and ZII− Purkinje cells differ in their role in synaptic plasticity (Nagao et al., 1997; Wadiche and Jahr, 2005; Paukert et al., 2010). Similarly, the calcium binding protein parvalbumin (PV) has been implicated in cerebellar plasticity (Schwaller et al., 2002; Bastianelli, 2003; Schwaller, 2007, 2009). PV is expressed in stellate, basket, and Purkinje cells (see Bastianelli, 2003 for review). There are reports in both mammals and birds, that PV is heterogeneously expressed in Purkinje cells such that there are parasagittal stripes of PV+ and PV− Purkinje cells (Braun et al., 1986; Fortin et al., 1998). In these studies, neither the extent to which this expression occurs in uniform stripes nor the distribution of stripes across folia and lobules are discussed. This prevents any extrapolation of comparisons to other molecular markers, mossy and/or climbing fibre projections, and one cannot determine whether this pattern is conserved across vertebrate species. In this report, we therefore provide a detailed description of PV expression across the avian cerebellar cortex and then directly compared the distribution of ZII and PV expression to determine the extent to which both ZII and PV overlap. To determine the generality of our observations, we used a variety of distantly related birds. These included pigeons (*Columba livia*) and hummingbirds (*Calypte ana, Selasphorus rufus*) because we have previously described the pattern of ZII expression in these species (Pakan et al., 2007; Iwaniuk et al., 2009b). We also used the zebra finch (*Taeniopygia guttata*). Braun et al. (1986) showed heterogeneity of PV expression in Purkinje cells in the developing cerebellum of zebra finch. Because Purkinje cell heterogeneity that occurs during development may be lost in adulthood (e.g. Sotelo and Wassef, 1991) and Rogers (1989) did not mention PV− Purkinje cells in chicken (*Gallus domesticus*) cerebellum, we felt wise to include the zebra finch as a species for comparison.

EXPERIMENTAL PROCEDURES

The procedures describing the use of animals for exprerimental purposes conformed to the guidelines established by the Canadian Council for Animal Care and were approved by the BioSciences Animal and Policy Care Committee at the University of Alberta. Brains were obtained from pigeons (*Columba livia*), hummingbirds (*Calypte anna* and *Selasphorus rufus*), and zebra finches (*Taeniopygia guttata*). Adult pigeons obtained from a local supplier, were deeply anesthetized with sodium pentobarbital (100 mg/kg, i.p.) and transcardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (PFA, pH=7.4). The brains were then removed and postfixed by immersion in the same fixative for several days. The brains from hummingbirds were kindly provided to us by Drs. Kenneth Welch Jr. and Raul Suarez (University of California, Santa Barbara). All of the hummingbird brains were immersion fixed in 4% PFA (same as above) for several weeks prior to processing. The brains from zebra finches were sent to ANI from Ákos Pogány (Eötvös Loránd University, Hungary) and Gergely Zachar (Semmelweiss University, Hungary) as part of an unrelated project. As with the hummingbirds, the finch brains were immersion fixed in PFA for several days. Prior to sectioning, all brains were cryoprotected in sucrose (30% in phosphate buffered saline (PBS), pH=7.4), embedded in gelatin, and sectioned on a freezing stage microtome in the coronal plane at a thickness of 40 μm. Serial sections through the cerebellum were collected into several series (in 0.1 M PBS).

Immunohistochemistry for parvalbumin and zebrin II

Free floating sections were washed several times 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 1–2 h at room temperature. Sections were then incubated for 24–48 h at room temperature in PBS containing 0.1% Triton X-100 and a primary antibody to PV (1:2000; either mouse anti-PV (P3088, Sigma, St. Louis, MI, USA) or rabbit anti-PV (ab11427, abcam, Cambridge, MA, USA)). Sections were then rinsed in PBS, and incubated in solutions containing the appropriate secondary antibody; either DyLight 488-conjugated donkey anti-mouse, or Cy2 conjugated donkey anti-rabbit (Jackson Immunoresearch Laboratories: diluted 1:200 in PBS, 2.5% normal donkey serum, and 0.4% Triton X-100) for 2–24 h at room temperature. The tissue was finally rinsed in PBS and mounted onto gelatinized slides for viewing.

For ZII immunoreactivity, after washing and blocking as described above, the sections were incubated in the primary antibody (1:400) for 48–72 h at room temperature. We used a monoclonal mouse anti-zebrin antibody kindly provided by Dr. R. Hawkes, which is produced by immunization with a crude cerebellar homogenate from the weakly electric fish Apteronotus (Brochu et al., 1990). This antibody recognizes a single polypeptide band in mouse with an apparent molecular weight of 36 kDa that cloning studies have identified as the isoenzyme aldolase C (Ahn et al., 1994; Hawkes and Herrup, 1995). The sections were then rinsed several times in PBS and incubated in DyLite 594 or Cy3 conjugated donkey anti-mouse secondary antibody (Jackson Immunoresearch Laboratories: 1:200 in PBS, 2.5% donkey serum, and 0.4% Triton X-100) for 2–3 h at room temperature. Following several rinses in PBS, the sections were then mounted onto gelatinized slides.

Double-labeling was performed by first incubating the tissue in the rabbit anti-PV primary antibody followed by the Cy2 donkey anti-rabbit secondary, and then incubated in mouse anti-ZII followed by the Cy3 donkey anti-mouse secondary. The procedures for blocking, dilution factors, incubation periods, and the primary and secondary antibodies used are as described above.

Serial sections were viewed with a compound light microscope (Leica DMRE; Leica Microsystems, Wetzlar, Germany) equipped with the appropriate fluorescence filters (i.e. fluorescein isothiocyanate (FITC) and rhodamine to visualize the PV and ZII, resepectively). 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). The images were compiled with PTGui v 6.0.3 (Rotterdam, Netherlands) and manipulated using Adobe Photoshop (San Jose, CA, USA) to compensate for brightness and contrast.

Nomenclature

The lateral view of the pigeon cerebellum is shown in Fig. 1A and midsaggital sections are shown for a zebra finch and hummingbird in Fig. 1C, E, respectively. In comparison to mammals, the avian cerebellum is best described as a vermis without hemispheres (Larsell, 1967), although small rudimentary hemispheres may exist (Pakan et al., 2007). As in the mammalian vermis, the avian cerebellum is divided into 10 lobules that are referred to as “folia”,

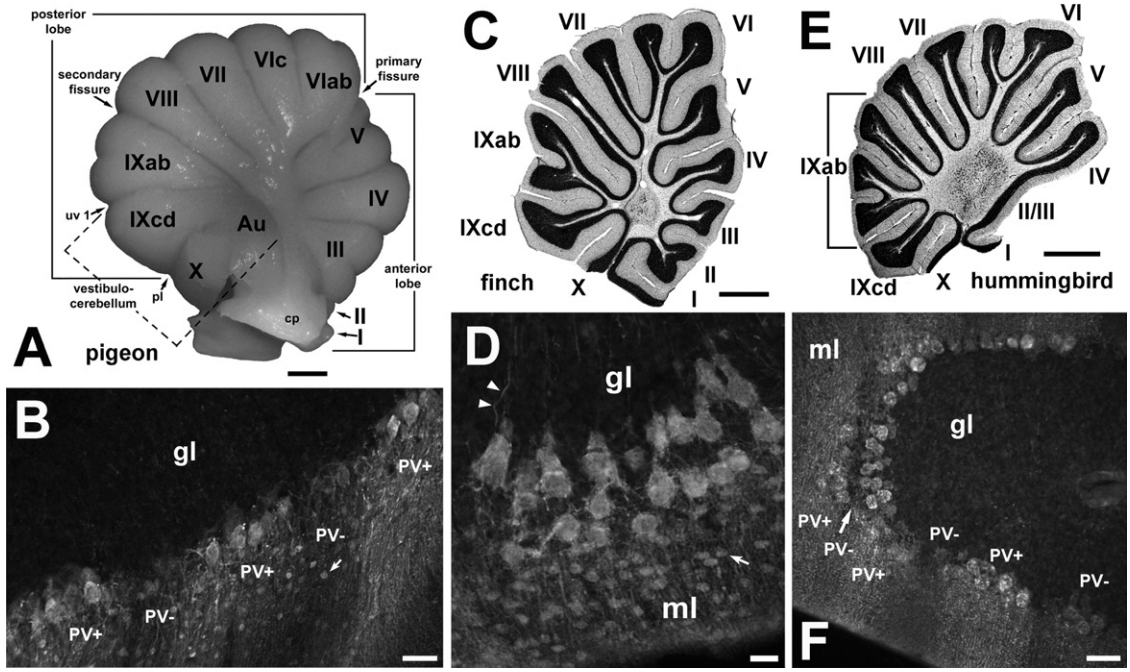

Fig. 1. (A, C, E) Illustrate the gross morphology of the cerebellum for pigeon (*C. livia*), finch (*T. guttata*), and hummingbird (*G. hirsute*), respectively. (A) Shows a lateral view of a wholemount of the pigeon cerebellum indicating major regions and the folia, whereas (C, E) show Nissl-stained midsagittal sections for the finch and hummingbird. The folia are labeled with Roman numerals (I–X) in an anterior to posterior direction. Subfolia are then named alpha-numerically (e.g. VIc). (B, D, F) Show parvalbumin (PV) expression in the avian cerebellar cortex for pigeon (B), finch (D), and hummingbird (F). Note that PV labeling is much heavier across the molecular layer (ml) compared to the granular layer (gl). In the ml many interneurons are labeled, which appear to be stellate and basket cells (see small arrow in B and D). Purkinje cell somae are labeled, as are the dendrites extending into the ml, and in some cases labeled axons are clearly labeled (triangles in D). In (B, F), PV+ and PV− refer to the alternating PV immunopositive and immunonegative "stripes". Scale bars, 1 mm in (A, C, E); 50 μm in (B); 20 μm in (D); 100 μm in (F). Au=auricle; uv 1=uvular sulcus; pl=posterolateral fissure.

and labeled using Roman numerals I–X (anterior to posterior). Also like the mammalian vermis, the avian cerebellum is divided into an anterior lobe (folia I-V), a posterior lobe (VI–IX) and the nodulus (X). Possible homologies between parts of the avian and mammalian cerebellum are discussed in Pakan et al. (2007) and Larsell (1967).

With respect to gross morphology, the pigeon and finch cerebella can be considered representative for the majority of avian species. Variation in cerebellar morphology among birds is discussed extensively by Iwaniuk et al. (2006a,b, 2007, 2009a). The hummingbird cerebellum is very unique in that there is a reduction in the anterior lobe such that folia II and III are greatly reduced or absent (see Fig. 2E and Iwaniuk et al., 2006b). Nonetheless, the pattern and number of ZII stripes in the anterior lobe of the hummingbird (Iwaniuk et al., 2009b) is essentially the same as that seen in pigeon (Pakan et al., 2007) and chick (Marzban et al., 2010).

RESULTS

Distribution of parvalbumin immunoreactivity

As expected, we observed PV immunoreactivity throughout the cerebellar cortex in all cases. Generally, there was more PV immunoreactivity in the Purkinje cell layer and molecular layer compared to the granular layer (see Fig. 1). In the molecular layer, interneurons were labeled, and based on their position, appearance and previous studies (Celio, 1990; Fortin et al., 1998; Bastianelli, 2003), we assumed they were basket and stellate cells (Fig. 1B, D). Often, the labeling of these interneurons was masked by the heavy labeling of Purkinje cell somae and dendrites (see Fig. 1B, F). In Fig. 1D we have indicated what appear to be labeled PV immunoreactive Purkinje cells axons in the granular layer, although it is possible that some of these are labeled climbing fibres (Fortin et al., 1998).

The PV immunoreactivity in Purkinje cells was clearly heterogeneous: some Purkinje cells were strongly immunoreactive whereas others showed little or no immunoreactivity (see Fig. 1B, F). For convenience we refer to these as PV+ and PV− Purkinje cells. The PV immunoreactivity was distributed across the cortex such that there were "stripes" or "bands" of PV+ cells alternating with PV− Purkinje cells (Fig. 1B, F). The stripes had clear borders and were quite distinct in the hummingbirds and finch, but less so in the pigeon (see Fig. 2H).

Anterior lobe

Fig. 2 shows the pattern of PV expression in the anterior lobe. Most Purkinje cells in the anterior lobe were PV+, with several PV− stripes spanning the folia that were only a few Purkinje cells wide (see Fig. 2C, E, F, I). Along the midline, there was invariably a PV− stripe and several more on either side of the midline out to the lateral margins of the folia (Fig. 2A, D, G, H). The exception was folium I (lingula) where PV± stripes were not observed. Rather, it

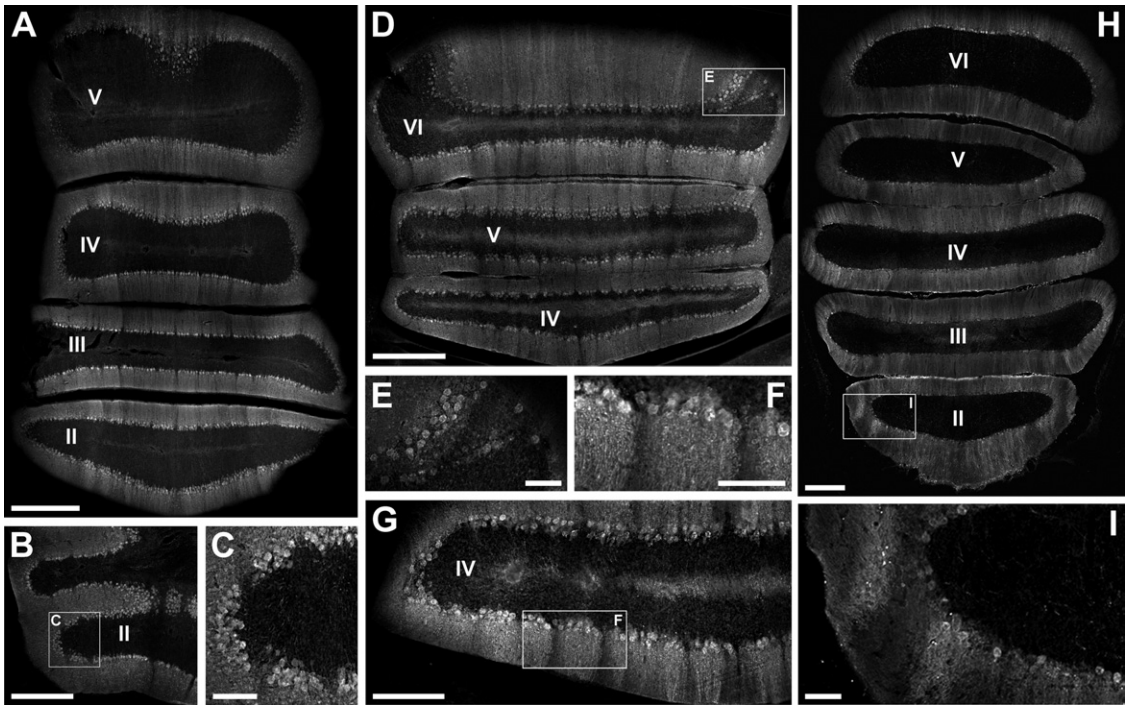

Fig. 2. Parvalbumin immunoreactivity in the anterior cerebellum. Coronal sections are shown through the anterior lobe of the cerebellum for zebra finch (A–C), hummingbird (D–G), and pigeon (H, I) to illustrate the "stripes" of PV+ and PV− Purkinje cells. Scale bars; 500 μm in (A, D, H); 400 μm in (B); 250 μm in (G); 100 μm in (C, E, F, I).

appeared that all Purkinje cells showed a weak to moderate level of PV expression (see Fig. 4E).

Posterior lobe and folium X

The posterior lobe is shown in Fig. 3. As with the anterior lobe, most Purkinje cells were PV+, alternating with thinner PV− stripes (e.g. Fig. 3D) and there was invariably a PV− band at the midline (Fig. 3A–C, E). In the posterior lobe, there were a few PV− bands that were wide in comparison with the anterior lobe. For example, in Fig. 3C, the lateral part of IXcd is largely PV−. Note also the large PV− stripes in IXab and IXcd in Fig. 3A. In the caudal margin of IXcd, shown in Fig. 3B, E (finch and hummingbird), wider PV+ and PV− stripes were obvious, especially in the ventral lamella. The stripes persisted through folia IXab and VIII (Fig. 3D), although the PV− stripes were thinner than those in IXcd. Stripes were still visible in the lateral margins of caudal VII, but most Purkinje cells in VII, and the caudal margin of VI, expressed PV weakly, if at all (see Fig. 4F). In folium X, PV stripes were only apparent in the rostolateral margin, where two and sometimes three PV− stripes were seen (see Fig. 5F). More medially and caudally in X, generally, PV− stripes were not apparent as most Purkinje cells were PV+ (see Fig. 5E, G).

Differences among species

The pattern of the distribution of PV immunoreactivity applies to all species we studied as we could discern no differences among the four species. This is apparent in the PV immunoreactivity of both the anterior lobe (e.g. Fig. 2A, D, H) and the posterior lobe (Fig. 3A, C). The similarity of the distribution of PV immunoreactivity across the species is best illustrated by comparing the photomicrographs of caudal folium IXcd in Fig. 3B (finch), with Figs. 3E and 6A, B (hummingbird) and Fig. 5A, B (pigeon).

A comparison of the distribution of parvalbumin and zebrin II immunoreactivity

The alternating sagittal PV+ and PV− bands were similar to the ZII stripes that were described previously in the avian cerebellum (Pakan et al., 2007, 2010; Iwaniuk et al., 2009b; Pakan and Wylie, 2008; Marzban et al., 2010), however, there is more polarity with the ZII stripes and the boundaries are easier to demark (see Figs. 4–6).

Generally speaking, there was a tendency for the labeling to be complementary: PV+ stripes tended to be ZII−, and vice versa (Figs. 4–6). This was most evident in folia II–IV, VIII, and IX. However, such an organization was not absolute. Fig. 4A shows the lateral margin of folium III in the pigeon where there are several thin ZII+ stripes. In the overlay, these appear to be concordant with the PV−areas. This pattern of ZII+/PV− stripes is reinforced in Fig. 4B with the magnification of the two ZII+ stripes in the ventral lamella (see also Fig. 4C). In folium VI, the overall level of ZII expression in high, but numerous thin ZII± stripe pairs can be seen, as can the complimentary expression of PV and ZII. This is shown in Fig. 4D, with a section from the lateral margin of VI. The PV+ cells are lateral to the ZII+ cells, although there are many double-labeled Purkinje cells. ZII expression is also strong in VII, but PV expression is very weak (Fig. 4F). In

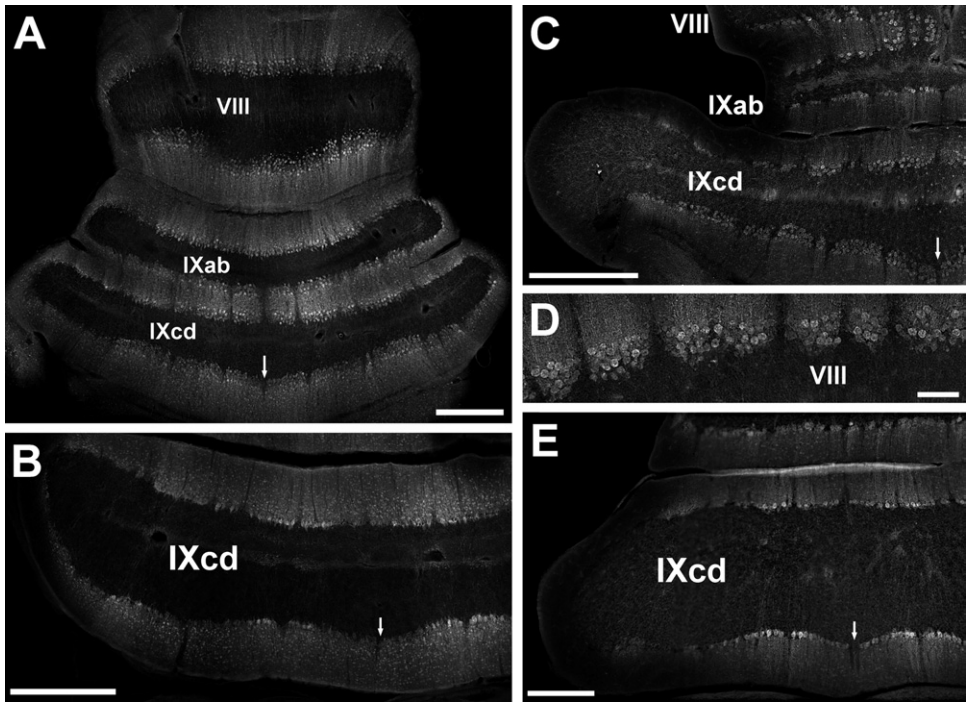

Fig. 3. Parvalbumin immunoreactivity in the posterior cerebellum. Coronal sections are shown through the posterior lobe of the cerebellum for zebra finch (A, B) and hummingbird (C–E) to illustrate the "stripes" of PV+ and PV− Purkinje cells. The vertical arrows indicate the midline. Scale bars; 500 μm in (A–C); 250 μm in (E); 100 μm in (D).

folium I, ZII and PV stripes are absent. Most Purkinje cells would be described as ZII+, but PV immunoreactivity is comparatively weak (Fig. 4E).

Fig. 5 shows coronal sections through folia IXcd and X (the vestibulocerebellum) in a pigeon reacted for both ZII (red) and PV (green). Our previous studies in pigeons (and hummingbirds) have shown that there are seven ZII± stripe pairs spanning IXcd, which are labeled Z1± to Z7± from medial to lateral (Pakan et al., 2007, 2010, 2011; Pakan and Wylie, 2008; Iwaniuk et al., 2009b). Fig. 5A, B shows a section from caudal IXcd, where the Z1+, Z1−, and Z2+ stripes are apparent. The complimentary expression of ZII and PV is evident. Fig. 5C shows a section from a more rostral level, to illustrate the expression in the regions lateral to Z2+. Z2− was PV+ except at the most lateral edge which was PV− and ZII− (see rightmost arrow in Fig. 5C overlay). Z3+ was usually entirely PV+ although the Purkinje cells in the lateral half appeared to show higher PV immunoreactivity. Z3−, which is a thin stripe, was invariably PV− (middle arrow in Fig. 5C). The Z4± and Z5± stripes had a similar organization to one another. The ZII−stripes were PV+, but the PV+ Purkinje cells extended into the medial regions of the ZII+ stripes. This is shown for the Z5± stripes in Fig. 5D. The boundary between the Z5+ and Z5−stripes can be difficult to demarcate at times, but the PV+ cells, although they showed the strongest immunoreactivity in the Z5− stripe, clearly extended into the lateral half of the Z5+ stripe and several cells were ZII+ and PV+. At the lateral edge of the Z4− stripe, there was always a stripe that was PV− (see leftmost arrow in Fig. 5C, and arrows in 5D). Such a stripe was sometimes observed at the lateral edge of Z5−.

Although folium X is contiguous with IXcd, X is largely ZII+, and ZII− stripes are absent until the most rostral regions where folium IXcd and X join to form the auricle (Pakan et al., 2007, 2010; Pakan and Wylie, 2008; Iwaniuk et al., 2009b). In this rostro-lateral region of folium X, we observed a generally complementary expression of ZII and PV (Fig. 5F). Throughout the remainder of X (i.e. medially and caudally), PV and ZII stripes were not observed; instead the folium appeared largely PV+. Although most Purkinje cells in folium X would be described as both PV+ and ZII+ (Fig. 5G), some single labeled PV+ and ZII+ Purkinje cells were seen (Fig. 5E).

Fig. 6 shows a coronal section through folia VIII–IXcd in a hummingbird reacted for both ZII (red) and PV (green). Overall, the complementary distribution of ZII and PV immunoreactivity was clearly apparent as shown in the insets in Fig. 6C (folium VIII) and Fig. 6D (ventral IXab and dorsal IXcd), although there were some Purkinje cells double labeled for both PV and ZII (see arrow in Fig. 6C overlay). As was seen the pigeon (Fig. 5A, B), in the caudal margin of the ventral lamella of IXcd, the complementary pattern was also quite evident in the hummingbird (Fig. 6A, B). Although there were some double-labeled Purkinje cells (arrows in Fig. 6B overlay).

In summary, the expression of PV and ZII was generally complementary, such that the cerebellum consisted of PV+/ZII− and PV−/ZII+ stripes. This was especially ev-

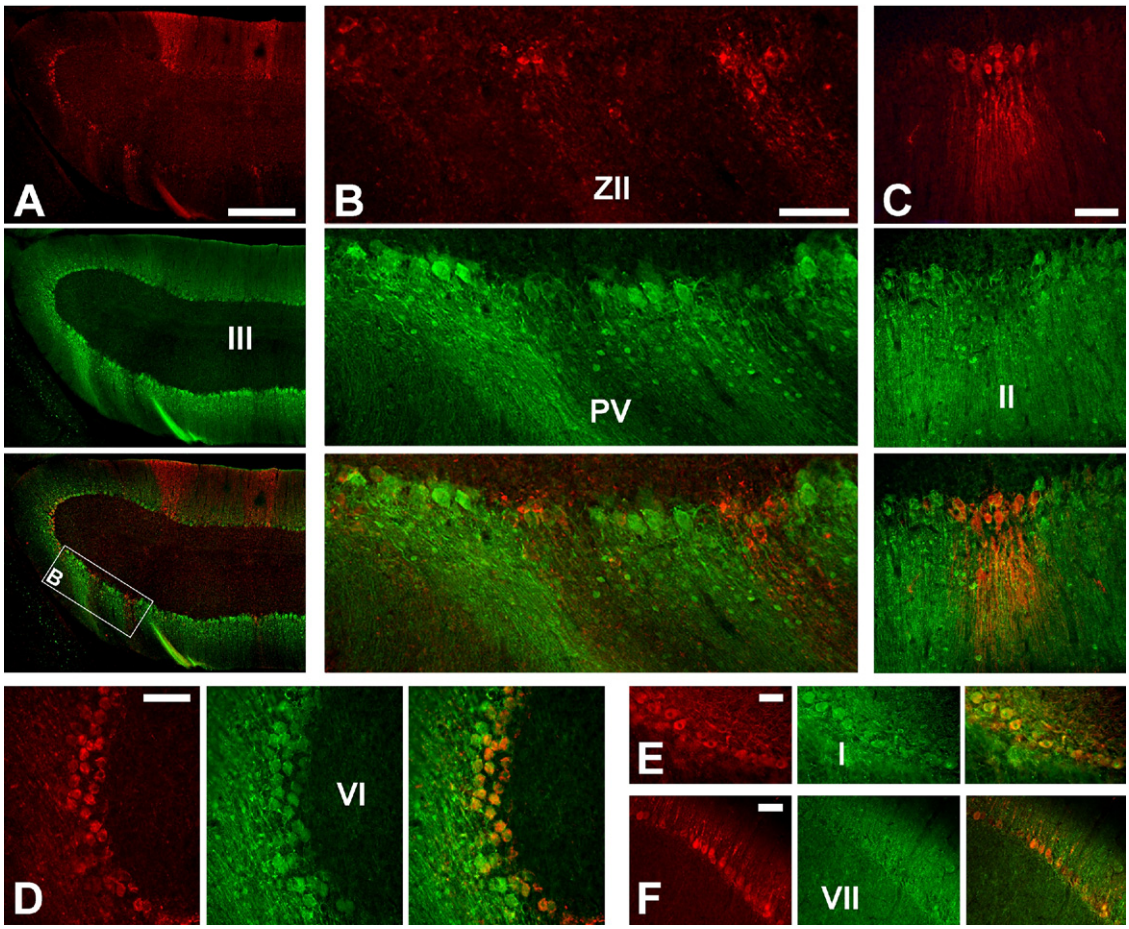

Fig. 4. A comparison of parvalbumin and zebrin II immunoreactivity in the pigeon cerebellum. Coronal sections though folia III (A, B), II (C), VI (D), I (E) and VII (F) are shown. In this and subsequent figures, the data are shown as trypticks of ZII (red), PV (green), and the overlay. Scale bars, 500 μm in (A); 100 μm in (B, C, D, F); 50 μm in (E).

ident in folia II–IV, and VIII–IXcd. However, this was far from absolute, and there were Purkinje cells that co-expressed PV and ZII, especially in folia I and X.

DISCUSSION

In the present study, we showed that PV is expressed heterogeneously in Purkinje cells in the avian cerebellum as a series of sagittal stripes. Stripes of Purkinje cells that strongly express PV (PV+) alternate with stripes that weakly express PV (PV−). Moreover, the expression of PV was, generally, complementary to that of ZII.

Do PV+ and PV− Purkinje cells have differential function roles?

The restricted expression of the calcium-binding proteins in neuronal populations in the central nervous system has been described in several species (e.g. Celio, 1990; Van Brederode et al., 1990; Resibois and Rogers, 1992; Pfeiffer and Britto, 1997; Pritz and Siadati, 1999). Calcium-binding proteins have numerous cellular functions (Kohr et al., 1991; Yamaguchi et al., 1991; Baimbridge et al., 1992; Schwaller et al., 2002; Schwaller, 2007), although their role from a systems perspective is still not well understood. That calcium binding proteins are expressed in Purkinje cells is not surprising; they are important for buffering calcium and thus preventing excitotoxicity that might occur with calcium influx. Calbindin, another calcium binding protein, is expressed uniformly in Purkinje cells (Schwaller et al., 2002; Bastianelli, 2003). Why PV is differentially expressed in Purkinje cells is unknown. PV is said to be a "slow" buffer, compared to calbindin, thus one would expect that PV+ and PV− cells would behave differently. Bastianelli (2003) outlines how PV helps maintain the synapse at its resting level during sustained excitation. This would allow Purkinje cells to transmit information linerally to the postsynaptic neuron throughout its firing range.

Schwaller (2009) emphasizes that PV is important for short-term plasticity in the cerebellum. It has also been suggested that ZII+ and ZII− Purkinje cells differ in their role in synaptic plasticity (Nagao et al., 1997; Wadiche and Jahr, 2005; Paukert et al., 2010). The cerebellum is well known for its role in behavioral plasticity (e.g. Thompson and Steinmetz, 2009; Zheng and Raman, 2010) and the

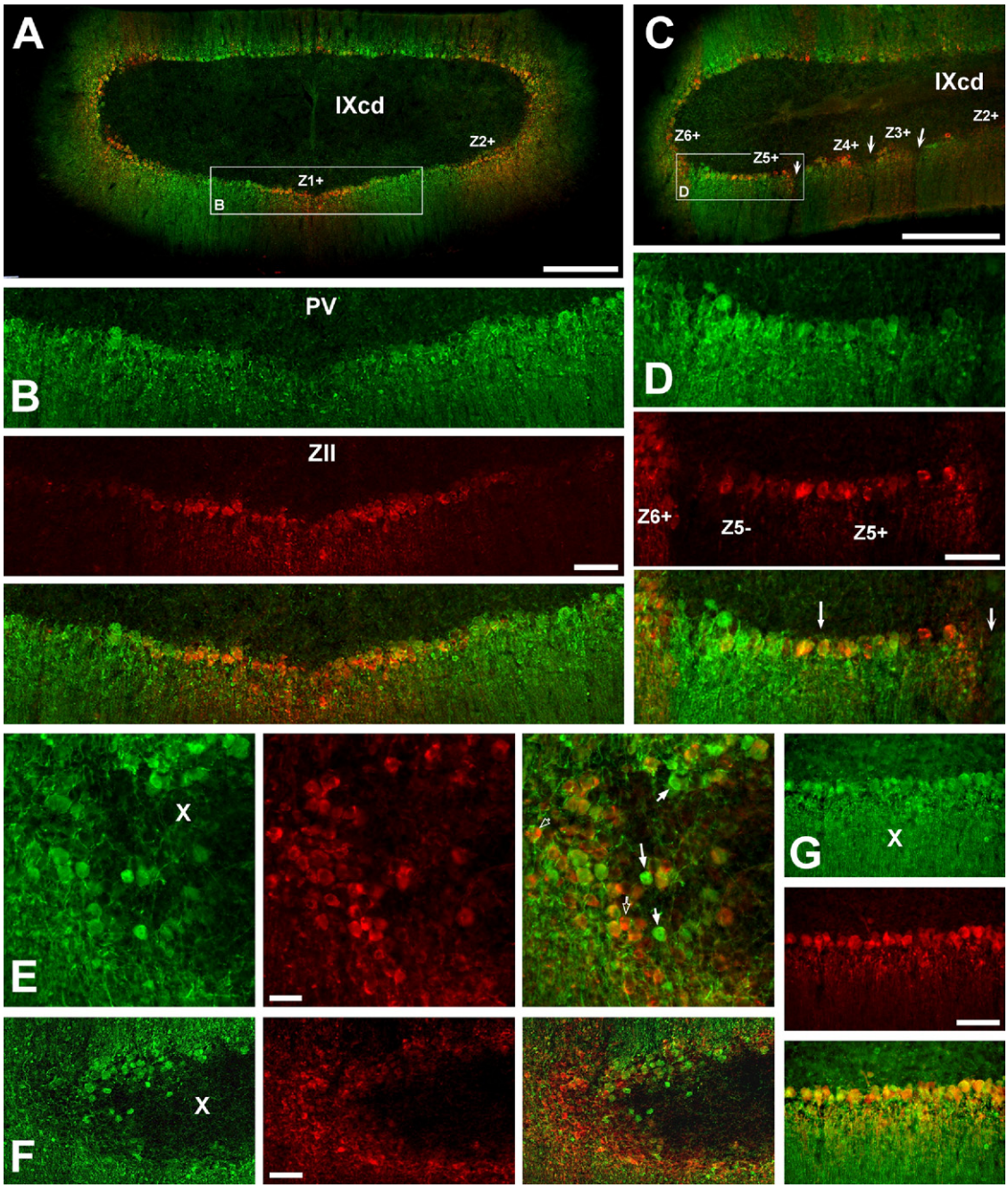

Fig. 5. A comparison of parvalbumin and zebrin II immunoreactivity in the vestibulocerebellum of the pigeon. (A–D) Show sections through folium IXcd, whereas (E–G) show sections through folium X. (A) Shows the overlay of PV (green) and ZII (red) from a section through the caudal part of IXcd. (C) Shows the overlay from a more rostral section highlighting the lateral margin of IXcd. (B, D) Show triptychs (PV, ZII, overlay) from the areas indicated in (A, C). In (C, D), the stylized arrows indicate spaces in the Purkinje cell layer lacking Purkinje cell bodies labeled for either PV or ZII. The simple arrow in (D) indicates some double labeled cells. (E–G) Respectively show sections through the caudo-lateral, rostro-lateral, and medial regions of folium X. Closed and open arrows indicate Purkinje cells single-labeled for either PV or ZII, respectively. Scale bars, 500 μm in (A, C); 100 μm in (B, D, F, G); 50 μm in (E).

flocculus in particular is involved in the plasticity of the vestibulo-ocular reflex (Miles and Lisberger, 1981). From studies of PV knock-out mice, one could surmise that PV− and PV+ Purkinje cells would show different activity. In fact, PV− Purkinje cells in such mice show a decrease in the duration of the complex spike, a decrease in the Purkinje cell pause, and an increase in the firing rate of simple spikes (Servais et al., 2005). These effects might be offset by the presence of ZII. ZII+ Purkinje cells tend to have a longer duration complex spike with more wavelets (Paukert et al., 2010). Nonetheless, differences in neuronal firing activity would surely translate into different roles in plas-

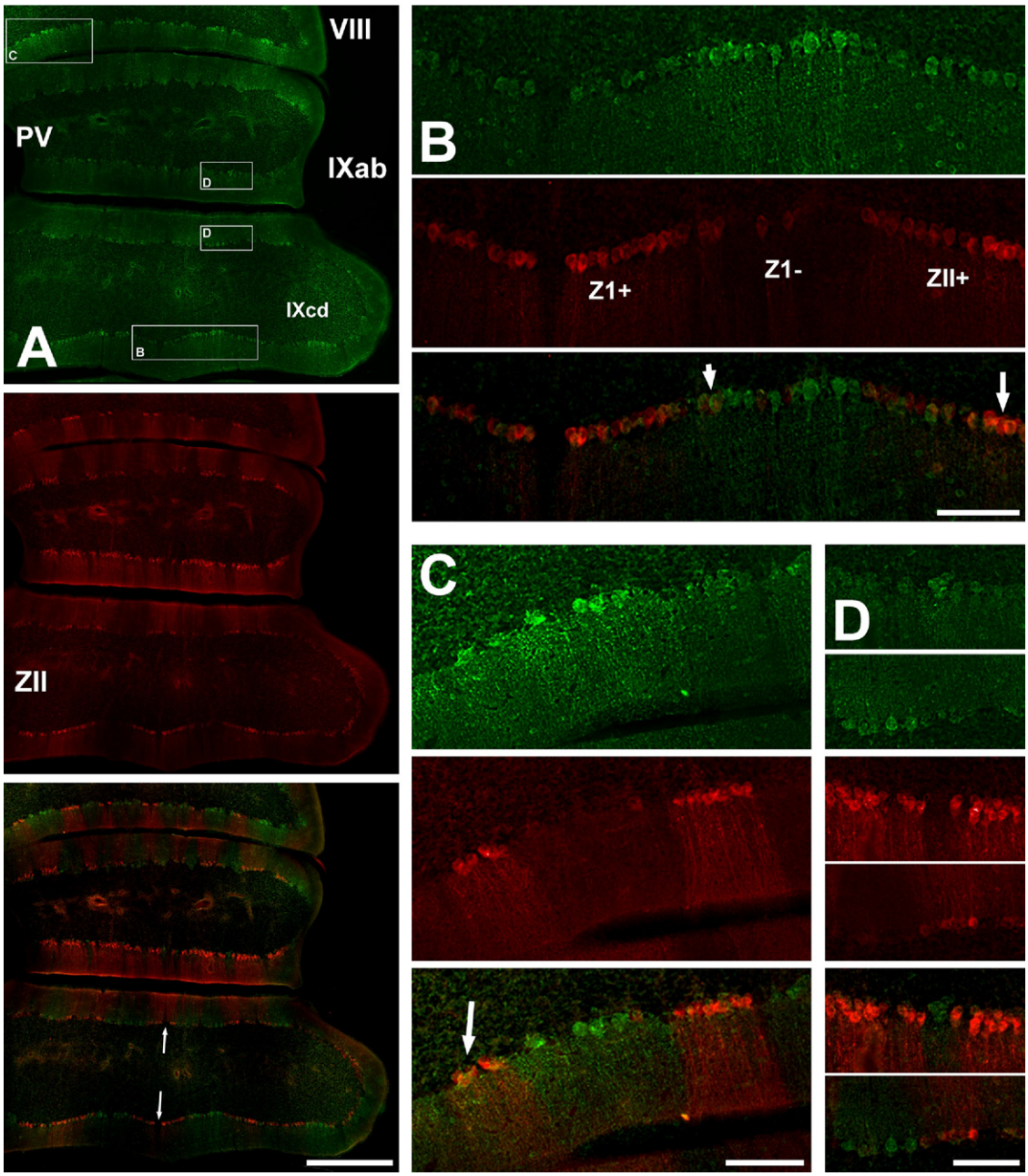

Fig. 6. A comparison of parvalbumin and zebrin II immunoreactivity in the posterior cerebellum of hummingbird. Photomicrographs from a single coronal section through posterior lobe are shown. In (A), the arrows in the overlay indicate the midline. The arrows in (B, C) indicate some double labeled Purkinje cells. Scale bars, 500 μm in (A); 100 μm in (B–D).

ticity, and it might be that the ZII+/PV− and ZII−/PV+ zones have differential roles in cerebellar plasticity. Whatever the role of heterogeneous PV expression in the cerebellar cortex, the fact that the pattern of expression is conserved across three highly divergent species suggests that its role is critical for cerebellar function.

Complementary and noncomplementary ZII and PV stripes

Throughout Figs. 4–6, it is clear that ZII+ stripes are generally PV− and vice versa. Thus, the overall pattern of ZII and PV expression is complementary. As we have mentioned several times already, this complementarity is not, however, absolute. There were numerous instances of ZII+ cells being PV+ and ZII− cells being PV−. That is, within a ZII+ stripe, although most cells were PV−, a smaller number of these ZII+ cells were PV+. In this respect, PV is similar to phospholipase cβ4 (PCβ4). PCβ4 expression is complementary to ZII, in general, with ZII+ stripes alternating with PCβ4+ stripes, but some PCβ4+ cells are ZII+ (Iwaniuk et al., 2009b; Marzban et al., 2010). An important difference between PCβ4 and PV, however,

is that PCβ4 is found primarily in the soma of Purkinje cells whereas PV is also found in Purkinje cell dendrites, cell bodies, and axons.

It remains to be seen what other markers can be used to characterize the compartmentalization of the avian cerebellar cortex. Some markers, such as corticotropin-releasing factor, are heterogeneously expressed in some climbing and mossy fibre afferents, but the pattern is not as simple as coincident or complementary with ZII (Bishop et al., 2000; Sawada et al., 2008). Other markers are heterogeneous in mammals, but not in birds. For example, heat shock protein 25 does not reveal stripes in the pigeon cerebellum (Pakan et al., 2007), in stark contrast to the mammalian cerebellum (Armstrong et al., 2000). To obtain a complete picture of how the "seemingly simple" cerebellar cortex is neurochemically organized, it will be necessary to undertake further double and triple labeling studies and map out the distribution of multiple neurochemical markers relative to one another as well as hodological connections.

The vestibulocerebellum

The vestibulocerebellum (VbC) in birds is comprized of folia IXcd and X, and is involved in the generation of compensatory eye movements (Waespe and Henn, 1987). Much is known about the organization of IXcd in pigeons from; (i) electrophysiological recordings of Purkinje cell response properties (Wylie and Frost, 1991, 1999; Wylie et al., 1993, 1998); (ii) studies of efferent and afferent projections of the VbC (Lau et al., 1998; Wylie et al., 1999, 2003a,b; Crowder et al., 2000), and (iii) studies showing the relationship between the ZII stripes and climbing and mossy fibre inputs to IXcd (Pakan and Wylie, 2008; Pakan et al., 2010). Pukinje cells in the VbC respond to optic flow, which is the pattern of motion that occurs across the retina during self-motion (Wylie et al. 1998). In the medial half of IXcd, which is referred to as the uvula, cells respond to patterns of optic flow that results from self-translation (Wylie et al., 1993; Wylie and Frost, 1999). In the lateral half of IXcd, which is referred to as the flocculus, cells respond best to patterns of optic flow that results from self-rotation about one of two axes: either the vertical axis (VA cells), or a horizontal axis oriented at 45° azimuth (HA cells) (Simpson et al., 1981; Graf et al., 1988; Wylie and Frost, 1993). As shown in several species, VA and HA cells are organized into parasagittal zones (Voogd and Wylie, 2004). In pigeons, the flocculus contains four sagittal zones that span folia IXcd and X: two VA zones are interdigitated with two HA zones. Previously, we showed that the ZII stripes in the flocculus are related to visual climbing fibre inputs in folium IXcd (Pakan and Wylie, 2008). The VA zones receive input from the caudal half of the medial column of the inferior olive (mcIO), whereas the HA zones receive input from the rostral mcIO (Wylie et al., 1999; Winship and Wylie, 2003; Pakan et al., 2005). Pakan and Wylie (2008) injected anterograde tracer into the caudal mcIO and found two distinct bands of CFs spanning IXcd and X. In IXcd the labeled CFs were in the P4± and P6± stripes. After injections of anterograde tracer into the rostral mcIO, Pakan and Wylie (2008) found two distinct bands of CFs spanning IXcd and X. In IXcd, the labeled CFs were in the P5± and P7± stripes. It therefore appears that each zone in the flocculus consists of two subzones defined by ZII immunoreactivity.

With data from the present study, we can also add that the subzones are defined by PV immunohistochemistry (although the complementary pattern of ZII and PV labeling was not absolute). Thus, each zone in the flocculus consists of a ZII+/PV− subzone and a ZII−/PV+ subzone. We also know that each subzone receives different visual mossy fibre inputs. These originate in two retinal-recipient nuclei: the nucleus of the basal optic root (nBOR) and the pretectal nucleus lentiformis mesencephali (LM) (Clarke, 1977; Brecha et al., 1980). Pakan et al. (2010) showed that after injection of antergade tacer in nBOR and LM the majority (>80%) of the terminal rosettes were in the granular layer adjacent to ZII+ stripes in IXcd. If one assumes that this translates to heavier input from the nBOR and LM to the ZII+/PV− Purkinje cells via granule cell axons, then the simple spike activity of Purkinje cells in the ZII+/PV− subzones in IXcd would be more responsive to optic flow stimuli than those in the ZII−/PV+ subzones. This assumption, however, is challenged by data from Barmack and Yakhnitsa (2008). They made microinjections of retrograde tracer in the molecular layer of the mouse uvula, and found that retrogradely labeled granule cells were widespread. As such, there may be little topographic specificity between the mossy fibre afferents and Purkinje cell simple spike activity (see also Barmack and Yakhnitsa, 2003, 2011). This paradox, that there is a sagittal distribution of at least some mossy fibre afferents, yet no conclusive evidence that this topography is maintained with the granule cell to Purkinje cell projection, must be resolved before definitive conclusions can be made regarding the relationship between Purkinje cell function and neurochemically defined stripes.

Acknowledgments—For graciously providing hummingbird and zebra finch brains, the authors thank Raul Suarez and Ken Welch Jr and Ákos Pogán and Gergely Zachar, respectively. The authors also wish to thank Richard Hawkes for providing the ZII antibody and two anonymous reviewers for their comments on the manuscript. Funding for this study was provided by grants to ANI and DRW from NSERC and to DRW from the Canada Research Chairs Program and CIHR.

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.

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

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

Akintunde A, Eisenman LM (1994) External cuneocerebellar projection and Purkinje cell zebrin II bands: a direct comparison of parasag-

ittal banding in the mouse cerebellum. J Chem Neuroanat 7:75–86.
Apps R, Hawkes R (2009) Cerebellar cortical organization: a one-map hypothesis. Nat Rev Neurosci 10:670–681.
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.
Baimbridge KG, Celio MR, Rogers JH (1992) Calcium-binding proteins in the nervous system. Trends Neurosci 15:303–308.
Barmack NH, Yakhnitsa V (2011) Topsy turvy: functions of climbing and mossy fibers in the vestibulo-cerebellum. Neuroscientist 17:221-236.
Barmack NH, Yakhnitsa V (2008) Distribution of granule cells projecting to focal Purkinje cells in mouse uvula-nodulus. Neuroscience 156:216–221.
Barmack NH, Yakhnitsa V (2003) Cerebellar climbing fibers modulate simple spikes in Purkinje cells. J Neurosci 23:7904–7916.
Bastianelli E (2003) Distribution of calcium-binding proteins in the cerebellum. Cerebellum 2:242–262.
Bishop GA, Seelandt CM, King JS (2000) Cellular localization of corticotropin releasing factor receptors in the adult mouse cerebellum. Neuroscience 101:1083–1092.
Braun K, Schachner M, Scheich M, Claus CW (1986) Cellular localization of the Ca^{2+}-binding protein parvalbumin in the developing avian cerebellum. Cell Tissue Res 243:69–78.
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.
Celio MR (1990) Calbindin D-28k and parvalbumin in the rat nervous system. Neuroscience 35:375–475.
Chan-Palay V, Nilaver G, Palay SL, Beinfeld MC, Zimmerman EA, Wu JY, O'Donohue TL (1981) Chemical heterogeneity in cerebellar Purkinje cells: existence and coexistence of glutamic acid decarboxylase-like and motilin-like immunoreactivities. Proc Natl Acad Sci 78:7787–7791.
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.
Cummings SL (1989) Distribution of corticotropin-releasing factor in the cerebellum and precerebellar nuclei of the cat. J Comp Neurol 289:657–675.
Cummings SL, Young WS 3rd, Bishop GA, De Souza EB, King JS (1989) Distribution of corticotropin-releasing factor in the cerebellum and precerebellar nuclei of the opossum: a study utilizing immunohistochemistry, in situ hybridization histochemistry, and receptor autoradiography. J Comp Neurol 280:501–521.
Dehnes Y, Chaudhry FA, Ullensvang K, Lehre KP, Storm-Mathisen J, Danbolt NC (1998) The glutamate transporter EAAT4 in rat cerebellar Purkinje cells: a glutamate-gated chloride channel concentrated near the synapse in parts of the dendritic membrane facing astroglia. J Neurosci 18:3606–3619.
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.
Fortin M, Marchand R, Parent A (1998) Calcium-binding proteins in primate cerebellum. Neurosci Res 30:155–168.
Fujita H, Oh-Nishi A, Obayashi S, Sugihara I (2010) Organization of the marmoset cerebellum in three-dimensional space: lobulation, aldolase C compartmentalization and axonal projection. J Comp Neurol 518:1764–1791.
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.
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.
Iwaniuk AN, Hurd PL, Wylie DRW (2006a) Comparative morphology of the avian cerebellum: I. Degree of foliation. Brain Behav Evol 68:45–62.
Iwaniuk AN, Hurd PI, Wylie DR (2006b) The comparative morphology of the cerebellum in caprimulgiform birds: evolutionary and functional implications. Brain Behav Evol 67:53–68.
Iwaniuk AN, Hurd PL, Wylie DR (2007) Comparative morphology of the avian cerebellum: II. Relative size of folia. Brain Behav Evol 69:196–219.
Iwaniuk AN, Lefevbre L, Wylie DR (2009a) The comparative approach and brain-behaviour relationships: a tool for understanding tool use. Can J Exp Psych 63:150–159.
Iwaniuk AN, Marzban H, Pakan JMP, Watanabe M, Hawkes R, Wylie DRW (2009b) Compartmentation of the cerebellar cortex of hummingbirds (Aves: Trochilidae) revealed by the expression of zebrin II and phospholipase Câ4. J Chem Neuroanat 37:55–63.
Jaarsma D, Levey AI, Frostholm A, Rotter A, Voogd J (1995) Light-microscopic distribution and parasagittal organisation of muscarinic receptors in rabbit cerebellar cortex. J Chem Neuroanat 9:241–259.
King JS, Madtes P Jr, Bishop GA, Overbeck TL (1997) The distribution of corticotropin-releasing factor (CRF), CRF binding sites and CRF1 receptor mRNA in the mouse cerebellum. Prog Brain Res 114:55–66.
Kohr G, Lambert CE, Mody I (1991) Calbindin-D28K (CaBP) levels and calcium currents in acutely dissociated epileptic neurons. Exp Brain Res 85:543–551.
Larsell O (1967) The cerebellum: from myxinoids through birds. Minnesota: The 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.
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, Chung SH, Pezhouh MK, Feirabend H, Watanabe M, Voogd J, Hawkes R (2010) Antigenic compartmentation of the cerebellar cortex in the chicken (*Gallus domesticus*). J Comp Neurol 518:2221–2239.
Marzban H, Chung S, Watanabe M, Hawkes R (2007) Phospholipase Cbeta4 expression reveals the continuity of cerebellar topography through development. J Comp Neurol 502:857–71.
Marzban H, Sillitoe RV, Hoy M, Chung SH, Rafuse VF, Hawkes R (2004) Abnormal HNK-1 expression in the cerebellum of an N-CAM null mouse. J Neurocytol 33:117–130.
Miles FA, Lisberger SG (1981) Plasticity in the vestibulo-ocular reflex: a new hypothesis. Annu Rev Neurosci 4:273–299.
Nagao S, Kwak S, Kanazawa I (1997) EAAT4, a glutamate transporter with properties of a chloride channel, is predominantly localized in Purkinje cell dendrites, and forms parasagittal compartments in rat cerebellum. Neuroscience 78:929–933.
Pakan JMP, Graham DJ, Gutiérrez-Ibáñez C, Wylie DR (2011) Organization of the cerebellum: correlating zebrin immunochemistry

with optic flow zones in the pigeon flocculus. Vis Neurosci 28:163–174.
Pakan JM, Graham DJ, Wylie DR (2010) Organization of visual mossy fiber projections and zebrin expression in the pigeon vestibulocerebellum. J Comp Neurol 518:175–198.
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.
Pakan JM, Wylie DR (2008) Congruence of zebrin II expression and functional zones defined by climbing fiber topography in the flocculus. Neuroscience 157:57–69.
Paukert M, Huang YH, Tanaka K, Rothstein JD, Bergles DE (2010) Zones of enhanced glutamate release from climbing fibers in the mammalian cerebellum. J Neurosci 30:7290–7299.
Pfeiffer CP, Britto LR (1997) Distribution of calcium-binding proteins in the chick visual system. Braz J Med Biol Res 30:1315–1318.
Pritz MB, Siadati A (1999) Calcium binding protein immunoreactivity in nucleus rotundus in a reptile, *Caiman crocodilus*. Brain Behav Evol 53:277–287.
Resibois A, Rogers JH (1992) Calretinin in rat brain: an immunohistochemical study. Neuroscience 46:101–134.
Rogers JH (1989) Immunoreactivity for calretinin and other calcium-binding proteins in cerebellum. Neuroscience 31:711–721.
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.
Sarna JR, Marzban H, Watanabe M, Hawkes R (2006) Complementary stripes of phospholipase Cbeta3 and Cbeta4 expression by Purkinje cell subsets in the mouse cerebellum. J Comp Neurol 496:303–313.
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 1222:106–117.
Sawada K, Sakata-Haga H, Fukui Y (2010) Alternating array of tyrosine hydroxylase and heat shock protein 25 immunopositive Purkinje cell stripes in zebrin II-defined transverse zone of the cerebellum of rolling mouse Nagoya. Brain Res 1343:46–53.
Schwaller B (2009) The continuing disappearance of "pure" Ca^{2+} buffers. Cell Mol Life Sci 66:275–300.
Schwaller B (2007) Emerging functions of the "Ca^{2+} buffers" parvalbumin, calbindin D-28k and calretinin in the brain. In: Handbook of neurochemistry and molecular neurobiology. Neural protein metabolism and function, Vol. 7 (Lajtha A, Banik N, eds), pp 197–222. New York: Springer.
Schwaller B, Meyer M, Schiffmann S (2002) 'New' functions for 'old' proteins: the role of the calcium-binding proteins calbindin D-28k, calretinin and parvalbumin, in cerebellar physiology. Studies with knockout mice. Cerebellum 1:241–258.
Servais L, Bearzatto B, Schwaller B, Dumont M, De Saedeleer C, Dan B, Barski JJ, Schiffmann SN, Cheron G (2005) Mono- and dual-frequency fast cerebellar oscillation in mice lacking parvalbumin and/or calbindin D-28k. Eur J Neurosci 22:861–870.
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, 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.
Sillitoe RV, Vogel MW, Joyner AL (2010) Engrailed homeobox genes regulate establishment of the cerebellar afferent circuit map. J Neurosci 30:10015–10024.
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.
Sotelo C, Wassef M (1991) Cerebellar development: afferent organization and Purkinje cell heterogeneity. Philos Trans R Soc Lond B Biol Sci 331:307–313.
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, 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 (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(**36**):9696–9710.
Thompson RF, Steinmetz JE (2009) The role of the cerebellum in classical conditioning of discrete behavioral responses. Neuroscience 162:732–755.
Van Brederode JF, Mulligan KA, Hendrickson AE (1990) Calcium-binding proteins as markers for subpopulations of GABAergic neurons in monkey striate cortex. J Comp Neurol 298:1–22.
van den Dungen HM, Groenewegen HJ, Tilders FJ, Schoemaker J (1988) Immunoreactive corticotropin releasing factor in adult and developing rat cerebellum: its presence in climbing and mossy fibres. J Chem Neuroanat 1:339–349.
Voogd J, Bigaré F (1980) Topographical distribution of olivary and cortico nuclear fibers in the cerebellum: a review. In: The inferior olivary nucleus: anatomy and physiology (Courville J, de Montigny C, Lamarre Y, eds), pp 207–234. New York: Raven.
Voogd J, Broere G, van Rossum J (1969) The medio-lateral distribution of the spinocerebellar projection in the anterior lobe and the simple lobule in the cat and a comparison with some other afferent fibre systems. Psychiatr Neurol Neurochir 72:137–151.
Voogd J, Glickstein M (1998) The anatomy of the cerebellum. Trends Neurosci 21:370–375.
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.
Waespe W, Henn V (1987) Gaze stabilization in the primate. The interaction of the vestibulo-ocular reflex, optokinetic nystagmus, and smooth pursuit. Rev Physiol Biochem Pharmacol 106:37–125.
Winship IR, Wylie DR (2003) Zonal organization of the vestibulocerebellum in pigeons (*Columba livia*): I. Climbing fibre 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, Bischof WF, Frost BJ (1998) Common reference frame for neural coding of translational and rotational optic flow. Nature 392:278–282.
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, Lau KL, Lu X, Glover RG, Valsangkar-Smyth M (1999) Projections of Purkinje cells in the translation and rotation zones of the vestibulocerebellum in pigeon (*Columba livia*). J Comp Neurol 413:480–493.

Yamaguchi T, Winsky L, Jacobowitz DM (1991) Calretinin, a neuronal calcium binding protein, inhibits phosphorylation of a 39 kDa synaptic membrane protein from rat brain cerebral cortex. Neurosci Lett 131:79–82.

Zheng N, Raman IM (2010) Synaptic inhibition, excitation, and plasticity in neurons of the cerebellar nuclei. Cerebellum 9:56–66.

(Accepted 6 April 2011)
(Available online 13 April 2011)