

When physiology meets anatomy: the tale of cerebellar compartments

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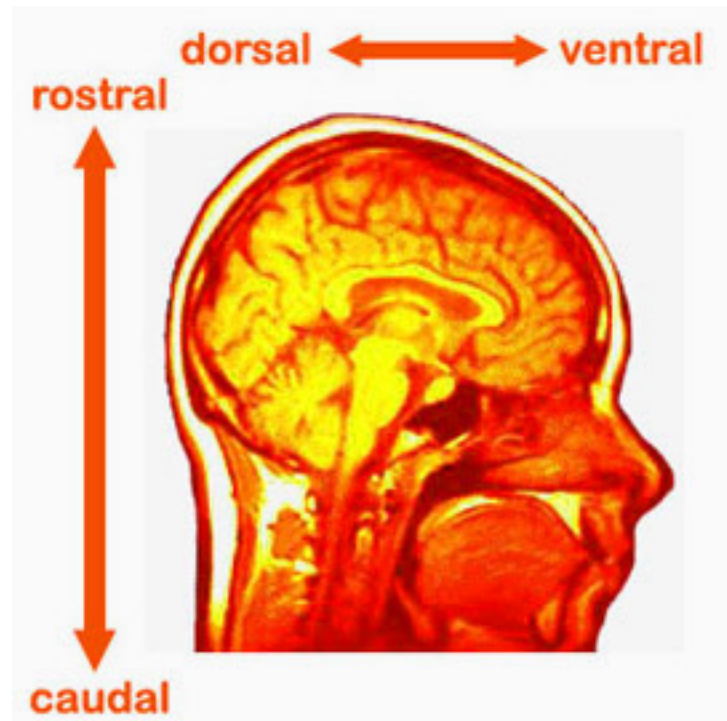
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Introduction

During the last forty years neuroanatomists have promoted the notion of a complex topographic map of the cerebellum consisting of a system of rostrocaudally oriented afferent fiber projections (especially the climbing fibers) and efferent cortico-nuclear projections (the Purkinje cell axons) in register with cortical parasagittal bands of chemically heterogeneous Purkinje neurons. The gross order of the cerebellar axonal projections was already known from the studies of Jansen & Brodal (1958), although sagittal banding of myelinated fibers in the cerebellar white matter was first demonstrated by Voogd (1964) with histochemical stains. Banded climbing fiber projections then became most clearly apparent with radioautographic tract-tracing methods and electro-anatomical investigations in the following three decades (Buisserat-Delmas & Angaut, 1993). Chemical heterogeneity

of Purkinje cells, that also has a long history (Scott, 1964), was demonstrated most strikingly by “zebrin” antibodies (Hawkes & Leclerc, 1987) and proven to occur in most vertebrate species (the turtle is a notable, but still unexplained exception).

The Purkinje cells are the main elements of the cortical map; their flat dendritic trees are orientated sagittally (i.e. at right angles to the course of the cortical folia) and receive input from individual climbing fibers on the sparse spines of the proximal domain and from myriad granule cell axons (ascending portions and parallel fibers) on the spines of the distal domain. The



The inhibitory Purkinje cell axons project fan-like to the cerebellar and vestibular nuclei, which in turn form the output of the cerebellum. The notion of a chemical heterogeneity of Purkinje cells, which is also accompanied by less conspicuous chemical heterogeneity of the cerebellar afferents, runs against the dogma of the stereotypic histological organization of the cerebellar cortex, but this did not deter microscope-based students from producing exacting and excruciatingly detailed cerebellar maps with cell class specific antibodies and neuroanatomical tract-tracers. This chemical heterogeneity included the enzymes 5'-nucleotidase, acetylcholinesterase, NADPH-diaphorase, zebrin II (or brain specific glycolytic isoenzyme aldolase C), cytochrome oxidase, nitric oxide synthase, protein kinase C δ , Oxa 2 (a β lactamase), Cdk5 activator p39, sphingosine kinase (SPHK), and extended to the adhesion molecule neuroplastin, the small heat shock protein HSP25, metabotropic receptors mGluR1b and GABA_B, the low affinity NGF

receptor, the neuronal glutamate transporter EAAT4, and perhaps also glutamate decarboxylase (GAD) the neuropeptide motilin and the amino acid taurine (reviewed by Vood *et al*, 1996; Herrup & Kuermele, 1997; Mugnaini, 2000). In most cases the on-off bands are not an all-or nothing phenomenon, but reflect the relative abundance of protein or antigen, possibly resulting from quantitative differences in transcription or regulated translation of the messages. That the cerebellar framework respects strict laws and is indeed quite complex is also indicated by the occurrence of minor Zebrin/EAAT4 bands (“satellite bands”), which are inserted at points within the major bands and have reproducibly been demonstrated in different animals (Voogd & Ruigrok, 2004).

Developmental studies clearly pointed out that banding of chemically heterogeneous Purkinje cells and climbing fiber compartments are linked in the establishment of the cerebellar topographic framework (Sotelo, 2003); they also presented a new way of looking at the internal organization of the inferior olivary complex (Apps, 1990; Voogd & Ruigrok, 2004). Re-examination of cerebellar pathology further indicated that selective death of Purkinje cells is related to the cortical topography (Sarna & Hawkes, 2003). However, the lack of a strong physiological underpinning for the chemical non-homogeneity of Purkinje cells seem to have diminished the impact of many neuronatomical investigations.

This situation might finally have turned, as a recent study by Wadiche & Jahr (2005) for the first time provides elegant, mechanistic evidence for a functionally testable relation between the activity patterns of Purkinje cells situated in different sagittal compartments. The experiments were carefully thought out and their results clearly link the heterogeneity of EAAT4 Purkinje cells with the phenomenon of long-term depression (LTD), arguably a fundamental aspect of cerebellar operation first demonstrated by Masao Ito, that has been implicated in cerebellar learning (Ito, 1984).

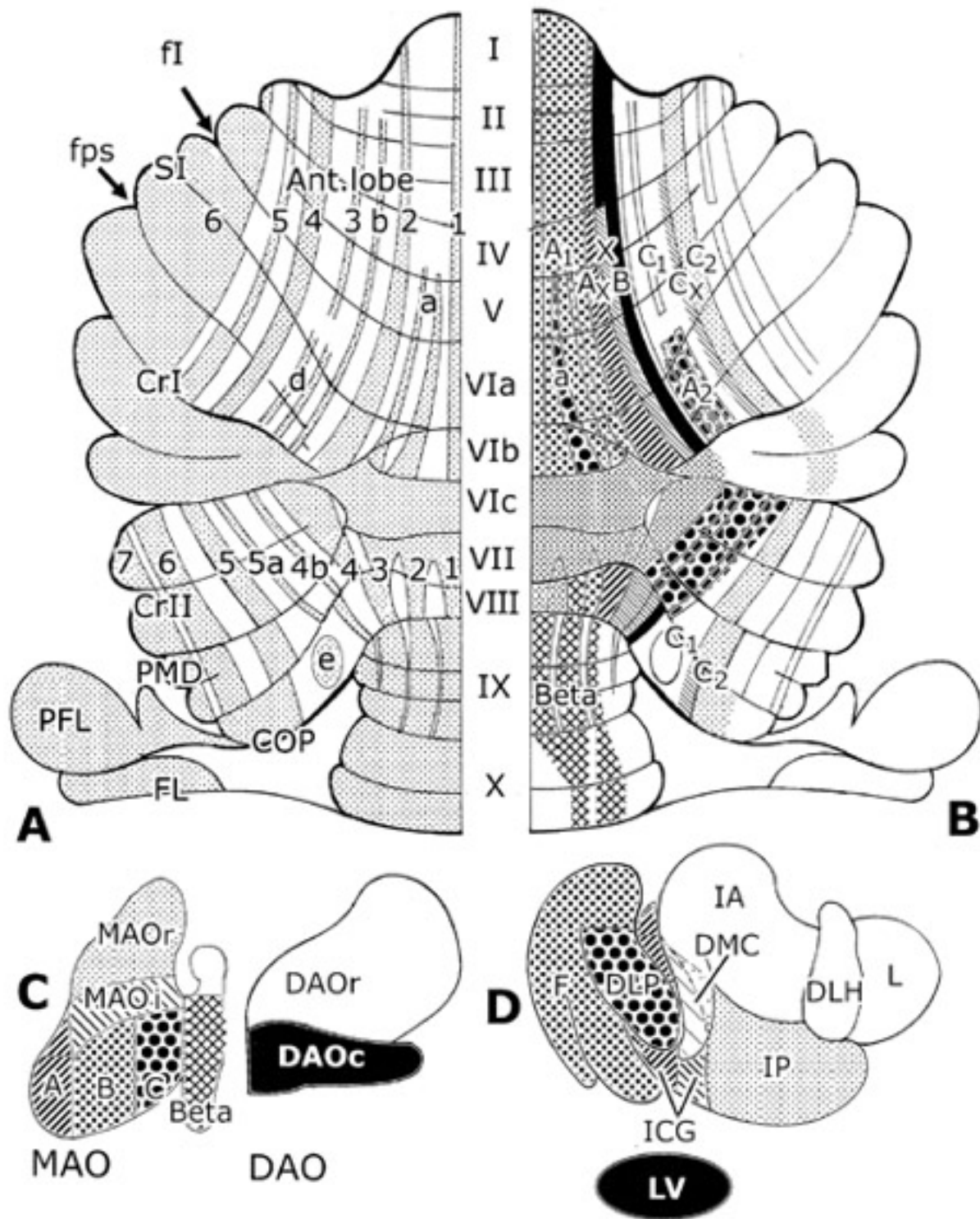


Figure 1. Diagram summarizing the localization of projection zones in the vermis of the cerebellum of the rat, their afferent and efferent connections, and their relation to the zebrin pattern. (A) Diagram of the zebrin pattern. (B) Projection zones are indicated with different symbols. The borders of the zebrin-positive zones are retained. The projection of the group Beta is based on a previous study (Voogd *et al.*, 1996b). (C) The origin of the olivocerebellar

projection shown in horizontal projections of the medial and dorsal accessory olive. (D) Diagram of the target nuclei of the projection zones. *Reproduced with permission of the Journal of Neurocytology*, © Springer-Verlag

	olivary subnucleus	MAOc B	MAOc A	MAOi	DAOc	MAOc C	Beta	?	MAOr
E	zone	A ₁	A _X	X C _X	B	A ₂	Beta		C ₂
	target nucleus	F	F/ICG	ICG/ IP	LV	DLP	F	F	IP
	zebrin zone anterior	P1-	P2+	P2-/ P3-	P2-	d bands			P4+
	zebrin zone posterior	P2-	P3+	P3-/ P4+	P4-	P4- P4b+/- P5a+/-	P1+/ P3+		P5+

After Voogd & Ruigrok TJH. (C) *Journal of Neurocytology* 2004 33:5-21.

(E) Key to the symbols indicating the various zones, and summary of their connections. Abbreviations: 1-7, zebrin-positive zones 1-7; a, satellite bands a; A, group A of caudal medial accessory olive; b, satellite band b; B, group B of caudal medial accessory olive; Beta, group Beta; C, group C of caudal medial accessory olive; COP, copula pyramidis; Crl, crus 1 of the ansiform lobule; CrII, crus II of the ansiform lobule; d, satellite bands d; DAOc, caudal portion of dorsal accessory olive; DAOr, rostral portion of dorsal accessory olive; DLH, dorsolateral hump; DLP, dorsolateral protuberance; DMC, dorsomedial crest; DMCC, dorsomedial cell column; e, satellite band e; F, fastigial nucleus; £1, primary fissure; FL, flocculus; fps, posterior superior fissure; IA, anterior interposed nucleus; ICG, interstitial cell groups; IP, posterior interposed nucleus; I-X, lobules I-X; L, lateral cerebellar nucleus; LV, lateral vestibular nucleus; MAOc, caudal portion of medial accessory olive; MAOi, intermediate portion of medial accessory olive; MAOr, rostral portion of medial accessory olive; PFL, paraflocculus; PMD, paramedian lobule; SI, lobulus simplex.

The study of Wadiche & Jahr rests on the solid quantitative analysis

by Dehnes *et al.* (1998), who showed that EAAT4 is distributed in Zebrin II-positive parasagittal bands, in contrast to the glial glutamate transporters GLAST, GLT, and the neuronal EAAT3, all of which are distributed evenly. In the Purkinje dendritic membrane EAAT4 is enriched at the spiny branchlets and its concentration is low at the postsynaptic density of the parallel fiber synapse, while is very high in the parts of the dendritic and spine membranes facing astrocytes (which express GLAST and GLT), indicating a relation of EAAT4 with the glial glutamate transporters. Notably, EAAT4 has much higher affinity for glutamate than the glial transporters (Arriza *et al.*, 1994).

To facilitate comparisons between functional properties of Purkinje neurons from different histochemical compartments, Wadiche & Jahr chose distant, rather than side-by-side, recording sites, making use of the discovery by Richard Hawkes and coworkers that lobules I-III (anterior lobe vermis) are largely zebrin II-free, while lobule X (vestibulo-cerebellum) is zebrin II-positive and is completely devoid of Zebrin II-free bands (another zebrin II-positive region would be the entire hemispherical crus 1). The Vollum Institute researchers show that the amplitude of EAAT currents elicited by synaptic stimulation greatly differs between Purkinje neurons situated in lobule X and in lobule III. They come to the interesting conclusion that the observed difference in the transporter current size is not due to different amount of glutamate released by presynaptic terminals, but to a differential expression level of the transporter in Purkinje neurons in the two anatomical areas.

They show also that the differential availability of transporters between cells in lobule X and III is a key factor for the activation of metabotropic glutamate receptors by synaptically released glutamate. This fact, in turn, is a key regulator of synaptic plasticity and controls fundamental physiological processes such as SDI and LTD. In particular, they show that in lobule III pairing parallel fiber stimulation

and Purkinje neuron depolarization resulted in strong LTD; when the pairing protocol was delivered to cells in lobule X, however, the fast glutamate reuptake from the densely packed neuronal transporters prevented the activation of metabotropic glutamate receptors and no statistically significant LTD was observed. Interestingly, mGluR1 activation in Purkinje neurons can evoke slow EPSCs that have been shown to be mediated by TRPC channels (Kim *et al.* 2003). Because TRP channels are calcium permeable, the expression level of EAAT in Purkinje neurons could also regulate other calcium dependent processes such as apoptosis. On this line of thought, Wadiche & Jahr venture the idea that EAAT4 concentration may be related to synapse elimination, which has been shown to have an mGluR-dependent component (Kano *et al.* 1997). Postsynaptically, this class of metabotropic receptors mediate slow, long lasting changes initiated by glutamate neurotransmission. Interestingly, expression of mGluR1 isoforms in the cerebellar molecular layer shows a clear topographic variation: while the long splice variant, mGluR1a, is expressed in all Purkinje cells, the short splice variant, mGluR1b, is mostly found in Purkinje cells organized in zebrin-negative longitudinal bands. The two mGluR1 isoforms, which differ in the length of the cytoplasmic COOH-terminus, likely share postsynaptic signaling cascades only in part and can be differentially regulated, as recently shown (Pula *et al.*, 2005).

Now that one of the main functional implications of the Purkinje cell banding pattern has been clarified, questions about the relations between the activities of interconnected Purkinje cells, cerebellar (or vestibular) nuclear neurons, and inferior olivary neurons may seem more approachable than before. Perhaps, it is time that the parasagittal cortical bands are deliberately included in cerebellar network models (Apps & Garwicz, 2005), which so far have largely been built upon the notion of cerebellar homogeneity.

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