

Golgi Cell Dendrites Are Restricted by Purkinje Cell Stripe Boundaries in the Adult Mouse Cerebellar Cortex

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Despite the general uniformity in cellular composition of the adult cerebellar cortex, there is a complex underlying pattern of parasagittal stripes of Purkinje cells with characteristic molecular phenotypes and patterns of connectivity. It is not known whether interneuron processes are restricted at stripe boundaries. To begin to address the issue, three strategies were used to explore how cerebellar Golgi cell dendrites are organized with respect to parasagittal stripes: first, double immunofluorescence staining combining anti-neurogranin to identify Golgi cell dendrites with the Purkinje cell compartmentation antigens zebrin II/aldolase C, HNK-1, and phospholipase C β 4; second, zebrin II immunohistochemistry combined with a rapid Golgi–Cox impregnation procedure to reveal Golgi cell dendritic arbors; third, stripe antigen expression was used on sections of a GlyT2-EGFP transgenic mouse in which reporter expression is prominent in Golgi cell dendrites. In each case, the dendritic projections of Golgi cells were studied in the vicinity of Purkinje cell stripe boundaries. The data presented here show that the dendrites of a cerebellar interneuron, the Golgi cell, respect the fundamental cerebellar stripe cytoarchitecture.

Key words: zebrin II; HNK-1; neurogranin; compartmentation; glycine transporter 2-EGFP transgene; dendrites

Introduction

There are multiple Purkinje cell classes in the cerebellum, which form an elaborate array of transverse zones and parasagittal stripes [for review, see Hawkes et al. (1992), Voogd et al. (1996), Hawkes and Eisenman (1997), Hawkes (1997), Herrup and Kuebler (1997), Oberdick et al. (1998), and Armstrong and Hawkes (2000)]. The granular layer is also subdivided into stripes (for review, see Ozol and Hawkes, 1997) that also align with the overlying Purkinje cell compartments [e.g., mossy fiber terminal fields (Matsushita et al., 1991; Akintunde and Eisenman, 1994; Ji and Hawkes, 1994; Voogd et al., 2003; Pijpers et al., 2006)], and an elaborate mosaic of patches is revealed both in tactile receptive field maps (Welker, 1987) and by histological treatments (Hawkes et al., 1997, 1998). Finally, functional studies have also demonstrated parasagittal stripes in the cerebellum (Ekerot and Larson, 1973, 1980) (for review, see Voogd et al., 1996) with direct correlations with particular Purkinje cell stripes (Chockan and Hawkes, 1994; Chen et al., 1996; Hallem et al., 1999).

Thus, we arrive at a gap in our current understanding of cerebellar function. We understand cerebellar connectivity in some detail, and we have a growing picture of the global architecture,

but have little data to bridge the two. Specifically, how are the cerebellar inhibitory interneurons arranged with respect to Purkinje cell compartments? Are their synaptic fields constrained within modules, do they span modular boundaries, or is their organization independent of boundaries? There are valuable physiological data from Gao et al. (2006) showing functionally that cerebellar inhibitory interneurons respect Purkinje cell compartments: this study comprises the equivalent structural studies. We focus on Golgi cell dendrites. In principle, Golgi interneurons' apical dendrites could interact with cerebellar parasagittal stripes in any of four ways (Fig. 1A–D): (1) a Golgi cell might be restricted to the same stripe that contains its soma; (2) the dendrites might receive input from one compartment but have its soma in the neighboring compartment; (3) the dendritic arbor might straddle boundaries between compartments; or (4) they might represent an altogether different network (for example, related to the parallel fiber system) and have their dendrites distributed independently of Purkinje cell compartmentation. Each hypothesis has unique structural implications that can be distinguished experimentally. First, anti-neurogranin immunohistochemistry, to identify the dendritic arbors of Golgi cells (Singec et al., 2003), was combined with Purkinje cell stripe antigens, zebrin II/aldolase C (Brochu et al., 1990; Ahn et al., 1994), HNK-1 (Marzban et al., 2004), and phospholipase C β 4 (Sarna et al., 2006), to explore interneuron cytoarchitecture in the context of cerebellar topography. Next, Golgi–Cox impregnation was combined with anti-zebrin II immunohistochemistry to relate Golgi cell dendrites to stripe boundaries. Finally, we examined a second subset of Golgi cells, as revealed by a glycine transporter T2-EGFP transgene [GlyT2-EGFP (Zeilhofer et al., 2005; Simat et al., 2007)]. In each case, the data reveal that Golgi cell dendrites are restricted

Received June 7, 2007; revised Feb. 4, 2008; accepted Feb. 4, 2008.

This work was supported by a grant from the Canadian Institutes of Health Research (R.H.). R.V.S. was a recipient of a Canadian Institutes of Health Research Strategic Training Program Studentship in Genetics, Child Development, and Health. We thank Sejal Shah for assistance with the histology and Drs. Enrico Mugnaini and Ray Turner for advice.

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DOI:10.1523/JNEUROSCI.4145-07.2008

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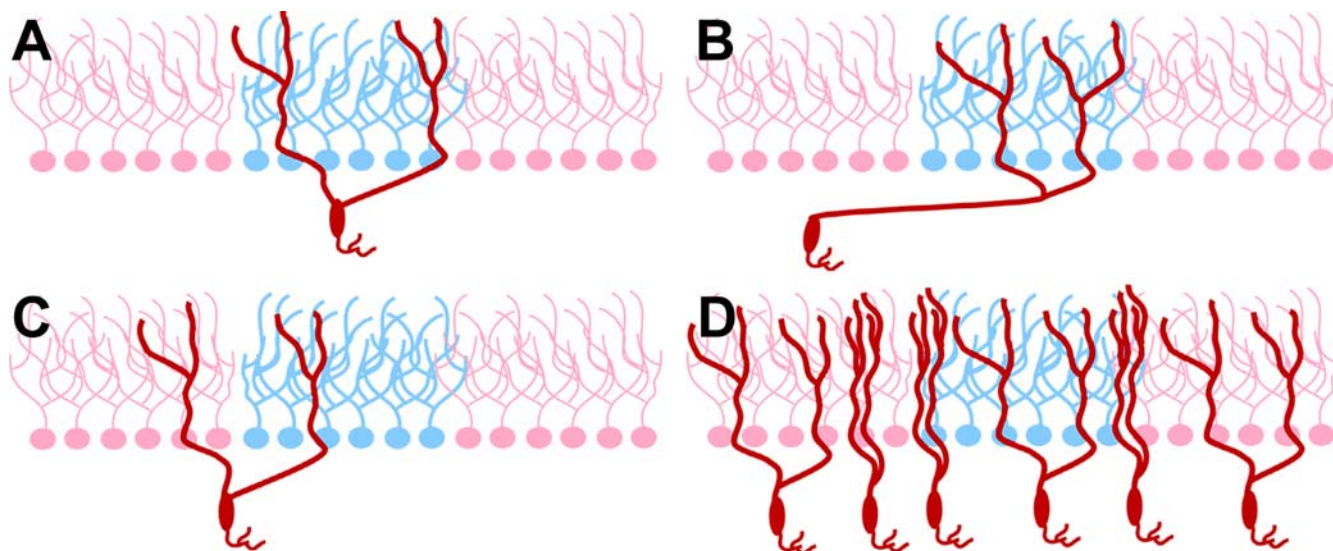


Figure 1. Schematic representation of four possible ways that Golgi cells may interact with Purkinje cell compartment boundaries. **A**, The somata and dendrites are restricted to one single compartment. **B**, The dendrites are restricted to one compartment, and the soma is located in a neighboring compartment. **C**, The dendrites of a single Golgi cell span different compartments. **D**, There is no restriction of Golgi cells with respect to Purkinje cell compartments.

such that they do not cross Purkinje cell stripe boundaries and that cerebellar interneurons are organized around cerebellar architecture.

Materials and Methods

All animal procedures conformed to institutional regulations and the *Guide to the Care and Use of Experimental Animals* from the Canadian Council for Animal Care. Adult CD1, BALB/c, and C57BL/6 mice (20–30 g; no strain-specific differences were noted) were obtained from Charles River Laboratories (St. Constant, Quebec, Canada) and maintained in the Animal Resource Centre at the University of Calgary. In addition, transgenic mice were used that specifically express enhanced green fluorescent protein under the control of the glycine transporter 2 promoter [GlyT2-EGFP (Zeilhofer et al., 2005)].

All antibodies were diluted in 10% normal goat serum in PBS. Anti-zebrin II is a mouse monoclonal antibody produced by immunization with a crude cerebellar homogenate from the weakly electric fish *Apteronotus* (Brochu et al., 1990) and subsequently shown to bind the respiratory isoenzyme aldolase C (Ahn et al., 1994; Hawkes and Herrup, 1996); it was used directly from spent hybridoma culture medium. Rabbit anti-neurogranin was raised against full-length recombinant rat neurogranin protein (Millipore, Billerica, MA; catalog #AB5620; used diluted 1:1000); it recognizes Purkinje cells in the neonatal cerebellum and Golgi cells in the adult cerebellum, as previously reported (Singec et al., 2003; Larouche et al., 2006), and on Western blots of newborn or postnatal day 20 cerebellar homogenates reveals a single polypeptide band of apparent molecular weight 10 kDa (Larouche et al., 2006), consistent with that of neurogranin/RC3 (Watson et al., 1990). Mouse hybridoma cell line TIB200 secreting monoclonal anti-HNK-1 (Abo and Balch, 1981) was obtained from the American Type Culture Collection (Rockville, MD). Culture supernatants were harvested every 2–3 d and used for immunocytochemistry without further purification (diluted 1:50). Rabbit anti-phospholipase C β 4 was raised against a synthetic peptide representing amino acids 15–74 of the mouse PLC β 4 protein fused to glutathione-S-transferase and expressed in bacteria; it was a generous gift from M. Watanabe (Hokkaido University, Sapporo, Japan). Control immunohistochemistry using either antibodies preabsorbed with antigen polypeptides or cerebellar sections from a PLC β 4 knock-out mouse yielded no significant immunostaining (Nakamura et al., 2004; Sarna et al., 2006; Marzban et al., 2007). Anti-PLC β 4 recognizes a single polypeptide band of 134 kDa apparent molecular weight on Western blots of mouse (Nakamura et al., 2004) and human (our unpublished data) cerebellar homogenates. The band is absent from Western blots of cerebellar

homogenates from a PLC β 4 null mouse (Jiang et al., 1996; Nakamura et al., 2004).

Perfusion and cryostat sectioning was as described by Sillitoe et al. (2003). Cerebellar sections were processed for single and double fluorescent immunohistochemistry as described previously (Sillitoe et al., 2003). Whole-mount cerebellar immunohistochemistry was described by Sillitoe and Hawkes (2002). Golgi–Cox staining was performed according to Glaser and Van der Loos (1981) as modified by Gibb and Kolb (1998). Subsequently, the cerebella were transferred to 30% sucrose in distilled water for >48 h, sectioned at 200–400 μ m on a Vibratome, and immunoperoxidase stained for zebrin II, with diaminobenzidine as the substrate (Eisenman and Hawkes, 1993). Photomicrographs were captured with a SPOT Cooled Color digital camera (Diagnostic Instruments, Sterling Heights, MI) and assembled in Adobe (San Jose, CA) Photoshop. The images were cropped and corrected for brightness and contrast but not otherwise manipulated. Images for deconvolution were captured from 200- μ m-thick transverse sections through lobule VIII of an GlyT2-EGFP (green) cerebellum that was immunofluorescence labeled by using anti-zebrin II, using a Retiga SRV camera mounted on a Leica (Bannockburn, IL) DM6000 microscope. Images were acquired and analyzed using Volocity software (version 4.1.0). Changing the light paths captured FITC and CY3 channels, and a total of 64 Z-slices (0.25 μ m step size) were collected by moving a “Leica Focus Drive.” The Z-stacks were rendered in Volocity using iterative restoration with the confidence limit set at 98% and the iterative limit set at 25. Three-dimensional images were assembled in Volocity and then imported into Adobe Photoshop CS2 and Adobe Illustrator CS2 to assemble the final figure. For cell counts, we focused on lobule VIII of the posterior zone of the cerebellum: the zebrin II/PLC β 4 stripe array was very clear here, and there is an alternating array of stripes of approximately equal widths (see Fig. 2) (however, our overall observations suggest strongly that the same conclusions apply throughout the cerebellar cortex). Golgi cells were assigned to a stripe based on the location of their somata. Stripe boundaries were defined by zebrin II/PLC β 4/HNK-1 expression. To visualize the GlyT2-EGFP-positive Golgi cell dendrites, we have exclusively used the posterior region (lobule VI–IX) of the cerebellar cortex, because it is hard to see the EGFP-immunoreactive dendrites in the anterior part of the cerebellum.

A Golgi cell apical dendrite was only analyzed if it reached at least one-half the thickness of the molecular layer; it was judged to cross a stripe boundary if it extended >10 μ m into the neighboring stripe.

Results

The mouse cerebellum is comprised of four transverse zones: anterior (\sim lobules I–V), central (\sim lobules VI–VII), posterior (\sim lobule VIII and dorsal lobule IX), and nodular [ventral lobule IX and lobule X (Ozol et al., 1999)]. Each zone is further divided into narrow parasagittal stripes. Zebrin II (Brochu et al., 1990) [for review, see Hawkes et al. (1992) and Hawkes and Herrup (1996)] [also known as the metabolic isoenzyme aldolase C (Ahn et al., 1994)] is expressed by all Purkinje cells in the central and nodular zones and in stripes of Purkinje cells in the anterior zone and posterior zone (Fig. 2*A,B*) (Ozol et al., 1999; Sillitoe and Hawkes, 2002).

Golgi cells (Golgi, 1883) are large interneurons with their cell bodies dispersed throughout the granular layer and numerous radiating dendrites (Palay and Chan-Palay, 1974). Golgi cell apical dendrites ramify through the molecular layer and are contacted by the axons of granule cells. In addition, a few basolateral dendrites extend in the granular layer (for review, see De Schutter and Bjaalie, 2001). In the adult mouse cerebellar cortex, neurogranin immunoreactivity is restricted to the somata and dendritic arbors of Golgi cells (Singec et al., 2003) [plus weak expression in a small subset of Purkinje cells, restricted primarily to the nodular zone, which is easily distinguished from Golgi cell immunoreactivity (Larouche et al., 2006)]. We have therefore used double-label neurogranin/zebrin II fluorescence immunohistochemistry to explore the behavior of Golgi cell apical dendrites at the boundaries between Purkinje cell compartments (Fig. 3*A*). Figure 3*B–D* shows three examples taken from lobule VIII of the vermis (the posterior zone) double labeled for zebrin II and neurogranin. In each case, a Golgi cell apical dendrite extended from the soma into the molecular layer, where it reached a boundary between a zebrin II⁺ and zebrin II[−] stripe but did not cross it. This is the case both for Golgi cells with their somata beneath a zebrin II⁺ stripe and those beneath a zebrin II[−] stripe. Restriction at stripe boundaries was seen both in the anterior zone and the posterior zone. In all, 4281 Golgi cell dendrites (primary and secondary branches) were examined in this manner [all from lobule VIII of the posterior zone, where alternating zebrin II^{+/−} stripes range from 100 to 230 μ m in width (Fig. 2); $n = 8$ animals]: 4164 (97.3%) were confined to a single compartment, and 117 (2.7%) appeared to cross into the neighboring compartment (with some appearing to straddle the boundary and others with their dendritic arbors preferentially in the neighboring stripe). Restriction of Purkinje cell dendrites is not confined to zebrin II^{+/−} boundaries: a similar pattern of Golgi cell restriction was also found both at boundaries revealed by HNK-1 expression in ventral lobule IX and lobule X [the nodular zone (Eisenman and Hawkes, 1993; Marzban et al., 2004)], where all Purkinje cells are zebrin II⁺ (Fig. 3*E*), and at boundaries of expression of PLC β 4 (see Fig. 5*A,B*) [unsurprisingly, because PLC β 4 expression is complementary to zebrin II and shares common stripe boundaries (Sarna et al., 2006)].

In a second approach, 200–400 μ m sections through the adult cerebellum were first Golgi–Cox impregnated and then immunoperoxidase counterstained for zebrin II (Fig. 4). Although

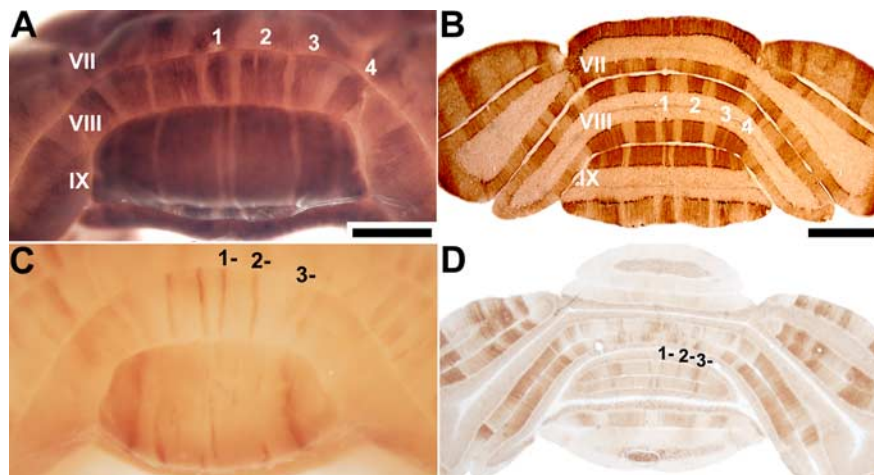


Figure 2. Zebrin II and PLC β 4 expression in the posterior zone (\sim lobule VIII) reveals an array of parasagittal stripes. *A*, Posterior view of a whole-mount cerebellum immunoperoxidase stained using anti-zebrin II. *B*, A transverse section cut through the posterior cerebellum immunoperoxidase stained for anti-zebrin II. *C*, Posterior view of a whole-mount cerebellum immunoperoxidase stained for PLC β 4. *D*, A transverse section cut through the posterior cerebellum immunoperoxidase stained for PLC β 4. The zebrin II-positive (P1+ to P4+) and PLC β 4 negative (P1− to P3−) stripes are labeled in all panels [for stripe terminology, see Sillitoe et al. (2002)]. The roman numerals refer to the lobules. Scale bars: *A* (for *A*, *C*), *B* (for *B*, *D*), 1 mm.

this rapid Golgi–Cox procedure gave less satisfactory cell fills than others, it proved better when combined with zebrin II immunohistochemistry. In all, 104 Golgi cells were studied. When Golgi cell dendritic morphology is viewed in the context of parasagittal Purkinje compartments, it is clear that the great majority of Golgi cell neurites do not cross Purkinje cell stripe boundaries ($n = 102/104$). The somata may lie under either zebrin II⁺ or zebrin II[−] stripes; in either case, the dendritic arbor of the Golgi cell extends into the molecular layer but does not cross over into the neighboring Purkinje cell compartment. In only two of these cases did we encounter Golgi cells whose dendritic arbors apparently spanned a Purkinje cell stripe boundary, both where significant curvature of the cortical surface occurred, with the result that zebrin II stripe boundaries were difficult to trace with confidence (Fig. 4*C*).

Finally, we examined a second subset of Golgi cells, those that express a GlyT2-EGFP transgene (Zeilhofer et al., 2005); Golgi cells that express neurogranin are GABAergic (and not glycinergic), whereas the Golgi cells devoid of neurogranin are either GABAergic/glycinergic or glycinergic only (Simat et al., 2007). In transverse sections through the GlyT2-EGFP cerebellum immunofluorescence stained for PLC β 4 or zebrin II, it was possible to examine these Golgi cell dendrites in the vicinity of Purkinje cell stripe boundaries by using a combination of epifluorescence [GlyT2-EGFP + PLC β 4 (Fig. 5)] and confocal [e.g., GlyT2-EGFP + zebrin II (Fig. 6)] microscopy. As in the previous cases, apical dendrites are restricted within a Purkinje cell stripe; dendrites from 68 Golgi cells were chosen that came within 20 μ m of a stripe boundary, and in no case was one seen to cross.

Discussion

Golgi cell dendrites are restricted by Purkinje cell stripe boundaries

The data presented here show for the first time that a cerebellar interneuron, the Golgi cell, respects the fundamental cerebellar stripe cytoarchitecture. Several limits on these data should be underscored. First, we have examined the apical dendritic arbors within individual sections (40 μ m for neurogranin/zebrin II and PLC β 4/GlyT2-EGFP immunohistochemistry; 200–400 μ m for

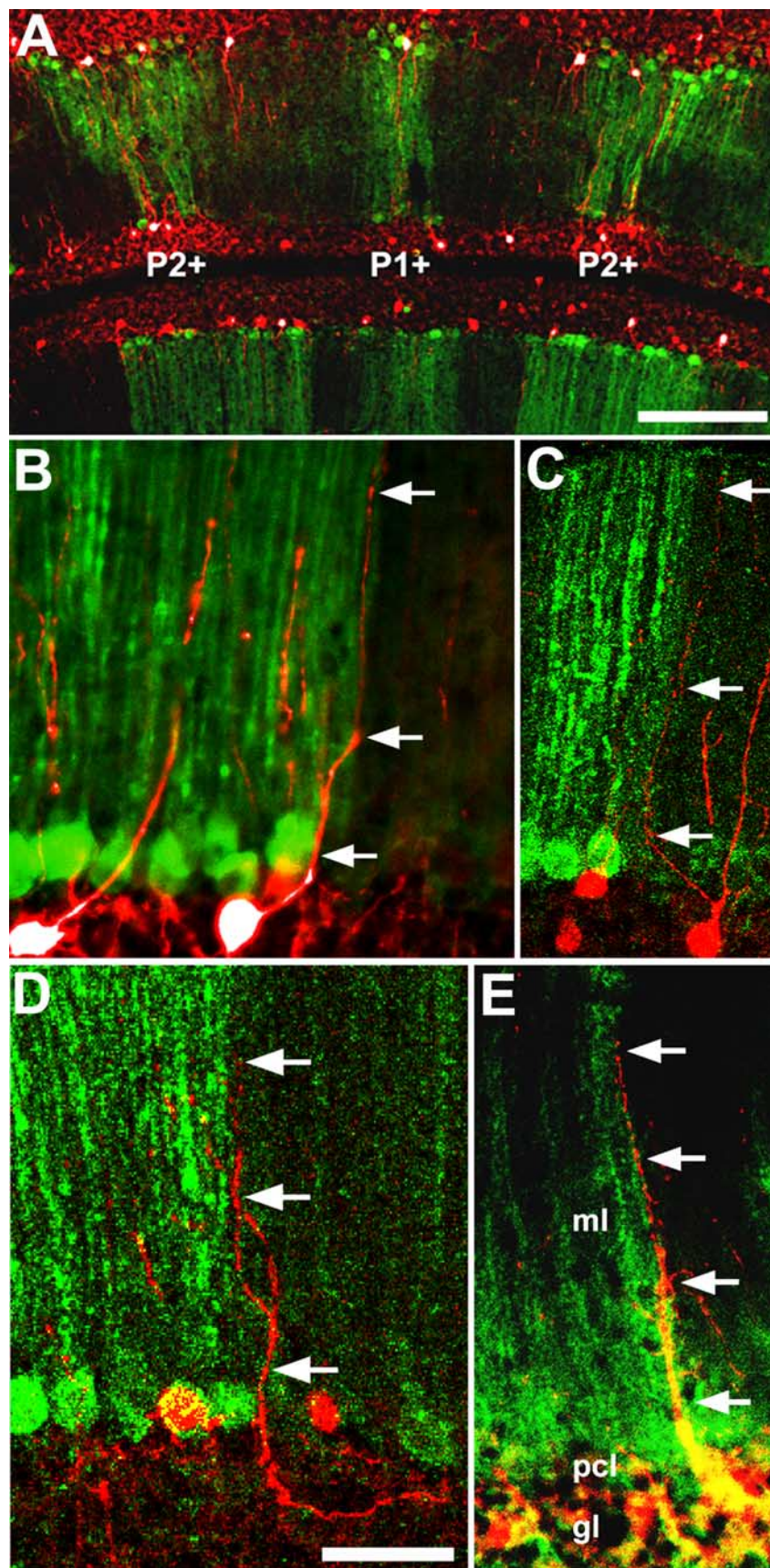


Figure 3. Golgi cell apical dendrites are restricted to parasagittal Purkinje cell compartments as defined by stripe antigens. **A**, Low-power view of a transverse section double-immunofluorescence stained for zebrin II (green; the P1⁺ and P2⁺ stripes are labeled) and neurogranin (red). It is likely that neurogranin-positive Golgi cells are more common beneath zebrin II-immunoreactive Purkinje cells. **B–D**, Three higher-power examples illustrating the restriction of neurogranin-

Golgi–Cox-stained tissue) but have not reconstructed the cells beyond this, because it proved too unreliable. In the future, intracellular fills might be used to overcome this shortcoming. Second, it is difficult to be sure that the dendrites are completely stained in either immunofluorescence or Golgi–Cox material: for example, some bleaching of the finest processes certainly occurred during confocal fluorescence microscopy, and the Golgi–Cox impregnations are likely incomplete. Therefore, the possibility that the most distal axonal and dendritic branches behave differently from the rest cannot be excluded (although there is no reason to assume that this is the case). Third, several Golgi cell phenotypes are known (Illing, 1990; Neki et al., 1996). Indeed, Simat et al. (2007) have recently shown that five distinct subtypes of Golgi cell can be characterized based on their neurotransmitter phenotype and morphology. We cannot be certain that all express neurogranin or the GlyT2-EGFP transgene, and although selective Golgi–Cox staining would seem improbable, we cannot exclude the presence of a small class of Golgi cells that plays a different functional role, for example to straddle compartment boundaries (as in Fig. 1B). Finally, technical limitations, both with immunohistochemistry and Golgi–Cox impregnation, made it impossible to examine whether Golgi cell axons are also restricted to compartments. Golgi cell axons are usually described as forming a dense plexus that extends in the granular layer beyond the dendritic expansion (Palay and Chan-Palay, 1974). Such axons as are impregnated by our Golgi–Cox procedure are close to the somata (Fig. 4), but the fills are incomplete and whether they cross stripe boundaries is uncertain. With these caveats, it is clear that Golgi cell apical dendritic arbors are strongly restricted to the same Purkinje cell stripe that contains their somata (Fig. 1A) [$\sim 3\%$ of apical dendrites were apparent exceptions (Fig. 4C); although these might reflect the selective inhibition of the neighboring stripe (Fig. 1B), we think it more likely that these were artifacts of folial curvature].

Functional implications

What might dendrite restriction imply functionally? If all granule cell input to Golgi cells were derived from parallel fiber synapses, it would seem to make little difference where the

← immunoreactive Golgi cell apical dendrites to zebrin II⁺ (**B**) and to zebrin II[−] (**C**, **D**) stripes. **E**, An example from a transverse section through lobule IX double labeled for HNK-1 (green) and neurogranin (red) showing the restriction of the apical dendrite at the HNK-1^{+/−} boundary. The arrows point to the Golgi cell dendrites at compartment boundaries. ml, Molecular layer; pcl, Purkinje cell layer; gl, granular layer. Scale bars: **A**, 200 μ m; **B–E** (in **D**), 50 μ m.

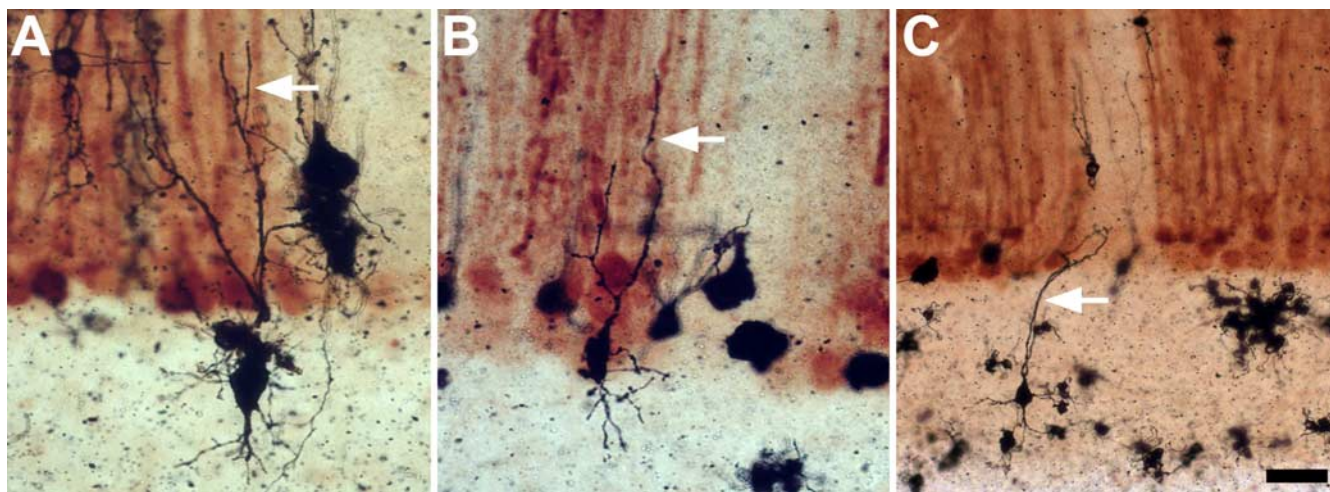


Figure 4. Three examples illustrating the restriction of Golgi–Cox-stained Golgi cells to zebrin II compartment boundaries. *A, B*, The Golgi cell dendrites are restricted to the same zebrin II compartment as the somata. *C*, The dendrites appear to be restricted to a neighboring zebrin II compartment (although the dendrite could not be traced far into the molecular layer). The arrows point to the Golgi cell dendrites at compartment boundaries. Scale bar, 50 μ m.

Golgi cell dendrites terminate, because most parallel fibers extend widely across the molecular layer (Ito, 1984), and thus all regions of the molecular layer are equivalent. However, at least two factors may modify that conclusion. First, there may be an important contribution from the ascending portions of the granule cell axons (Gundappa-Sulur et al., 1999), thereby favoring mossy fiber–granule cell inputs from the mossy fiber pathway that terminate in the stripe. Second, although the same parallel fiber input may be received by all stripes, it may be modulated locally, for example the same mechanisms that induce long-term depression at parallel fiber→Purkinje cell synapses might also at parallel fiber→Golgi cell synapses [especially given evidence for the role of nitric oxide-dependent long-term potentiation at parallel fiber→stellate cell synapses (Rancillac and Crépel, 2003) and at mossy fiber→granule cell synapses (Maffei et al., 2003)]. By this hypothesis, the restriction of Golgi cell dendrites to within a stripe would allow patterns of inhibition to be customized to specific patterns of mossy fiber activity. If the axonal arbors of Golgi cells were similarly restricted, this would imply that Golgi cells feed back on the same mossy fiber pathway by which they are preferentially stimulated. This is similar to the suggestion of Ito (1984) that Golgi cells act as “codon size regulators,” and that a Golgi cell compartment is a functional unit of the mossy fiber input pathway. It is also consistent with the suggestions of Sugihara et al. (1999) that climbing fiber collaterals could terminate on the Golgi cells beneath the Purkinje cell stripe that they innervate. Finally, these data are also consistent with optical imaging results showing that molecular layer inhibition is compartmentalized into zebrin II parasagittal domains that differentially modulate the spatial pattern of cerebellar cortical activity (Gao et al., 2006).

Possible developmental mechanisms

The main conclusion of this study is that the dendrites of cerebellar Golgi interneurons do not cross Purkinje cell compartment

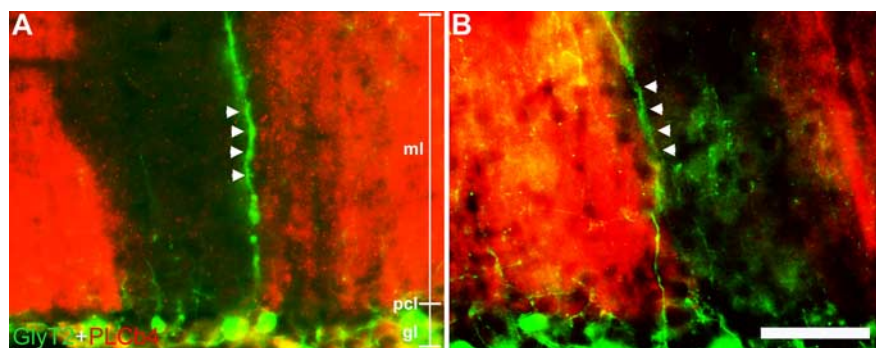


Figure 5. Double immunofluorescence with a GlyT2-EGFP transgene (Golgi cell dendrites) and anti-PLC β 4 (Purkinje cell stripes) reveals that Golgi cell dendrites are restricted at Purkinje cell stripe boundaries. Two examples of GlyT2-EGFP⁺ Golgi cell dendrites (green; arrowheads) in the vicinity of a PLC β 4^{+/−} stripe boundary (red); in 68 cases examined, the dendrite never crossed between stripes. ml, Molecular layer; pcl, Purkinje cell layer; gl, granular layer. Scale bar, 50 μ m.

boundaries. What is the chance that such a result would occur purely by chance? It is not straightforward to estimate how often Golgi cell dendrite restriction would occur randomly. In our sample, the average transverse displacement of the Golgi cell apical dendrite from its soma was $82 \pm 28 \mu$ m (SEM; $n = 40$) in the case in which the dendrite did not cross a boundary, and $90 \pm 41 \mu$ m ($n = 22$) when it did. Therefore, for example, if we take the width of a stripe in lobule VIII (either zebrin II⁺ or zebrin II[−]) as $\sim 180 \mu$ m, and set the lateral extent of a Golgi cell apical dendrite from its soma at 90μ m, then one-half of all Golgi cell apical dendrites would cross a stripe boundary. In the present data, the proportion is $<3\%$. This implies a mechanism for restriction. Restriction might come about because Purkinje cell compartments are separated by neurite-impervious boundaries [similar to the inhibitory boundaries described elsewhere in compartmentalized neural structures: e.g., whisker barrel fields in the somatosensory cortex (Faissner and Steindler, 1995)]. However, no such barriers to growth have been reported in the cerebellum, and neurite restriction at compartment boundaries is not a general feature of the cerebellum because parallel fibers cross parasagittal compartments with impunity. Alternatively, Golgi cell dendritic arbors may be restricted because they develop in concert with the Purkinje cell dendrites (Hekmat et al., 1989; Nagata and Nakatsuji, 1991) [as proposed to explain the topographical

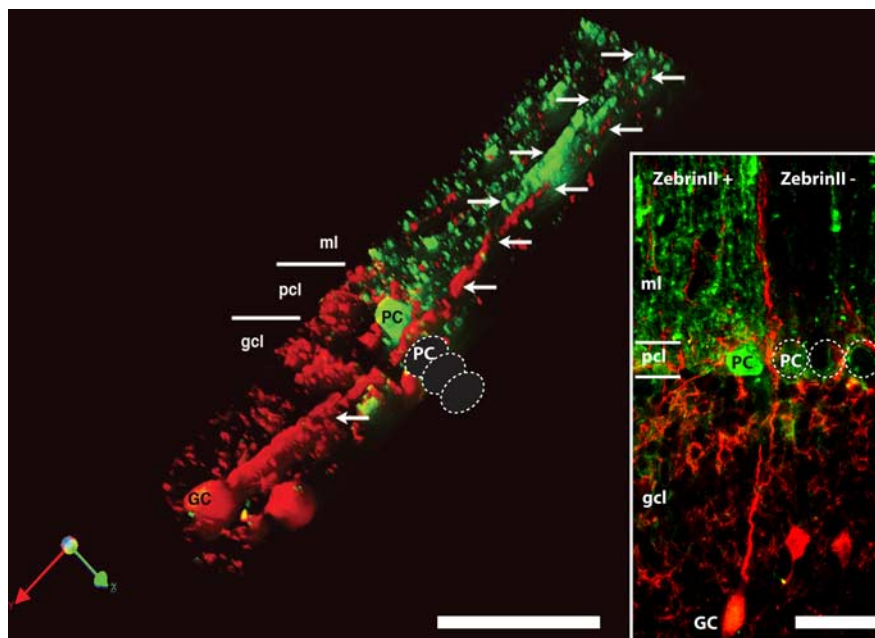


Figure 6. GlyT2-EGFP-expressing Golgi cell dendrites are restricted at zebrin II^{+/−} parasagittal stripe boundaries. Deconvolution analysis of a 200 μ m stained section cut transversely through lobule VIII of the adult GlyT2-EGFP mouse cerebellum and immunofluorescence labeled for zebrin II. The Golgi cell dendrites (red) respect the zebrin II^{+/−} Purkinje cell boundary (green; arrows). In the three-dimensional (3-D) rendered image, the GlyT2-EGFP⁺ Golgi cell dendrite does not invade the zebrin II⁺ stripe. The 3-D image has been rotated to better appreciate the relationship between the dendrites and Purkinje cells. The green arrow (x) represents the longitudinal plane (the arrowhead indicates the right side). The red arrow (y) shows the top and bottom position (the arrowhead indicates the bottom). The inset is a single slice taken from the same experimental slide acquired by normal stack microscopy demonstrating the surface of the tissue; the Golgi cell dendrite can be traced from deep within the granular layer into the molecular layer, where it tracks along the zebrin II^{+/−} border. The positions of the neighboring zebrin II[−] Purkinje cell somata are outlined with dotted lines. ml, Molecular layer; pcl, Purkinje cell layer; gcl, granule cell layer; GC, Golgi cell; PC, Purkinje cell. Scale bars, 50 μ m.

association between mossy fiber terminal fields and specific Purkinje cell stripes (Sotelo and Wassef, 1991; Ji and Hawkes, 1995)]. In such a model, no compartment-specific restrictions are required: the newly born Golgi cells would migrate via the white matter tracts (Zhang and Goldman, 1996) into the embryonic Purkinje cell clusters. There they would affiliate with the local Purkinje cells and extend their dendrites along the nascent Purkinje cell dendrites. Subsequently, as the granule cells begin to innervate the Purkinje cells, the Golgi cell dendrites would be displaced and would synapse with their parallel fiber and ascending axon synaptic partners locally, while retaining their original topographical restriction. Hence, as the Purkinje cell clusters disperse into a monolayer perinatally, individual Golgi cell dendritic arbors would automatically be restricted to one side of a boundary.

References

- Abo T, Balch CM (1981) A differentiation antigen of human NK and K cells identified by a monoclonal antibody. *J Immunol* 127:1024–1029.
- Ahn AH, Dziennis S, Hawkes R, Herrup K (1994) The cloning of zebrin II reveals its identity with aldolase C. *Development* 120:2081–2090.
- Akintunde A, Eisenman LM (1994) External cuneocerebellar projection and Purkinje cell zebrin II bands: a direct comparison of parasagittal banding in the mouse cerebellum. *J Chem Neuroanat* 7:75–86.
- Armstrong CL, Hawkes R (2000) Pattern formation in the cerebellar cortex. *Biochem Cell Biol* 78:551–562.
- 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.
- Chen G, Hanson CL, Ebner TJ (1996) Functional parasagittal compart-

ments in the rat cerebellar cortex: an in vivo optical imaging study using neutral red. *J Neurophysiol* 76:4169–4174.

- 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.
- De Schutter E, Bjaalie JG (2001) Coding in the granular layer of the cerebellum. *Prog Brain Res* 130:279–296.
- 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.
- Ekerot CF, Larson B (1973) Correlation between sagittal projection zones of climbing and mossy fiber paths in cat cerebellar anterior lobe. *Brain Res* 64:446–450.
- Ekerot CF, Larson B (1980) Termination in overlapping sagittal zones in cerebellar anterior lobe of mossy and climbing fiber paths activated from dorsal funiculus. *Exp Brain Res* 38:163–172.
- Faissner A, Steindler D (1995) Boundaries and inhibitory molecules in developing neural tissues. *Glia* 13:233–254.
- Gao W, Chen G, Reinert KC, Ebner TJ (2006) Cerebellar cortical molecular layer inhibition is organized in parasagittal zones. *J Neurosci* 26:8377–8387.
- Gibb R, Kolb B (1998) A method for Vibratome sectioning of Golgi-Cox whole rat brain. *J Neurosci Methods* 79:1–4.
- Glaser EM, Van der Loos H (1981) Analysis of thick brain sections by obverse-reverse computer microscopy: application of a new, high clarity Golgi-Nissl stain. *J Neurosci Methods* 4:117–125.
- Golgi C (1883) Sulla fina anatomia degli organi centrali del sistema nervoso IV. Sulla fina anatomia delle circonvoluzioni cerebellari. *Rivista Sperimentale di Freniatria* 9:1–17.
- Gundappa-Sulur G, De Schutter E, Bower JM (1999) Ascending granule cell axon: an important component of cerebellar cortical circuitry. *J Comp Neurol* 408:580–596.
- Hallem JS, Thompson J, Gundappa-Sulur S, Hawkes R, Bjaallie 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 (1997) An anatomical model of cerebellar modules. *Prog Brain Res* 114:39–52.
- Hawkes R, Eisenman LM (1997) Stripes and zones: the origins of regionalization of the adult cerebellum. *Perspect Dev Neurobiol* 5:95–105.
- Hawkes R, Herrup K (1996) Aldolase C/zebrin II and the regionalization of the cerebellum. *J Mol Neurobiol* 6:147–158.
- Hawkes R, Brochu G, Doré L, Gravel C, Leclerc N (1992) Zebrins: molecular markers of compartmentation in the cerebellum. In: *The cerebellum revisited* (Llinás R, Sotelo C, eds), pp 22–55. New York: Springer.
- Hawkes R, Gallagher E, Ozol K (1997) Blebs in the cerebellar granular layer as a sign of structural inhomogeneity. I. Anterior lobe vermis. *Acta Anatomica* 158:205–214.
- Hawkes R, Gallagher E, Ozol K (1998) Blebs in the cerebellar granular layer as a sign of structural inhomogeneity. II. Posterior lobe vermis. *Acta Anatomica* 163:47–55.
- Hekmat A, Kunemund V, Fischer G, Schachner M (1989) Small inhibitory cerebellar interneurons grow in a perpendicular orientation to granule cell neurites in culture. *Neuron* 2:1113–1122.
- Herrup K, Kuemerle B (1997) The compartmentalization of the cerebellum. *Annu Rev Neurosci* 20:61–90.

- Illing R-B (1990) A subtype of cerebellar Golgi cells may be cholinergic. *Brain Res* 522:267–274.
- Ito M (1984) *The cerebellum and neural control*. New York: Raven.
- Ji Z, Hawkes R (1994) Topography of Purkinje cell compartments and mossy fiber terminal fields in lobules II and III of the rat cerebellar cortex: spinocerebellar and cuneocerebellar projections. *Neuroscience* 61:935–954.
- Ji Z, Hawkes R (1995) Developing mossy fiber terminal fields in the rat cerebellar cortex may segregate because of Purkinje cell compartmentation and not competition. *J Comp Neurol* 359:197–212.
- Jiang H, Lyubarsky A, Dodd R, Vardi N, Pugh E, Baylor D, Simon MI, Wu D (1996) Phospholipase C β 4 is involved in modulating the visual response in mice. *Proc Natl Acad Sci USA* 93:14598–14601.
- Larouche M, Che P, Hawkes R (2006) Neurogranin expression identifies a novel array of Purkinje cell parasagittal stripes during mouse cerebellar development. *J Comp Neurol* 494:215–227.
- Maffei A, Prestori F, Shibuki K, Rossi P, Taglietti V, D'Angelo E (2003) NO enhances presynaptic currents during cerebellar mossy fiber-granule cell LTP. *J Neurophysiol* 90:2478–2483.
- 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.
- Marzban H, Chung S, Watanabe M, Hawkes R (2007) Phospholipase C β 4 expression reveals the continuity of cerebellar topography through development. *J Comp Neurol* 502:857–871.
- Matsushita M, Ragnarson B, Grant G (1991) Topographic relationship between sagittal Purkinje cell bands revealed by monoclonal antibody to zebrin I and spinocerebellar projections arising from the central cervical nucleus in the rat. *Exp Brain Res* 84:133–141.
- Nagata I, Nakatsuji N (1991) Rodent CNS neuroblasts exhibit both perpendicular and parallel contact guidance on the aligned parallel neurite bundle. *Development* 112:581–590.
- Nakamura M, Sato K, Fukaya M, Araiishi K, Aiba A, Kano M, Watanabe M (2004) Signaling complex formation of phospholipase C β 4 with metabotropic glutamate receptor type 1 α and 1,4,5-triphosphate receptor at the perisynapse and endoplasmic reticulum in the mouse brain. *Eur J Neurosci* 20:2929–2944.
- Neki A, Ohishi H, Kaneko T, Shigemoto R, Nakanishi S, Mizuno N (1996) Metabotropic glutamate receptors mGluR2 and mGluR5 are expressed in two non-overlapping populations of Golgi cells in the rat cerebellum. *Neuroscience* 75:815–826.
- Oberdick J, Baader SL, Schilling K (1998) From zebra stripes to postal zones: deciphering patterns of gene expression in the cerebellum. *Trends Neurosci* 21:383–390.
- Ozol K, Hayden JM, Oberdick J, Hawkes R (1999) Transverse zones in the vermis of the mouse cerebellum. *J Comp Neurol* 412:95–111.
- Ozol KO, Hawkes R (1997) The compartmentation of the granular layer of the cerebellum. *Histol Histopathol* 12:171–184.
- Palay SL, Chan-Palay V (1974) *Cerebellar cortex: cytology and organization*. Berlin: Springer.
- 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.
- Rancillac A, Cr  pel F (2003) Synapses between parallel fibres and stellate cells express long-term changes in synaptic efficacy in rat cerebellum. *J Physiol (Lond)* 554:707–720.
- Sarna JR, Marzban H, Watanabe M, Hawkes R (2006) Complementary stripes of phospholipase C β 3 and C β 4 expression by Purkinje cell subsets in the mouse cerebellum. *J Comp Neurol* 496:303–313.
- 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, Benson MA, Blake DJ, Hawkes R (2003) Abnormal dysbindin expression in cerebellar mossy fiber synapses in the mdx mouse model of Duchenne muscular dystrophy. *J Neurosci* 23:6576–6585.
- Simat M, Parpan F, Fritschy JM (2007) Heterogeneity of glycinergic and gabaergic interneurons in the granule cell layer of mouse cerebellum. *J Comp Neurol* 500:71–83.
- Singec I, Knott R, Ditter M, Frotscher M, Volk B (2003) Neurogranin expression by cerebellar neurons in rodents and non-human primates. *J Comp Neurol* 459:278–289.
- Sotelo C, Wassef M (1991) Cerebellar development: afferent organization and Purkinje cell heterogeneity. *Phil Trans R Soc Lond B* 331:307–313.
- Sugihara I, Wu H, Shinoda Y (1999) Morphology of single olivocerebellar axons labeled with biotinylated dextran amine in the rat. *J Comp Neurol* 414:131–148.
- Voogd J, Jaarsma D, Marani E (1996) The cerebellum: chemoarchitecture and anatomy. In: *Handbook of chemical neuroanatomy* (Swanson L, Bj  rklund A, H  kfelt T, eds), pp 1–369. Amsterdam: Elsevier Science.
- 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.
- Watson JB, Battenberg EF, Wong KK, Bloom FE, Sutcliffe JG (1990) Subtractive cDNA cloning of RC3, a rodent cortex-enriched mRNA encoding a novel 78 residue protein. *J Neurosci Res* 26:397–408.
- Welker W (1987) Spatial organization of somatosensory projections to granule cell cerebellar cortex: functional and connectional implications of fractured somatotopy (summary of Wisconsin studies). In: *New concepts in cerebellar neurobiology* (King JS, ed), pp 239–280. New York: Alan R. Liss.
- Zeilhofer HU, Studler B, Arabadzisz D, Schweizer C, Ahmadi S, Layh B, Bosl MR, Fritschy JM (2005) Glycinergic neurons expressing enhanced green fluorescent protein in bacterial artificial chromosome transgenic mice. *J Comp Neurol* 482:123–141.
- Zhang L, Goldman JE (1996) Generation of cerebellar interneurons from dividing progenitors in white matter. *Neuron* 16:47–54.