Cerebellar cortical organization: a one-map hypothesis

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Abstract | The fundamental architecture of the cerebellum is concealed within a terminological forest — transverse zones and stripes, longitudinal zones and microzones, patches, etc. To make things worse, the same term is used in different contexts to describe quite different patterns of spatial localization. Here we consider the possibility that this complexity hides the fact that the cerebellar cortex contains only one map, which has been charted in various ways.

Inferior olivary complex

Collection of subnuclei located in the ventral medulla oblongata which are the sole source of climbing fibre afferents to the cerebellum.

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A systematic spatial representation of anatomical pathways, physiological activity and/or molecular features projected onto the cerebellar cortical surface.

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Correspondence to R.A. e-mail: <u>r.apps@bristol.ac.uk</u> doi:10.1038/nrn2698 The cerebellum has two separate parts — the cerebellar cortex, built around the Purkinje cells and the focus of this Review, and the cerebellar nuclei, including some vestibular nuclei. The cortex can be thought of as a sheet of Purkinje cells (~160,000 in mice) and each Purkinje cell is influenced by two major types of input — direct action of climbing fibres from the inferior olivary complex, and indirect action of mossy fibres (from a range of sources throughout the CNS) via granule-cell-parallel fibres and cortical interneurons. In turn, Purkinje cell axons are the sole output of the cerebellar cortex and these target the cerebellar nuclei. Anatomical, molecular and physiological approaches have been used to map the organization of these connections. As a consequence, at least three different viewpoints have emerged of cerebellar cortical topography, based on the anatomy and physiology of climbing fibre inputs and Purkinje cell outputs, the physiology of mossy fibre inputs, restriction boundaries in gene expression and regional differences in Purkinje cell phenotype.

Each of these different experimental approaches yields a 'map' of the cerebellar cortex with its own nomenclature (TABLE 1). This could imply that each map is independent of the others, but, as discussed later, this is not necessarily the case, and the relationship between the different maps is becoming clearer. This is not just a matter of semantics as progress in understanding cerebellar function will require experiments involving multiple techniques applied to the same (accurately identified) parts of the cerebellar cortex in a range of species.

In this Review we consider a number of the more commonly used terms to describe cerebellar cortical organization with a view to providing some clarification. Overarching this, we present the case for a 'onemap hypothesis' in which the different terminologies are in fact unnecessary because studies in both developing and adult animals suggest that the different mapping techniques — anatomical, physiological and molecular — reveal different facets of a common topography. The architecture that emerges is highly reproducible, conserved through evolution, involves all features of the cerebellum and shows an extraordinary level of resolution, with topography in mouse specified down to fewer than a hundred Purkinje cells.

We start with the Purkinje cells and review evidence that these encode an intricate topography — an array of 'transverse zones', each subdivided into 'stripes'. Although it goes beyond the scope of this short Review, inhibitory interneurons of the cerebellar cortex also show restriction within the same cerebellar map (for example, see REF. 1). Next, we argue that the Purkinje cell array is the scaffold that guides the distributions of climbing fibre and mossy fibre afferents during development to generate 'longitudinal zones'. Finally, we review evidence that both the climbing fibre and mossy fibre afferent systems and Purkinje cell stripes have a common organization.

Macroscopic division of the cerebellum

The best-established terminology partitions the cerebellum on each side of the midline into three longitudinal regions that run along the rostral to caudal plane: the vermis (medial cerebellum), the paravermis (intermediate cerebellum or pars intermedia) and the hemisphere (lateral cerebellum)^{2,3} (FIG. 1). Each of these regions is folded into lobules (and each lobule is subdivided into folia, not shown in FIG. 1). For non-human studies there are two cerebellar nomenclatures for lobules. In some cases the names are interchangeable (for example, lobule I of Larsell^{4,5} is the same as the lingula, and lobule VI is the same as the declive), whereas in other instances the relationship is more complicated (for example, lobules IV and V are the same as the culmen, and lobule VII can

Aldolase C

A brain-specific glycolytic isoenzyme that converts p-fructose 1,6-bisphosphate into glycerone phosphate and p-glyceraldehyde 3-phosphate. be interchanged with folium plus tuber). The two terminologies also conceal a fundamental disagreement regarding the relation of the vermis to the hemispheres (for further details see REF. 6).

The relationship between lobulation and the underlying molecular topography is complex. On the one hand, although lobulation is under strong genetic control7 and is clearly conserved across species8, transverse zone boundaries defined by expression patterns (see below) do not correspond to groups of lobules9; furthermore, in mutant animals¹⁰ the expression boundaries can shift with respect to lobulation. On the other hand, defective Purkinje cell migration in embryogenesis¹⁰⁻¹³ leads to abnormal lobulation, which indicates a developmental link between the formation of Purkinje cell stripes and lobules. Finally, it is not clear that lobules are functional units. Indeed, electrophysiological mapping in a range of species suggest that the fissures between lobules are not consistent boundaries between functionally identified subregions^{14,15} (although correlations have been found in some cases - for example, lobules VIc and VII in primates correspond to the oculomotor vermis¹⁶). Although it is clear that lobulation provides useful anatomical landmarks, we think it is mainly the cerebellum's solution to the problem of packing a large surface area into a small space.

Transverse zones

Although anatomical, physiological and behavioural studies have emphasised a longitudinal organization within the cerebellar cortex (see later), developmental studies^{17,18} suggest that the development of the fundamental cerebellar architecture begins with the subdivision of the cerebellar cortex into five (or six in birds) transverse zones¹⁷ (FIG. 2). Based on patterns of gene expression (see Supplementary information S1 (table)) these transverse zones are: the anterior zone (AZ: ~lobules I-V in mouse); the central zone (CZ), further divided into the anterior CZa (~lobule VI) and the posterior CZp (~lobule VII^{19,20}); the posterior zone (PZ: ~lobules VIII to dorsal IX); and the nodular zone (NZ: ~ventral lobule IX and lobule X) (for examples, see REFS 9,21). Transverse zones overlap extensively. For example, the interdigitation of AZ and CZ in the mouse occupies most of the caudal face of the primary fissure (lobules V and VI17). Thus, on each side

of the midline there are at least five transverse zones in the vermis with matching zones in the hemisphere, each comprising, in mouse, fewer than 10,000 Purkinje cells.

The analysis of transverse zones as functional groupings is still in its infancy, but presumably they underpin to some extent the traditional compartmentalization of the cerebellum (that is, into the vestibulocerebellum, spinocerebellum and pontocerebellum⁶, although, with the exception of the vestibulocerebellum, we consider this terminology misleading and best avoided, see the legend in FIG. 1).

Stripes

Every transverse zone is subdivided into a series of stripes (or 'bands') oriented along the rostrocaudal axis and which are defined by the restricted expression of molecular markers (FIG. 2). The most comprehensively studied molecular marker is zebrin II²², which cloning studies revealed to be the metabolic enzyme aldolase C^{23,24}. Zebrin II is expressed by a subset of Purkinje cells (zebrin II+) that alternate with Purkinje cells that do not express this marker (zebrin II-), thus forming zebrin II+/- stripes (FIG. 2). The zebrin II+/stripes are symmetrically distributed across the midline, highly reproducible between individuals^{22,25,26} and conserved across species (reviewed in REF. 27). Since the first stripe marker was identified over 40 years ago (5'-nucleotidase)²⁸ numerous other markers have been identified. Those co-expressed with zebrin II include zebrin I^{25,26} sphingosine kinase 1a (SPHK1a)²⁹, the CDK5 P39 activator³⁰, phospholipase CB3 (REF. 31), excitatory amino-acid transporter 4 (EAAT4)³², metabotropic glutamate receptor 1a (mGluR1a; also known as GRM1A)³³, integrin β 1 (REF. 34) and the $GABA_{B2}$ receptor³⁵. There are also positive markers of zebrin II-Purkinje cells (for example, phospholipase $C\beta4$ (REF. 31); neuroplastin³⁶; EBF2 (REF. 37)).

The stripe pattern of Purkinje cells differs between transverse zones. Thus, the AZ and PZ have alternating zebrin II+/- stripes, whereas the CZ and NZ are uniformly zebrin II+. Stripes are discontinuous across transverse zone boundaries, so it is wrong to assume, for example, that the first zebrin II+ stripe from the midline in the AZ is the same as the P1+ stripe in the NZ (for stripe terminology, see REE 21).

Table 1 Different cerebellar nomenclatures					
Topographical unit	Description	Experimental basis			
Longitudinal zone	A rostrocaudally extended array of Purkinje cells within the cerebellar cortex with specific olivocerebellar and cortico-nuclear connections and climbing fibre input relayed through a common set of olivocerebellar pathways	Anatomy and physiology			
Module	A longitudinal zone of Purkinje cells together with its olivo-cortico-nuclear connections and associated recurrent pathways	Anatomy			
Microzone	Subdivision of a longitudinal zone in which Purkinje cells have similar climbing fibre receptive fields	Physiology			
Transverse zone	Region of cerebellar cortex identified by mediolateral gene expression boundaries	Gene expression patterns			
Purkinje cell stripe/ band	Longitudinally oriented subregion of a transverse zone in which Purkinje cells have the same phenotype	Gene expression patterns			
Patch	Region of cerebellar cortex with similar mossy fibre receptive fields in the granular layer	Physiology			

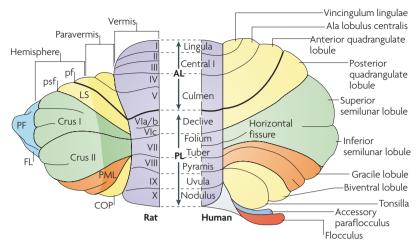


Figure 1 | Gross morphology of the cerebellum. Dorsal view of the rat cerebellum to the left and simplified dorsal view of the human cerebellum to the right to indicate the comparative nomenclature. Equivalent regions in the rat and human cerebellum are given the same colour. On the left the three longitudinal compartments are indicated (the vermis, the paravermis and the hemisphere). On the left, lobules in the vermis are numbered according to Larsell's schema, whereas on the right, the corresponding nomenclature as described by Bolk is shown. For both views the primary fissure (pf) dividing the anterior and posterior lobes is highlighted in bold. For the large-scale division of the cerebellum, student textbooks also still frequently use the terms archicerebellum, paleocerebellum and neocerebellum (also known, respectively, as spinocerebellum, vestibulocerebellum and pontocerebellum). However, in our view this terminology is confusing. Although the 'vestibulocerebellum' is justly named because of its major connections with the vestibular system, the terms 'spinocerebellum' and 'pontocerebellum' are misleading and best avoided as they imply that the relevant regions of cerebellar cortex only receive these particular types of input, which is certainly not the case (see for example REFS 15,143–145). AL, anterior lobe; COP, copula pyramidis; Crus I and Crus II, ansiform lobule; FL, flocculus; LS, lobulus simplex; PF, paraflocculus; PL, posterior lobe; PML, paramedian lobule; psf, posterior superior fissure. Figure is modified, with permission, from REF. 6 © (1998) Elsevier. Additional information is derived from REF. 4.

The expression of Zebrin II does not, however, reveal the full complexity of the cerebellar cortex there are more Purkinje cell subtypes than simply those based on expression of zebrin II. First, several markers reveal stripes in the transverse zones that are uniformly zebrin II+. For example, heat shock protein 25 (HSP25) and human natural killer antigen 1 (HNK1) are expressed by subsets of the zebrin II+ population of the CZ and NZ^{9,38}. Secondly, individual zebrin II+/- stripes can be further subdivided. For example, the expression of phospholipase Cβ4 reveals that the first and second zebrin II- stripes from the midline (P1- and P2-) of the AZ in mice each comprise three embryonic sub-stripes, which fuse during development³⁹ (see also FIG. 3), and expression of an L7/pcp2-lacZ transgene in the AZ is restricted selectively to the medial subdivision of P2-(REF. 17). Thus, all transverse zones are divided into Purkinje cell stripes but the boundaries of the stripes depend on the marker that is used to visualize them. So far, no single molecular marker has been found that visualizes all stripes.

When both multiple transverse zones and multiple stripes per zone are considered, several hundred anatomical units are present in the cerebellar cortex. In mice, a typical stripe therefore comprises only a few hundred Purkinje cells.

What does development tell us?

Embryology casts an informative light on the adult cerebellar map (FIG. 3). Purkinje cells are the first neurons of the cerebellar cortex to be born (in mouse, for example, they develop on embryonic day 10 (E10)-E13 (REF. 40)). Postmitotic Purkinje cells migrate from the fourth ventricle to form a stereotyped array of clusters⁴¹⁻⁴³. At the time of Purkinje cell birth the transverse zone and stripe topography is already established⁴⁴. Whether the molecular identity of each stripe is determined at this time is unclear³⁷, but by the time a mouse is born the molecular identity of individual Purkinje cells seems to be set and independent of cerebellar connectivity⁴². Because the identity of Purkinje cells is established early in development the stripe pattern is highly resistant to experimental manipulation^{9,37,45-48}. For instance, in some mouse mutants, Purkinje cells are ectopic in adults, which results in an abnormal stripe pattern, but in all cases the organization of the transverse zone and stripes is still present and the molecular phenotypes of the ectopic Purkinje cells are normal⁴⁹⁻⁵².

The embryonic Purkinie cell clusters are the targets of the developing climbing fibres and mossy fibres. During this matching, it is believed that the Purkinje cells form a template around which afferent topography is constructed^{53–55}. The embryonic Purkinje cell clusters disperse postnatally primarily along the rostrocaudal axis to form stripes, mediated by reelin signalling^{11-13,56}. Sotelo in particular has argued persuasively that afferent topography is secondary to Purkinje cell architecture⁵⁷. The development of Purkinje cell stripes and climbing fibre terminal fields is dominated by direct chemospecific interactions (a phenomenon known as the 'matching hypothesis'58), in which Eph receptors and Ephrins play a prominent part^{59,60}. Activity dependent processes have a role in refining the projection but not in establishing the striped topography⁶¹.

A close alignment between Purkinje cell stripes and climbing fibre terminal fields is easily understood because there is a direct synaptic connection. For the mossy fibre afferents this is less obvious, because they synapse on granule cells rather than Purkinje cells. However, Purkinje cell clusters seem to be the organizers in this case as well. First, rudimentary mossy fibre topography is established before most granule cells are formed⁶², and is preceded by transient, possibly functional contact between mossy fibre afferents and Purkinje cells^{63,64} in specific embryonic clusters^{65,66}. As the embryonic clusters disperse into stripes the afferent terminals seem to move with them, thereby establishing the adult connectivity. During postnatal development, mossy fibres displace to the granular layer as the granule cells descend from the external granular layer, but they retain their fundamental topographical relationships with the Purkinje cells^{62,67}. Second, observations from mutant animals with agranular cerebella show that mossy fibre topography forms independently of synaptogenesis⁶⁷ (the peak of synaptogenesis in the granular layer is during the second and third postnatal weeks^{68,69}, by which time the topography is mature), and is organized into stripes despite the absence of a normal mossy-fibre–granule-cell–Purkinje-cell pathway^{70,71}. Third, mutant mice in which Purkinje cells are ectopic and in which the mutation directly affects migration of Purkinje cells (for example, reeler mutant mice^{72–74}) have mossy fibre afferents that terminate on the ectopic Purkinje cell clusters. Fourth, a study of mossy fibre projections in mice in which <u>engrailed 2</u> was ectopically expressed under the control of the *L7/pcp2* promoter indicated that altering the architecture of Purkinje cells led to a corresponding reorganization of mossy fibre afferents⁷⁵. Thus, Purkinje cells have a key role in guiding the spatial distribution of mossy fibre terminals. The data do not support a role for competition between mossy fibres in the establishment of afferent topography, but this does not rule out more subtle secondary, activity dependent refinement within the larger topographical units, perhaps as the motor system matures (such as from stripes to patches: for example, see REFS 76,77; although this is refuted by some, see REF. 67).

In summary, developmental studies of cerebellar topography suggest that there is a single map, built on a scaffold of Purkinje cell architecture. But, is this true?

Longitudinal zones

Numerous pathway tracing studies have revealed 'longitudinal zones' within the cerebellar cortex: narrow, rostrocaudally elongated regions that run perpendicular to the long axis of the lobules^{3,78–80} (FIG. 4). Longitudinal zones were originally defined by their topographically

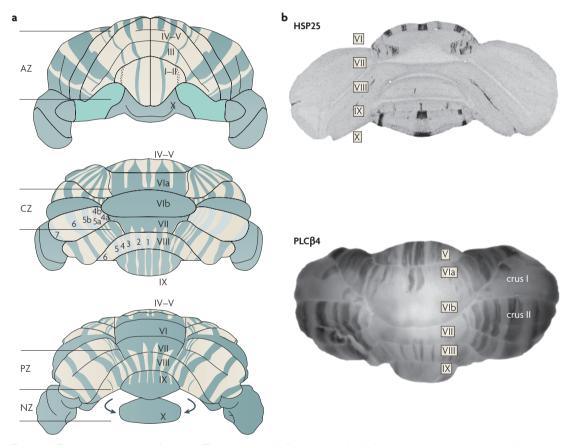


Figure 2 | Transverse zones and stripes. The mouse cerebellar cortex is divided into transverse zones, and each zone is subdivided into parasagittal stripes. Zones and stripes are clearly seen in the expression patterns of multiple Purkinje cell antigens. a | The distribution of Purkinje cells that are immunoreactive to zebrin II in the cerebellum of the adult mouse, seen from anterior (top panel), dorsal (middle panel) and posterior (bottom panel) views²¹. The expression of zebrin II reveals a complex cytoarchitecture comprising four transverse zones in the vermis in both hemispheres: the striped anterior zone (AZ) and the posterior zone (PZ) alternating with the uniformly zebrin II immunopositive central zone (CZ) and nodular zone (NZ; partly reflected out from beneath lobule IX). Zebrin II+ stripes of Purkinje cells are referred to as P1+ to P7+ (numbered in the figure as 1-7 for clarity) from the midline laterally, and the intervening zebrin II- stripes (beige in the figure) are numbered with reference to the neighbouring (medial) zebrin II+ stripe (that is, P1-lies immediately lateral to P1+ etc.^{21,22}). Lobules in the vermis are indicated by Roman numerals. b | Two specific examples of stripes. In the top panel, heat shock protein 25 (HSP25) immunocytochemistry on a transverse section through the mouse cerebellum reveals Purkinje cell stripes in the vermis that are restricted to lobule VI (part of the CZ) and lobules IX/X (the NZ). In the bottom panel, immunocytochemical staining for PLCB4 reveals stripes of Purkinje cells in the AZ (for example, lobule V) and PZ (for example, lobule VIII) but not in the CZ (for example, lobule VIb) or NZ (for example, lobule IX). Part a reproduced, with permission, from REF. 21 © (2002) Histochemical Society. The top panel of part b is reproduced, with permission, from REF. 9 © (2000) Wiley-Liss. The bottom panel of part **b** is reproduced, with permission, from REF. 31 © (2006) Wiley-Liss.

organized Purkinje cell output to different territories within the cerebellar and vestibular nuclei (the socalled A, B, C1, C2, C3, D1 and D2 longitudinal zones of Voogd; for a review of the earlier literature see REF. 2); additional longitudinal zones have subsequently been added, for example, X, CX and D0 (FIG. 4). A longitudinal zonal arrangement also holds true for the anatomy of olivocerebellar climbing fibre projections⁸¹ and for the pattern of termination of physiologically characterised spino-olivocerebellar pathways (as described originally by Oscarsson and co-workers⁸²). In general, the maps of olivocerebellar connections defined by anatomical and physiological methods coincide, for example, see REF. 83. For this reason the use of upper and lower case letters to distinguish between anatomically and physiologically defined longitudinal zones has largely been discontinued. The exception is the distinction between the physiologically defined d2 zone and the anatomically defined D2 zone, as they receive their climbing fibre input from the dorsal accessory olive and the principal olive, respectively⁸⁴. To overcome this problem the physiologically defined zone has been renamed Y85.

Anatomical tract tracing has also been used to describe the way in which olivocerebellar (climbing fibre)

afferents and corticonuclear (Purkinje cell) efferents are linked to form discrete complexes, the cortical component of each of these 'modules' being a longitudinal zone of Purkinje cells^{2,79}. Anatomically defined modules have been extended to include other connections, notably the nucleo-olivary and reciprocal olivo-nuclear projections⁸⁶. In the same way that Purkinje cell stripes are evolutionarily conserved, cerebellar modules are remarkably similar in a range of species (see Supplementary information S1 (table)). The behavioural significance of these modules is beyond the scope of this Review but their conservation implies that each subserves a similar function in different species (for example, regulation of spinal reflexes and limb movements by the paravermal modules⁸⁷; and control of compensatory eye movements by modules in the flocculus⁸⁸).

Some longitudinal zones can be further split into smaller units called 'microzones'^{82,89}. In particular, highresolution electrophysiological mapping from medial to lateral across the width of the vermal B zone or the paravermal C3 zone shows small groups of Purkinje cells with distinct climbing fibre receptive fields that arise from different body parts. Each Purkinje cell group typically occupies a narrow, rostrocaudally oriented strip

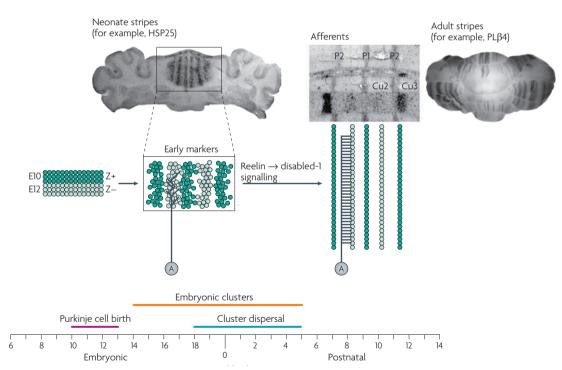


Figure 3 | **The development of Purkinje cell stripes in mice.** Purkinje cells are born between embryonic day 10 (E10) and E13. The zebrin II expressing (Z+) Purkinje cells are born earliest. They migrate from the fourth ventricle into the cerebellar anlage, where they form a reproducible array of clusters with distinct molecular phenotypes (detectable from E14–PND5 (postnatal day 5)), revealed by 'early' markers such as L7/pcp2-lacZ (REF. 48); cadherins¹⁴⁶; neurogranin¹⁴⁷; heat shock protein 25 (HSP25 — shown at PND3 (REF. 148)). Clusters are the targets of ingrowing climbing fibre and mossy fibre afferents (A). The clusters disperse between E18 and PND5 under the influence of reelin-to-disabled-1 signalling to form the adult stripes (for example, phospolipase C β 4 (PLC β 4)+/– (REF. 31) etc.). In many cases, a direct link can be made between specific clusters in the embryo and specific stripes in the adult as revealed by late markers (for example, through PLC β 4 expression³⁹). The afferent climbing fibres and mossy fibre disperse along with their Purkinje cell targets, and thus remain in register (see cuneocerebellar (Cu) mossy fibre projections to and Zebrin II+/– Purkinje cell stripes in the AZ). Top left image is reproduced, with permission, from REF. 67 © (1995) Wiley-Liss. Top right image is reproduced, with permission, from REF. 61 © (2006) Wiley-Liss.

of cortex within the broader longitudinal zone (each zone \sim 1 mm and each microzone \sim 100–300 µm wide). Microzones mapped in the vermal B zone form a regular array of olivo-cortico-nuclear 'microcomplexes' that are thought to control different aspects of the motor functions handled by the broader module⁸². However, the most extensively studied microzones lie within a small part of the C3 longitudinal zone in the paravermis of lobule V in cats, and their organization seems to be rather different⁸⁹. Whereas individual microzones in the B zone are thought to extend the entire rostrocaudal length of the zone (more than 100 mm in cat)⁸², microzones in C3 tend to be much shorter, usually extending across no more than a few adjacent cerebellar folia. In addition, microzones located in different parts of the paravermal cortex can have the same climbing fibre receptive field characteristics (for example, there are at least four separate 'eyeblink' microzones in each paravermis90). This has led to the concept that spatially separated collections of microzones with common climbing fibre input — termed 'multizonal microcomplexes' — may be important for the parallel processing and integration of information from mossy fibre inputs derived from multiple sources^{3,89}. Some anatomical data are consistent with such a possibility (for example, see REF. 91), but physiological studies to fully test this hypothesis are currently lacking.

Anatomical tracer studies have also revealed a correspondingly detailed map within the inferior olive, with subgroups of olivary cells providing climbing fibres to different parts of the same longitudinal zone (for example, see REF. 92 and FIG. 4). In some cases the resolution of the anatomical mapping has been sufficient to reveal this connectivity at a level that might correspond to microzones^{93,94}. Longitudinal zones are therefore most probably composite entities, and the basic operational unit of the cerebellar cortex is narrower, possibly an individual microzone or, in the case of the paravermis, an assembly of microzones forming a multizonal microcomplex³.

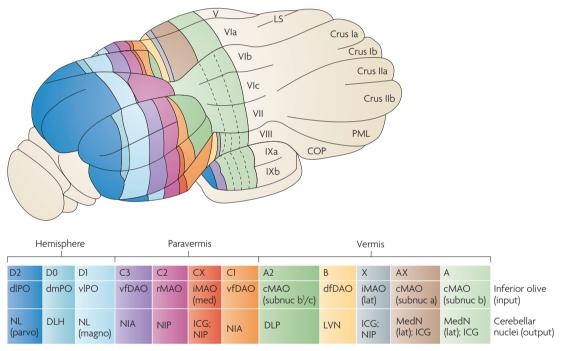


Figure 4 | Longitudinal zones. Dorso-posterior view of the rat cerebellum, indicating the approximate location of different longitudinal zones on the cerebellar surface on the left hand side. Each longitudinal zone is defined by its inferior olive climbing fibre input and Purkinje corticonuclear output. From the medial to the lateral plane (right to left in the figure) are shown: the A, AX, X, B and A2 zones (in the vermis), the C1, CX, C2 and C3 zones (in the paravermis), and the D1, D0 and D2 zones (in the hemisphere). Longitudinal zones in the paraflocculus and flocculus are not shown. In the simplified block diagrams below, matching colours show, for individual cerebellar cortical zones, the sites of origin of climbing fibres in the contralateral inferior olive, and the corresponding Purkinje cell corticonuclear output targets in the ipsilateral cerebellar and vestibular nuclei. Note that some longitudinal zones are not necessarily present in all cerebellar lobules in the adult animal (for example, the X and B zones). Figure is based on data from REFS 14,79,80,94,97,149,150. cMAO (subnuc a), subnucleus a of caudal medial accessory olive; cMAO (subnuc b), subnucleus b of caudal medial accessory olive; cMAO (subnuc b¹/c), subnucleus b¹ and c of caudal medial accessory olive; COP, copula pyramidis; dfDAO, dorsal fold of dorsal accessory olive; DLH, dorsolateral hump; DLP, dorsolateral protuberance of medial nucleus; dIPO, dorsal lamella of the principal olive; dmPO, dorsomedial subnucleus of the principal olive; ICG, interstitial cell group; iMAO (lat), lateral part of intermediate medial accessory olive; iMAO (med), medial part of intermediate medial accessory olive; LVN, lateral vestibular nucleus; LS, lobulus simplex; MedN (lat), lateral part of medial nucleus; MedN (med), medial part of medial nucleus; NIA, nucleus interpositus anterior; NIP, nucleus interpositus posterior; NL (magno), magnocellular part of lateral nucleus. NL (parvo), parvocellular part of lateral nucleus; PML, paramedian lobule; rMAO, rostral medial accessory olive; vfDAO, ventral fold of dorsal accessory olive; vlPO, ventral lamella of the principal olive.

Table 2 Relationship between longitudinal zones and Purkinje cell stripes.												
	Hemi	sphere		Parav	ermis			Vermis				
Longitudinal zone	D2	D0	D1	C3	C2	CX	C1	A2	В	Х	AX	А
Anterior Lobe: Purkinje cell stripes in the AZ	P6+	P5-	P5+	P4-	P4+	P3-*	P3+/- b+/-	P2b– c+/– d +/–	P2-	P2-*	P2+	P1+/- a+/-
Posterior lobe: Purkinje cell stripes in the PZ	P7+	P6-	P6+	P5-	P5+	e2-	P4– e+/– P4b–	P4a– P4b+/– P5a+/–	P4	P3-	P3+	P1+/- P2+/-

Table 2 Relationship between	longitudinal	zones and Purki	nje cell stripes.
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*P3- in lobules IV-V. Authors from REF. 94 named this band P3b- to distinguish it from P3-, ‡P2- in lobules V-VI. Authors from REF. 94 named this band P2a- to distinguish it from P2-. AZ, anterior transverse zone; PZ, posterior transverse zone. Data derived from REFS 80,94,97.

The relationship between longitudinal zones and molecular stripe markers has revealed extensive co-localization of longitudinal zones and stripes of zebrin II+/- Purkinje cells^{54,80,95-98} (TABLE 2). For example, the C2 zone in the PZ co-localizes with the P5+ stripe97. However, because both zebrin II+/- stripes and longitudinal zones are often composite structures the exact relationships can be more complex; in some cases, an apparently homogeneous longitudinal zone may co-localize with several zebrin II+/- stripes. For instance, the A2 zone in the PZ comprises the P4a-, P4b+/- and P5a+/- stripes94,97. In other cases, individual longitudinal zones can subdivide an individual zebrin II stripe (for example, the X and B zones in the AZ subdivide P2- (REF. 97); P2- is also shown to be composite in developmental studies³⁹). Nevertheless, the general observation is that longitudinal zones and Purkinje cell stripes are aligned, consistent with the one-map hypothesis.

The mossy fibre projection

The pattern of termination in the cerebellar cortex has been charted for mossy fibres from a range of different sources (for example, those arising from the spinal cord^{96,99}, pontine¹⁰⁰, cuneate^{96,101} and lateral reticular nuclei¹⁰²). Mossy fibres tend to terminate bilaterally, forming multiple, longitudinally oriented stripes. These mossy fibre terminal field stripes are usually most prominent in the vermis, but they are generally broader and less well defined than longitudinal zones¹⁰², although a degree of correspondence has been noted (described later and in REFS 99,103). Climbing fibres arising from a specific subnucleus of the olive are mainly directed to one or two longitudinal zones^{85,104}, but mossy fibres from a given source display more extensive transverse branching and diverge to terminate in multiple longitudinal zones. Also, studies of pontocerebellar projections suggest that mossy fibre terminations are in register with regions of the cerebellar cortex that are defined by their climbing fibre input from different body parts rather than with longitudinal zones per se^{105,106}. On the other hand, mossy fibre terminal fields in the granular layers of the vermis have a reproducible relationship with the overlying Purkinje cell zebrin II stripes⁹⁶. In some cases the boundaries align precisely, but in other cases mossy fibre terminal fields split Purkinje cell stripes into narrower

sub-stripes (for example, cuneocerebellar and spinocerebellar projections to the AZ⁹⁶). Importantly, however, there is no anatomical evidence that mossy fibre terminal fields straddle or ignore a Purkinje cell stripe boundary.

In the crowns of folia in the posterior hemispheres the term 'patch' has also been used to describe tactile mossy fibre inputs to the granular layer (FIG. 5). Patches are synonymous with the 'fractured somatotopy' of body part representations first described by Welker and colleagues¹⁰⁷ and these can vary considerably in size and shape (FIG. 5). This interpretation of map organization is consistent with tactile receptive field maps in which functionally identified patches both closely correspond to the zebrin II labelling patterns (for example, in rat lobule IXa¹⁰⁸ or crus IIa¹⁰⁹; see also REFS 110,111), and reveal discontinuities that point towards the map being patchy rather than striped. Indeed, a 'patchy' interpretation of mossy fibre terminal fields is supported by patchy granular layer expression patterns seen throughout the cerebellar cortex (for example, acetylcholinesterase^{112,113}, neuronal nitric oxide synthase^{114,115}, cytochrome oxidase¹¹⁶, synaptophysin^{25,117}, 3-α-fucosyl-N-acetyl-lactosamine (CD15)¹¹⁸, the NR2C subunit of the NMDA receptor¹¹⁹ and dystrophin¹²⁰).

Perhaps the most extensive regionalization of the granular layer is seen when the cerebellum is ethanol-fixed, paraffin-embedded and sectioned. Upon rehydration, cerebellar sections wrinkle elaborately to expose an array of 'blebs' in the granular layer that is reproducible between individuals and is aligned with the overlying Purkinje cell stripes as revealed by expression of zebrin II¹²¹⁻¹²³. In mice, for example, this technique reliably divides the whole cerebellar cortex into several thousand reproducible units, each comprising, on average, around 100 Purkinje cells¹²¹. The structural basis of blebbing is not known, nor is its relationship to patches, but one possibility is that each bleb is the terminal field of a small number of mossy fibre afferents.

If mossy fibre terminal fields align with Purkinje cell stripe boundaries then a prediction of the one-map hypothesis is that they will also align with longitudinal zones. In some instances this seems to be the case, as patches and longitudinal zones can occupy similar territories and might well represent the same spatial areas of the cerebellar cortex (lower panel FIG. 6). At a finer level of resolution, detailed electrophysiological mapping¹²⁴ of climbing fibre responses of individual Purkinje cells and multiunit granule cell activity in the underlying granular layer in response to tactile stimulation of different body parts, has also revealed examples of elongated 'patches' that might be congruent with microzones (see the expanded view of the medial part of crus II shown in FIG. 6). Consistent with the one-map hypothesis, it is therefore possible that small patches defined by their mossy fibre input might correspond to individual microzones defined by their climbing fibre input. However, this possibility needs further study to show that such correspondence is the rule rather than the exception.

What underlying anatomy could account for such an arrangement? When the distribution of mossy fibre and climbing fibre connections associated with a particular longitudinal zone were mapped in the same experiment, it was found that wherever climbing fibre terminals were located in the cortex, labelled mossy fibre terminals were situated in the underlying granular layer^{97,125}. Moreover, labelled mossy fibre terminals were also located in other parts of the cortex, immediately below Purkinje cell stripes with the same zebrin II phenotype as the longitudinal zone under study. Such results are consistent with the original findings of Eccles and colleagues¹²⁶ and also more recent

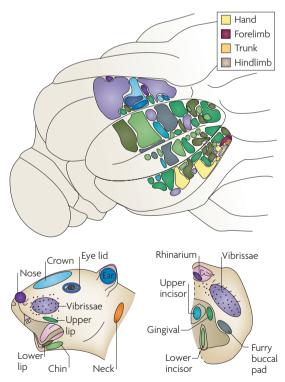


Figure 5 | **Patches.** Dorso-posterior view of the left hand side of the rat cerebellum showing the spatial distribution of receptive fields recorded in the granular layer in response to mechanical stimulation of different body parts as detailed in the panels below. Note the multiple representations of the same body parts to produce a 'fractured somatotopical' mosaic pattern of variable sized patches. Figure is based on data from REF. 107.

physiological studies¹²⁷ that show a close correspondence between inputs conveyed by climbing fibres to the molecular layer and those conveyed by mossy fibres to the underlying granular layer. Thus, an increasing body of evidence points to an alignment (anatomical and physiological) between mossy fibre and climbing fibre projections that target the same Purkinje cell stripes (see also REF. 128).

Nevertheless, the topography of mossy fibre afferent projections is sometimes interpreted as stripes and other times as patches. Is a single description appropriate, and if so, is it stripe or patch? One way to reconcile the two descriptions is to propose that Purkinje cell stripes are subdivided into smaller units that correspond to small patches, which in turn might correspond to individual microzones — that is, that anatomical stripes are subdivided functionally into chains of small patches or microzones, which can be revealed by differential gene expression or electrophysiological mapping.

Functional significance of the map?

What might be the functional significance of this elaborate architecture? The heterogeneity divides the cerebellar cortex into hundreds or thousands of reproducible units — in the mouse each comprising no more than a few hundred Purkinje cells. A detailed discussion would seem premature at this stage as the necessary data are mostly lacking, but among other functions these multiple units could be used for the parallel processing of sensorimotor commands, energy efficient information processing, positional coding of sensory inputs and/or molecular fine-tuning of local circuits for specific functions (for examples, see REFS 3,129–131).

Regarding molecular fine-tuning, recent studies suggest that molecular heterogeneity might have functional implications for motor learning. Long-term depression results from the coincident activation of climbing fibre and parallel fibre inputs that lead to activation of ionotropic and metabotropic glutamate receptors132. Several molecules involved in glutamatergic transmission are expressed in Purkinje cell stripes. For example, the mGluR1b splice variant is restricted to the zebrin II- Purkinje cell subpopulation³³ as is the synaptic glycoprotein neuroplastin³⁶. Conversely, protein kinase $C\delta^{133}$, the GABA_{P2} receptor³⁵ and excitatory amino-acid transporter EAAT4 (REFS 32,134) are all expressed preferentially by the zebrin II+ Purkinje cells, and phospholipase Cy2 (PLCy2) seems to be confined to the zebrin II+ Purkinje cells of the NZ¹³⁵.

In general, these data suggest the hypothesis that the mechanisms underlying long-term depression and the ways in which it is manifested differ between Purkinje cell stripes. There is also a close relationship between Purkinje cell complex spike synchrony (generated by activity in climbing fibre afferents) and zebrin II+ stripes in the lateral but not medial part of crus II¹³⁶. This suggests that information processing might differ between stripes, but clearly, studies are needed to explore this possibility further.

Conclusion: towards a unified map

Cerebellar cortical patterning can be viewed through different prisms, each of which reveals a characteristic map (TABLE 1): one map is based on patterns of Purkinje cell gene expression (transverse zones and Purkinje cell stripes), a second one on the compartmentalization of the granular layer into patches and a third is based on

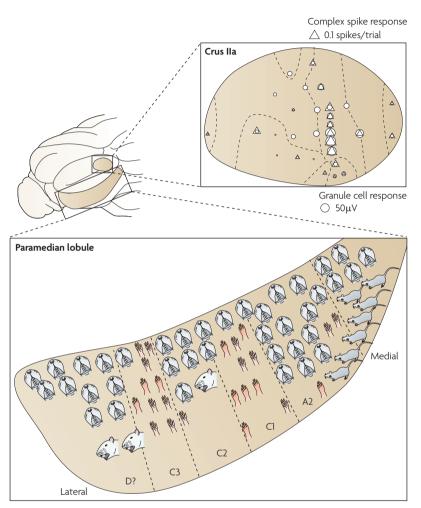


Figure 6 | Patches can have similar spatial distributions as longitudinal zones and microzones. Dorso-posterior view of the left hand side of the rat cerebellum with the paramedian lobule expanded in the lower panel to show multiple representations (patches) of the same body parts in different locations, defined by multiunit receptive field activity evoked in the granular layer (see also FIG. 5). Superimposed on this map are the approximate boundaries of longitudinal zones. Note the close correspondence between patches and different longitudinal zones defined by their principal climbing fibre input: A2 zone, contralateral face; C1 zone, ipsilateral forelimb; C2 zone, convergent input from different body parts; C3 zone, ipsilateral forelimb; D? zone, not tested. In the upper panel to the right, a medial part of crus IIa is expanded to show where Purkinje cell complex activity (generated by activity in climbing fibre afferents, triangles) and underlying granular layer multiunit activity (circles) was evoked by mechanical stimulation of the lower incisors. Each symbol indicates a recording site, with the largest circles and triangles indicating sites where the strongest activity was evoked. Dashed lines demarcate the boundaries of different granular layer patches defined by their perioral receptive field characteristics. Note the close correspondence between sites of strongest granular layer and complex spike activity and their spatial distribution in the rostrocaudal axis to form a small elongated 'patch' (equivalent to a 'microzone'). Bottom part is modified, with permission, from REF. 107 © (1978) Karger and from REF. 14 © (1997) Wiley-Liss. Top part is modified, with permission, from REF. 124 © (2001) Wiley-Liss.

patterns of olivocerebellar and corticonuclear connections (longitudinal zones and microzones). Taking cerebellar development also into account, we think that the following is the fundamental reality: early in cerebellar development, Purkinje cell topography is laid down as a set of transverse zones, each of which is subdivided into an array of longitudinal stripes. During embryogenesis, mossy and climbing fibre afferents use the Purkinje cell scaffold to organize their topography. As a consequence, the afferent maps and the Purkinje cell map coincide. In brief, transverse zones are subdivided into stripes (one or more stripes equals a longitudinal zone) (TABLE 2), and stripes are further segmented longitudinally into microzones that correspond to one or more small patches.

Several questions remain outstanding to verify the existence of a unitary cortical map. For example, do all molecular boundaries match functional boundaries? In particular, do climbing fibre receptive fields (microzones) correspond to Purkinje cell stripes as defined by molecular markers? Similarly, do all mossy fibre terminal fields (patches) align with Purkinje cell stripes? In most cases, a combination of high-resolution anatomical, molecular and physiological mapping techniques will begin to answer these questions. But an additional important question remains: what is the role of parallel fibres? The topography of the mossy fibre afferent projections is complicated by the way in which they reach the Purkinje cells. In adults the connection is indirect, through the granule cells and their parallel fibre axons. Parallel fibres can be very long (for example, ~5mm in rats137) and run orthogonal to the long axis of a cerebellar folium, thus intersecting multiple stripes. Does this imply that mossy fibre projections are tightly topographically focused to a particular Purkinje cell stripe, but that this precision is promptly discarded (perhaps parallel fibres are the solution to the problem that mossy fibre afferents are too restricted!)? In other words, do parallel fibres broadcast mossy fibre information to multiple patches or zones within the same lobule^{3,138,139}? Or is mossy fibre information relayed mainly to a local group of overlying Purkinje cells140-142, in which case, do parallel fibres modulate rather than transmit activity across multiple regions of the cortex? In any case, parallel fibres are a highly conserved feature of cerebellar anatomy from fish to mammals and must be important. They remind us that the cerebellum is not composed of hundreds of independent units, but rather, that it is a complex interconnected system of hundreds of units working in concert to achieve motor coordination.

In conclusion, we propose that early in cerebellar development topography is laid down as a set of embryonic Purkinje cell clusters. Then, perinatally, clusters disperse to become the adult transverse zones, each of which comprises an array of longitudinal stripes. During embryogenesis, the mossy fibre and climbing fibre afferents use the Purkinje cell clusters as a scaffold to organize their topography. In this way, the afferent terminal fields align with the Purkinje cell stripes to generate in the adult a unified cerebellar map.

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