Architecture and development of olivocerebellar circuit topography

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The cerebellum has a simple tri-laminar structure that is comprised of relatively few cell types. Yet, its internal micro-circuitry is anatomically, biochemically, and functionally complex. The most striking feature of cerebellar circuit complexity is its compartmentalized topography. Each cell type within the cerebellar cortex is organized into an exquisite map; molecular expression patterns, dendrite projections, and axon terminal fields divide the medial-lateral axis of the cerebellum into topographic sagittal zones. Here, we discuss the mechanisms that establish zones and highlight how gene expression and neural activity contribute to cerebellar pattern formation. We focus on the olivocerebellar system because its developmental mechanisms are becoming clear, its topographic termination patterns are very precise, and its contribution to zonal function is debated. This review deconstructs the architecture and development of the olivocerebellar pathway to provide an update on how brain circuit maps form and function.

Keywords: inferior olive, circuitry, topography, climbing fibers, cerebellum, zones

INTRODUCTION

It is well established that brain circuits are organized into spatial maps that control behavior (Hubel and Wiesel, 1979; Johnston, 1989; Friedman and O'Leary, 1996; Logan et al., 1996; Bozza et al., 2002; Huffman and Cramer, 2007; Leergaard and Bjaalie, 2007; Li and Crair, 2011; Suzuki et al., 2012). Yet, we have a limited understanding of how precise functional connections form during map development. Neural circuit connectivity is intensely studied in the cerebellum because its cellular networks are well understood and its developmental mechanisms are experimentally tractable. Cerebellar circuits have an established role in motor control and they are now also implicated in higher order functions such as cognition and emotion (Sacchetti et al., 2009; Strata et al., 2011). Two main types of afferents transmit information to the cerebellum: climbing fibers and mossy fibers. Climbing fibers arise only from neurons of the inferior olivary nucleus in the brainstem (**Figure 1**) and monoinnervate adult Purkinje cells (**Figure 2A**) whereas mossy fibers originate from numerous brain and spinal cord nuclei to innervate granule cells. Each climbing fiber elicits powerful Purkinje cell responses that sculpt cerebellar function (**Figures 2C,D**). Here, we discuss the development, organization, and function of the olivocerebellar projection and highlight the mechanisms that make this pathway an attractive model for understanding topographic brain circuitry.

CEREBELLAR SAGITTAL ZONES

The adult cerebellum is anatomically divided into distinct folds called lobules (**Figure 3A**; Larsell, 1952). Mammals and birds have 10 lobules that are separated from one another by a series of fissures. Because each fissure extends to a specific depth in the cerebellum, each lobule develops with a unique shape (**Figure 3A**). The invariance of lobule structure and their conservation across species support the idea that lobule/fissure formation is spatially and temporally controlled by complex morphogenetic programs (Sudarov and Joyner, 2007).

Strikingly, each lobule in the cerebellum is further compartmentalized along the medial-lateral axis into sagittal zones (**Figure 3**). Each set of zones is clearly delineated by the patterned expression of genes and proteins (Apps and Hawkes, 2009). The most comprehensively studied zonal marker is zebrin II (Brochu et al., 1990; **Figures 3B,C, 4D**), an antigen on the aldolase C protein (Ahn et al., 1994; Hawkes and Herrup, 1995). Zebrin II is expressed by alternating subsets of Purkinje cells (zebrin II+ adjacent to zebrin II−), thus forming complementary rows of biochemically distinct Purkinje cells (**Figures 3B,C, 4D**). The zonal organization of zebrin II is symmetrical about the cerebellar midline, highly reproducible between individuals, and conserved across species (Brochu et al., 1990; Sillitoe et al., 2005; Apps and Hawkes, 2009). The pattern of zebrin II has an intricate relationship to the expression of several other Purkinje cell proteins. For example, phospholipase Cβ3 (PLCβ3), sphingosine kinase 1a (SPHK1a), and excitatory amino-acid transporter 4 (EAAT4; Hawkes et al., 1985; Hawkes and Leclerc, 1987; Dehnes et al., 1998; Terada et al., 2004; Sarna et al., 2006) are all co-expressed with zebrin II. In contrast, phospholipase C β4 (PLCβ4; Armstrong and Hawkes, 2000; Sarna et al., 2006) is expressed selectively in zebrin II− zones. In addition to the complementary and corresponding relationships between zones, proteins such as neurofilament heavy chain (NFH) divide individual zebrin II zones into smaller sagittal units (Demilly et al., 2011).

FIGURE 1 | (A) Wholemount image of an adult brain showing the cerebellum (Cb) from a dorsal view. The dotted line indicates the level of the tissue section schematic in **(B)**. **(B)** Schematic of sagittal section cut through an adult cerebellum showing the cerebellum (red) and inferior olive (IO; blue).

FIGURE 2 | (A) Schematic of a simplified cerebellar microcircuit illustrating the two major sensory afferent pathways that project to the cerebellum: climbing fibers and mossy fibers. Climbing fibers (blue projection) terminate directly onto Purkinje cells whereas mossy fibers (yellow projection) terminate on granule cell dendrites (green). Granule cell axons called parallel fibers contact Purkinje cells (purple). Purkinje cells are the sole output of the cerebellar cortex and transmit signals to the cerebellar nuclei (red). **(B)** High power image of a climbing fiber expressing cocaine-and amphetamine-regulated transcript (CART) peptide [arrow; staining was performed according to Reeber and Sillitoe (2011)]. The target Purkinje cell is weakly immunoreactive for CART. **(C)** Example Purkinje cell spike train recorded *in vivo*. Recordings were performed in Ketamine/Xylazine anesthetized mice using 2–5 M Ohm Tungsten electrodes (Thomas Recording, Germany). Signals were band-pass filtered at 300–5000 Hz, amplified with an ELC-03XS amplifier (NPI, Germany), and recorded with Spike2 (CED, England). **(D)** Higher power view of the recording trace illustrating the clear distinction between a climbing fiber complex spike (cs) and simple spike (ss) responses in Purkinje cells. Asterisk in panel **(C)** indicates a complex spike. The layers of the cerebellum are indicated as molecular layer (ml), Purkinje cell layer (pcl), granular layer (gl), and white matter (wm). The cerebellar nuclei are located in the white matter. Scale bar in **(B)** = 25 μm.

Cumulatively, molecularly defined zonal compartments divide the cerebellar cortex into hundreds of reproducible units with each one containing up to several hundred Purkinje cells (Apps and Hawkes, 2009).

Purkinje cell zones may be used to divide the cerebellum into four transverse domains in the anterior–posterior axis (Ozol et al.,

1999). For example, in the vermis zebrin II expression reveals a specific pattern in lobules I–V and VIII/IX (**Figures 3B,C**, **4D**). In contrast, expression of the small 25 kDa heat shock protein HSP25 delineates distinct zonal patterns in lobules VI/VII and IX/X, which express zebrin II in all Purkinje cells (Armstrong et al., 2000). Afferent termination patterns mirror the topography

FIGURE 3 | (A) Schematic of a sagittal section cut through the cerebellar vermis revealing the stereotypical foliation pattern, which consists of 10 lobules [adapted with permission from White and Sillitoe (2013)]. The cerebellum can be further divided along the anterior–posterior axis into four transverse domains: anterior (blue; lobules I–V), central (green; lobules VI and VII), posterior (yellow; lobules VIII and anterior IX), and nodular (red; lobules posterior IX and X) (Ozol et al., 1999). **(B)** In the adult cerebellum, zebrin II/aldolase C expression, which is revealed using wholemount staining (Sillitoe and Hawkes, 2002; White et al., 2012), delineates zones of Purkinje cells. The transverse zones are color coded according to panel **(A)**. **(C)** A schematic representation of an unfolded vermis illustrating the full pattern of zebrin II zones (adapted with permission from Sillitoe and Joyner, 2007). Lobule numbers are indicated by Roman numerals. Anterior and posterior axes are denoted by A and P.

of Purkinje cell zones (**Figures 4B,E**). As a result, each transverse domain is innervated by a specific combination of functionally distinct afferent fibers. For instance, spinocerebellar mossy fibers project to lobules I–V and VIII/IX (Arsenio Nunes and Sotelo, 1985; Brochu et al., 1990; Sillitoe et al., 2010), whereas the vestibulocerellar mossy fibers project mainly to lobules IX and X (Jaarsma et al., 1997; Maklad and Fritzsch, 2003). In mouse, climbing fibers that express cocaine- and amphetamine-related transcript peptide (CART) terminate selectively in lobules VI/VII and IX/X (Reeber and Sillitoe, 2011), and corticotrophin releasing factor (CRF) expressing climbing fibers are expressed in a striking array of zones in lobules I–V and VIII/IX (**Figures 4C,E**) (Sawada et al., 2008).

The efferent side of the cortical circuit also respects the zonal topography. Sugihara and collaborators have mapped the trajectories of Purkinje cell axons from specific cerebellar cortical compartments onto the three sets of cerebellar nuclei. They revealed a close correspondence between adolase C expressing Purkinje cell terminals with subdivisions of cerebellar nuclei (Sugihara and Shinoda, 2007). Together, Purkinje cell zones, afferent topography, and Purkinje cell efferent projections to the cerebellar nuclei define the cerebellar module, the functional unit of the cerebellum (Apps and Hawkes, 2009; Ruigrok, 2011).

ANATOMICAL AND FUNCTIONAL ORGANIZATION OF OLIVOCEREBELLAR ZONES

Fine topological mapping using anterograde tracers injected into specific sub-nuclei of the inferior olive and the tracing of climbing fiber collateral projections labeled from injections into the cerebellar cortex of birds, rodents, and primates have shown that there is a strict and precise association between climbing fiber topography and zebrin II Purkinje cell zones (Voogd et al., 2003; Sugihara and Shinoda, 2004; Voogd and Ruigrok, 2004; Sugihara and Quy, 2007; Pakan and Wylie, 2008; Sugihara et al., 2009; Fujita et al., 2010). In addition, several studies have used climbing fiber markers to link the architecture of chemically distinct subsets of climbing fiber afferents to the adult pattern of Purkinje cell zones (**Table 1**). For example, CRF, an amino acid peptide, is expressed in a subset of climbing fibers that corresponds to specific Purkinje cell zones (Sawada et al., 2001, 2008) (**Figures 4C,E**). In addition, we recently showed that the expression of the CART 55–102 peptide (**Figure 2B**) is intricately patterned into a complex topographic map that respects HSP25 (mouse) and zebrin II (rat) Purkinje cell zone boundaries (Reeber and Sillitoe, 2011). The class III intermediate filament protein peripherin is also expressed in a subset of climbing fibers that are organized into parasagittal compartments, although it is not clear how peripherin labeled climbing fibers relate to Purkinje

FIGURE 4 | (A) Schematic illustrating climbing fibers projecting from the inferior olivary nucleus (IO) to the cerebellum (Cb) and their organization into a crude zonal pattern at E17. **(B)** A schematic of the adult brain showing climbing fibers projecting from the inferior olive (IO) to well-defined Purkinje cell zones in the cerebellum. **(C–E)** In the adult cerebellum corticotropin-releasing factor (CRF) is expressed in subsets of climbing fibers that align with zebrin II

Purkinje cell zones. Panels **(C)** and **(D)** show individual channels of CRF and zebrin II expression and panel **(E)** is a merged image showing the corresponding relationship between the two patterns. The staining pattern of CRF and its relationship to zebrin II zones was previously described (Sawada et al., 2008). CRF and zebrin II staining was carried out exactly as previously described (Sawada et al., 2008). Scale bar in **(E)** = 100 μm (applies to **C–D**).

cell zones (Errante et al., 1998). The precise topography of the olivocerebellar pathway raises the tantalizing possibility that zonal circuits may be functionally relevant. In this regard, two pressing questions have yet to be fully answered: (1) what is the functional significance of zones? and (2) what role do topographic circuits play during behavior?

Previous electrophysiological mapping studies suggested that parasagittal zones could be related to cerebellar function (Armstrong et al., 1974; Ekerot and Larson, 1980; Llinas and Sasaki, 1989; Chockkan and Hawkes, 1994; Sugihara et al., 1995; Chen et al., 1996; Hallem et al., 1999). However, it was only recently that modern optical imaging and electrophysiological approaches were exploited to uncover potential links between functional cerebellar circuits and zonal architecture (Ebner et al., 2012; Graham and Wylie, 2012). In their seminal paper, Wadiche and Jahr (2005) used molecular physiology approaches to demonstrate that synaptic plasticity may vary between zones. Accordingly, the level of glutamate that is released at climbing fiber terminals is zone dependent (Paukert et al., 2010) and climbing fiber inputs initiate synchronous firing in zones of Purkinje cells (Sasaki et al., 1989; Lang et al., 1999; Blenkinsop and Lang, 2006; Wise et al., 2010). These studies support the notion that there are fundamental differences in the physiology of Purkinje cell zones and suggest the possibility that climbing fibers contribute to the functional specificity of the zones.

The behavioral significance of zones remains elusive. However, surgically induced lesions and localized delivery of pharmacological agents into the inferior olive have provided some evidence that cerebellar zones may facilitate behavior (Watanabe et al., 1997; Seoane et al., 2005; Pijpers et al., 2008; Horn et al., 2010; Cerminara and Apps, 2011). For example, Llinas and collaborators found that when the neurotoxin 3-acetylpyridine (3AP) is injected intraperitoneally, the inferior olive is rapidly destroyed and severe ataxia emerges (Llinas et al., 1975). Similarly, injecting another neurotoxin called trans-crotononitrile (TCN) into rats inactivates the olive and induces profound motor deficits (Seoane et al., 2005; Cerminara and Apps, 2011). Ruigrok and colleagues used yet a different approach to inactivate the olive (Pijpers et al., 2008). They injected cholera toxin b conjugated to saporin into individual cerebellar cortical zones, which retrogradely transported the neurotoxin into the olive and induced dysfunction of specific modules. By targeting distinct modules they were able to demonstrated specific defects in the step phase-dependent modulation of cutaneously induced reflexes during locomotion (Pijpers et al., 2008; Cerminara and Apps, 2011). Moreover, inactivating specific olivary subdivisions in cats with the glutamate receptor blocker, CNQX, produced a series of unique motor deficits that were dependent on the particular sub-nucleus that was lesioned (Cerminara, 2010; Horn et al., 2010). What is far from clear is whether each zone encodes specific behaviors (or distinct aspects of a behavior), or

Table 1 | Molecular and genetic markers for studying olivocerebellar topography.

Transient expression in subsets of climbing fibers	
CGRP (zones in rat E16-P20)	Chedotal and Sotelo, 1992; Morara et al., 1992
Parvalbumin (zones in rat ~P0–P10)	Wassef et al., 1992; Chedotal and Sotelo, 1993

Topographic climbing fiber projections	
Calretinin (zones in cat)	Yan and Garey, 1996
CART (zones in mouse and rat)	Reeber and Sillitoe, 2011
CRF (zones in mouse and opossum)	Cummings et al., 1989; Sawada et al., 2008
DNPI/VGLUT2 (zones in mouse)	Paukert et al., 2010
NPY (zones in rat)	Ueyama et al., 1994
Peripherin (zones in rat)	Errante et al., 1998

Compartmentalization of the inferior olive	
BEN	Chedotal et al., 1996
Brn3a	Xiang et al., 1996
Brn3b	Xiang et al., 1996
CART	Reeber and Sillitoe, 2011
Cdh6	Suzuki et al., 1997
Cdh8	Suzuki et al., 1997; Redies et al., 2011
Cdh11	Suzuki et al., 1997
CRF	Yamano and Tohyama, 1994
Cx36	Belluardo et al., 2000; Weickert et al., 2005
Cx45	Van Der Giessen et al., 2006
Cx47	Weickert et al., 2005
Cx57	Zappala et al., 2010
DCC	Bloch-Gallego et al., 1999
EphA3	Nishida et al., 2002
EPHA4	Hashimoto et al., 2012
EphA5	Nishida et al., 2002
EphA6	Nishida et al., 2002
EPHA7	Hashimoto et al., 2012
ER81	Zhu and Guthrie, 2002; Hashimoto et al., 2012
FOXP2	Hashimoto et al., 2012
NPY	Ueyama et al., 1994; Morara et al., 1997
Nr-CAM	Backer et al., 2002
Pannexin1	Weickert et al., 2005
Pdh7	Redies et al., 2011
Pdh10	Redies et al., 2011
Unc-5H2	Bloch-Gallego et al., 1999
Unc-5H3	Bloch-Gallego et al., 1999
DNPI/VGLUT2	Hisano et al., 2002

Genetic markers for the inferior olive and/or climbing fibers	
CART-Cre	Madisen et al., 2010
CRF-Cre	Martin et al., 2010

(Continued)

Table 1 | Continued

Cx36-LacZ	Degen et al., 2004
Cx45-lacZ	Van Der Giessen et al., 2006
Npy-GFP	Nishiyama et al., 2007
Parvalbumin-Cre	Tanahira et al., 2009
Parvalbumin-CreER	Taniguchi et al., 2011

Note that the markers in each subsection are organized in alphabetical order and molecules of the same family are grouped together. The names of proteins are upper case and not italicized. mRNAs and transgenic mouse lines are italicized.

whether multiple zones interact during motor control. Perhaps one way to unravel what zones do is to uncover how they form. Indeed, developmental studies have raised two critical questions that are ultimately relevant to cerebellar behavior: (1) what are the cellular and molecular mechanisms that control Purkinje cell zone development? and (2) how do climbing fiber projections invade, recognize, and connect to their targets?

GENETIC LINEAGE, MIGRATION, AND AXONOGENESIS OF INFERIOR OLIVE CELLS

Several landmark studies have used the regulatory sequences of developmentally expressed genes to design genetic tools for tracking the fate of cerebellar and inferior olive cells from embryogenesis to adulthood (Rodriguez and Dymecki, 2000; Hoshino et al., 2005; Machold and Fishell, 2005; Pascual et al., 2007). Genetic fate-mapping studies using *Atonal homolog 1* (*Atoh1*, formerly known as *Math1*) and *Wnt1* regulatory elements revealed that inferior olive neurons emerge from a distinct progenitor pool in the lower rhombic lip of the hindbrain (Rodriguez and Dymecki, 2000; Landsberg et al., 2005; Wang et al., 2005; Nichols and Bruce, 2006). In accordance with these findings, genetic fate-mapping using a *pancreas specific transcription factor 1a-Cre* (*Ptf1a*$^{Cre/+}$) allele to drive *lacZ* reporter gene expression in *R26R* [*Gt(ROSA)26Sor*^{tm1sor}; Soriano, 1999] mice revealed that inferior olivary neurons are derived from a distinct *Ptf1a* domain (Hoshino et al., 2005; Yamada et al., 2007). Hoshino and colleagues determined that *Ptf1a* is required for the proper development of inferior olive neurons, because the inferior olivary complex is severely altered in *Ptf1a* null mutants (Yamada et al., 2007). Without *Ptf1a*, some inferior olive neurons do not differentiate while others migrate inappropriately. Moreover, a large number of apoptotic cells were observed in the *Ptf1a* mutants, and the fate of *Ptf1a*-dependent lineages adopted mossy fiber neuron characteristics (Yamada et al., 2007). Although *Ptf1a* appears to control the development of most, if not all, olivary neurons, it is not clear what upstream or downstream molecular pathways might be responsible for generating the sub-nuclei. Studies by Bloch-Gallego and colleagues provide some insight into this question. The authors determined that the absence of Rho-guanine exchange factor Trio impairs the organization of the inferior olivary nucleus into distinct lamellae (Backer et al., 2007). Additionally, in a recent elegant study, quail-chick chimaeras were used to provide evidence that each inferior olive sub-nucleus originates from

specific rhombomeres, developmental hindbrain units that are each restricted in their lineages (Hidalgo-Sanchez et al., 2012). It is intriguing that climbing fiber zones, which arise from distinct olivary sub-nuclei, may be specified early by rhombomere specific cues.

Inferior olive neurons are born dorsally in the lower rhombic lip and migrate circumferentially around the edges of the brainstem to their final location near the ventral midline (Altman and Bayer, 1987; Sotelo, 2004; Sotelo and Chedotal, 2005) (**Table 2**). Tritiated thymidine labeling (Altman and Bayer, 1987) and HRP axonal tracing *in vitro* (Bourrat and Sotelo, 1988, 1990b) revealed that inferior olivary neurons migrate along the lateral edges of the brainstem in a unique "submarginal stream" (Altman and Bayer, 1987; Bourrat and Sotelo, 1988, 1990b; Sotelo and Chedotal, 2005). Interestingly, the somata of olivary neurons do not cross the floor plate, whereas their axons do cross and project exclusively to the contralateral cerebellum (Altman and Bayer, 1987; Altman, 1997). The restriction of olivary neurons to one side of the midline is controlled by both chemoattractive and chemorepellent molecules (e.g., netrin-1/DCC and Slit/Robo; Bloch-Gallego et al., 1999; Causeret et al., 2002; de Diego et al., 2002; Marillat et al., 2004). Marillat et al. (2004) showed that Rig-1/Robo3 plays an essential role in controlling the migration of precerebellar neurons and the projection of axons across the midline. In *Rig1/Robo3* deficient mice, inferior olive neurons incorrectly send axons to the ipsilateral cerebellum in addition to sending the normal contralateral projection (Marillat et al., 2004).

The first climbing fibers arrive in the developing cerebellum at ~embryonic day (E) 14/15 in the mouse (Paradies and Eisenman, 1993) (**Table 2**) and are already organized in a crude zonal map at ~E15/16 (Sotelo et al., 1984; Chedotal and Sotelo, 1992; Paradies and Eisenman, 1993; Paradies et al., 1996), which is approximately when Purkinje cells begin to express parasagittal markers (e.g., *engrailed1/2* and *L7/Pcp2*) (Hashimoto and Mikoshiba, 2003; Wilson et al., 2011). By ~E17 in mice, olivocerebellar topography strongly corresponds with the nascent architecture of Purkinje cell zones (Paradies et al., 1996; **Figure 4A**).

FORMATION OF OLIVOCEREBELLAR ZONES

The almost perfect overlap between climbing fiber terminal field topography and Purkinje cell zones suggests that the spatial and temporal targeting of cerebellar afferent pathways is closely coordinated with Purkinje cell development. Purkinje cells become postmitotic between ~E10 and ~E13 and form symmetrical

Table 2 | Timeline of olivocerebellar development.

Developmental stage	Developmental event	References
~E12/13 rat (E10/11 mouse)	Inferior olive neurons are born	Pierce, 1973; Bourrat and Sotelo, 1990a, 1991; Sotelo, 2004
~E14/15 mouse	Climbing fibers arrive in cerebellum	Paradies and Eisenman, 1993
~E16–E18 rat (E14–16 mouse)	Inferior olive neurons settle in final position adjacent to the floor plate	Bourrat and Sotelo, 1990a; Sotelo, 2004
~E16 rat (E14 mouse)	Transient biochemical compartmentation of inferior olive and Purkinje cells (arising independently)	Wassef et al., 1992; Larouche et al., 2006
~E15/16 mouse	Climbing fibers organize into crude parasagittal clusters	Paradies and Eisenman, 1993
~E17 mouse	Climbing fiber topography corresponds clearly with nascent Purkinje cell zone	Paradies et al., 1996
~P0–P5 rat (P0–P3 mouse)	Olivocerebellar projections resolve into precise sagittal zones similar to the adult	Sotelo et al., 1984
~P0 rat (P0 mouse)	Creeper stage starts	Watanabe and Kano, 2011
~P0–P10 rat (P0–P8 mouse)	Critical period for olivocerebellar plasticity	Sherrard et al., 1986
~P3 mouse	Discrete climbing fiber mediated EPSCs recorded in Purkinje cells (all fibers induce similar amplitudes in perinatal Purkinje cells)	Hashimoto and Kano, 2003
~P5 rat (P3 mouse)	Pericellular nest stage starts	Watanabe and Kano, 2011
~P5 mouse	Development of climbing fiber terminal structure	Mason and Gregory, 1984
~P7 mouse	"Winner" climbing fiber is strengthened	Hashimoto and Kano, 2003
End of the first postnatal week	Climbing fiber complex spikes are first detected	Woodward et al., 1969
~P9 rat (P7 mouse)	Capuchon stage	Watanabe and Kano, 2011
~P12 rat (P10 mouse)	Dendritic stage commences	Watanabe and Kano, 2011
~P7–11 rat (P5–9 mouse)	Climbing fiber pruning and perisomatic synpase elimination: the early phase	Watanabe and Kano, 2011
~P12–17 rat (P10–15 mouse)	Climbing fiber pruning and perisomatic synpase elimination: the late phase	Watanabe and Kano, 2011

zonal "clusters" by ~E14 (Hashimoto and Mikoshiba, 2003; Hoshino et al., 2005; Sillitoe et al., 2009; Namba et al., 2011; Sudarov et al., 2011). Climbing fiber neurons are also born at ~E10/11 (Sugihara and Shinoda, 2007). Interestingly, when they arrive in the developing cerebellum they immediately project into clusters of Purkinje cells (Paradies and Eisenman, 1993). The predictable termination of climbing fibers into Purkinje cell zones suggests that the Purkinje cells may play an active role in instructing the pattern of olivocerebellar targeting.

Sotelo and collaborators postulated that the cerebellum and the inferior olive might have matching gene expression domains that establish bidirectional signaling to generate the olivocerebellar map (Sotelo and Wassef, 1991; Sotelo and Chedotal, 1997, 2005). Support for this hypothesis was first provided by using a combination of markers that labeled zones of Purkinje cells (calbindin, GMP-cyclic dependent protein kinase, Purkinje cell-specific glycoprotein, and PEP-19) and also marked corresponding subsets of inferior olive cells along with their projections [calbindin, parvalbumin, and calcitonin gene-related peptide (CGRP); **Table 1**]. The precision and reproducibility of zonal boundaries defined by these markers suggested the possibility that inferior olivary neurons might target Purkinje cell zones by recognizing positional cues (Sotelo and Wassef, 1991; Sotelo and Chedotal, 1997, 2005).

Eph/ephrin genes play a major role in establishing brain topography (Flanagan and Vanderhaeghen, 1998; Cang et al., 2008a,b; Allen-Sharpley and Cramer, 2012). In the cerebellum, eph/ephrin are expressed in distinct parasagittal domains (Karam et al., 2000, 2002). Nishida and coworkers (2002) provided compelling evidence for the involvement of eph/ephrin signaling in controlling the molecular matching between climbing fibers and Purkinje cells during olivocerebellar circuit formation (Nishida et al., 2002). They showed that altering ephA receptor and ephrin-A ligand expression in chick hindbrain explant cultures disrupted the anterior–posterior targeting of olivocerebellar axons. However, this study did not address whether eph/ephrin signaling controls the development of olivocerebellar zones (Nishida et al., 2002; Hashimoto and Hibi, 2012). Regardless, because specific ephA/ephrin-A manipulations can disrupt the global targeting of olivocerebellar axons, there is a possibility that other eph/ephrins and/or additional molecules likely cooperate to establish precise Purkinje cell-afferent interactions during map formation. Besides the eph/ephrins, possible candidates are the type-II classic cadherin and δ-protocadherin cell–cell adhesion molecules, which are expressed in a striking array of Purkinje cell sagittal zones (Suzuki et al., 1997; Neudert et al., 2008; Redies et al., 2011) and in specific subdivisions of the inferior olive (Suzuki et al., 1997; Neudert et al., 2008; Redies et al., 2011). Despite these clues, we still do not have a clear picture of what genes control the topographic connectivity of olivocerebellar zones nor do we understand the detailed mechanisms that initiate and maintain the physical interaction between specific Purkinje cells and climbing fibers. However, recent work demonstrates that starting from birth, inferior olive neurons spontaneously organize into clusters that fire synchronous Ca^{2+} transients in *in vitro* brain slice preparations (Rekling et al., 2012). Curiously, during early postnatal development spontaneous waves travel along

chains of axon collaterals that connect sagittal rows of Purkinje cells (Watt et al., 2009). Both phenomena were suggested as likely mechanisms contributing to the development of cerebellar compartments. However, whether the spontaneous waves of Purkinje cell activity are linked to the spontaneous activity of inferior olive neurons awaits further analysis. It will also be interesting to determine whether cerebellar spontaneous activity interacts with developmental gene function in a zone specific fashion.

POSTNATAL REMODELING OF CLIMBING FIBERS

Following the establishment of the crude zonal map, climbing fibers undergo extensive morphological changes and proceed through different stages of fiber remodeling to form functionally mature connections (Watanabe and Kano, 2011) (**Table 2**). The first phase of remodeling is the "creeper" stage (~P0 in rat) when climbing fibers are very thin and form transient synapses on immature Purkinje cell dendrites (Chedotal and Sotelo, 1993; Sugihara, 2005; Watanabe and Kano, 2011). Then, climbing fibers enter a "transitional" stage and exhibit characteristics that are intermediate between those of the creeper and nest stages (Sugihara, 2005). The "pericellular nest' stage (~P5) is defined by the dense terminal arbors ("nest") that surround Purkinje cell somata (Cajal, 1911; O'Leary et al., 1971; Mason et al., 1990; Sugihara, 2005; Watanabe and Kano, 2011). During this stage, each Purkinje cell receives polyneuronal input from more than five different climbing fibers. Climbing fibers are progressively displaced onto the developing dendritic stems of maturing Purkinje cells ("capuchon stage"; starting at ~P9). As the dendritic arbors develop, the climbing fibers leave their perisomatic and capuchon positions to occupy peridendritic positions (after ~P12; referred to as dendritic stage; Chedotal and Sotelo, 1992; Watanabe and Kano, 2011). During this period, climbing fibers translocate up the Purkinje cell dendrite to find their ultimate location within the basal two thirds of the molecular layer (Crepel et al., 1976; Mariani and Changeux, 1981; Hashimoto and Kano, 2005; Kano and Hashimoto, 2009; Watanabe and Kano, 2011).

The monoinnervation of adult climbing fibers onto Purkinje cells is achieved through massive pruning of climbing fibers during postnatal development. Previous studies have revealed systematic changes occurring in the relative synaptic strength of multiple climbing fibers when they polyinnervate a single Purkinje cell during postnatal development. These studies revealed that climbing fiber mediated excitatory postsynaptic currents (EPSCs) recorded in Purkinje cells have similar amplitudes until ~P3. In the second postnatal week, multiple EPSCs differentiate into one large EPSC and a few small EPSCs (Hashimoto and Kano, 2003). These results suggest that climbing fiber synaptic strengths are similar to one another during early postnatal development, and a single climbing fiber, the "winner," is selectively strengthened during the second postnatal week (~P7; Hashimoto and Kano, 2003; Bosman et al., 2008). Following these studies, Kano and colleagues used electrophysiological and morphological techniques to determine that competition between multiple climbing fibers occurs at the soma before climbing fibers form synapses with Purkinje cell dendrites (Hashimoto et al., 2009). Notably, the "winner" climbing fiber undergoes translocation to the dendrites

and simultaneously maintains synapses on the soma, while the weaker climbing fibers remain around the soma forming "pericellular nests" with the "winner" synapses (Hashimoto et al., 2009). After the strengthening of a single "winner" climbing fiber, pruning and perisomatic synapse elimination occur in two distinct phases: the early phase (∼P7–11), which is independent of parallel fiber synapses and the late phase (∼P12–17), which depends on activity between parallel fibers and Purkinje cells (Watanabe and Kano, 2011).

In three different mutant mice, *weaver*, *staggerer*, and *reeler*, Purkinje cells develop in the absence of granule cells but are permanently innervated by multiple climbing fibers (Crepel and Mariani, 1976; Mariani et al., 1977; Crepel et al., 1980; Mariani and Changeux, 1980; Steinmayr et al., 1998). Similarly, studies using experimentally-induced "hypogranular" cerebella (Woodward et al., 1974; Crepel and Delhaye-Bouchaud, 1979; Bravin et al., 1995; Sugihara et al., 2000) revealed that the presence of intact granule cells, normal parallel fiber-Purkinje cell synapses, and activity all play a role in climbing fiber synapse elimination.

The process of fiber elimination is mediated by several molecules including metabotropic glutamate receptor mGluR1, PLCβ4, Ca(v)2.1 P/Q-type Ca^{2+} channel, glutamate receptor Glurδ2, precerebellin (or Cbln1), and the GABA synthesizing enzyme GAD67 (Kano et al., 1995, 1997, 1998; Kashiwabuchi et al., 1995; Offermanns et al., 1997; Sugihara et al., 1999; Ichikawa et al., 2002; Miyazaki et al., 2004, 2010; Hirai et al., 2005; Uemura et al., 2007; Hashimoto et al., 2011; Nakayama et al., 2012; Uesaka et al., 2012). Mutations that alter the function of these proteins cause severe defects in climbing fiber synapse development and elimination (Kano et al., 1995, 1997, 1998; Kashiwabuchi et al., 1995; Offermanns et al., 1997; Sugihara et al., 1999; Ichikawa et al., 2002; Miyazaki et al., 2004, 2010; Hirai et al., 2005; Uemura et al., 2007; Hashimoto et al., 2011; Nakayama et al., 2012; Uesaka et al., 2012). Interestingly, Kano and colleagues developed an organotypic co-culture preparation to recapitulate *in vivo* climbing fiber remodeling and with this system identified neuroligin-2 as a key player of climbing fiber elimination in Purkinje cells (Uesaka et al., 2012). Thus, synaptogenesis in the olivocerebellar projection starts relatively early during brain circuit formation, occurs over a protracted period of time, and requires both genetic control and neural activity (Chedotal and Sotelo, 1992; Sotelo, 2004). However, it is not clear whether developmental remodeling plays a role in generating climbing fiber compartments: although one can imagine that the precise zonal boundaries emerge as supernumerary axons are pruned away.

PLASTICITY OF OLIVOCEREBELLAR ZONE CONNECTIVITY

In contrast to the adult central nervous system which has a limited capacity for axonal regeneration, the immature central nervous system is capable of some axonal regrowth (Nicholls and Saunders, 1996). However, regrowth during development frequently occurs through an alternative pathway that is distinct from the normal one. The olivocerebellar pathway is an excellent example of a system in which regrowth establishes a new pathway. Various groups have used the pedunculotomy approach to stimulate transcommissural olivocerebellar reinnervation to determine the temporal properties of afferent-target interactions during development (Angaut et al., 1985; Sherrard et al., 1986; Zagrebelsky et al., 1997; Sugihara et al., 2003; Dixon et al., 2005; Willson et al., 2007). Following unilateral early postnatal transsection of an inferior cerebellar peduncle (which carries the climbing fibers), the contralateral inferior olive degenerates and new axons, arising from the remaining inferior olive, grow into the denervated hemicerebellum (Zagrebelsky et al., 1997). The innervation of these transcommissural axons precisely aligns with Purkinje cell expression zones and mirrors the distribution of the "unaltered" projections on the intact side (Zagrebelsky et al., 1997). Sugihara and colleagues (2003) have shown that the newly formed projections develop normal climbing fiber arborizations and form functional synapses onto Purkinje cells. Remarkably, olivocerebellar reinnervation can compensate for motor deficits (Dixon et al., 2005) and rescue the cerebellums influence over spatial learning (Willson et al., 2007). Similar to what might occur during normal development, reinnervation may be regulated by position-dependent cues that mediate the precise connectivity between climbing fibers and Purkinje cells (Dixon and Sherrard, 2006; Willson et al., 2008).

NOVEL TOOLS TO STUDY OLIVOCEREBELLAR DEVELOPMENT, CONNECTIVITY, AND FUNCTION

Neuronal tracing using viruses and genetically encoded fluorescent reporters are now widely used for unraveling circuit connectivity (Wickersham et al., 2007; Marshel et al., 2010; Wall et al., 2010). Retrograde transneuronal infection of rabies virus reveals the organization of multi-synaptic neuronal networks (Coulon et al., 1989; Ugolini, 1995; Kelly and Strick, 2000; Graf et al., 2002). Genetically modified viruses have also allowed control over which cells are initially infected, extent of viral spread, and direction of the spread (Callaway, 2008). Recently, the use of a deletion-mutant rabies virus allowed the spread of the virus to be restricted to monosynaptic connections for selectively revealing first-order presynaptic neurons (Wickersham et al., 2007, 2010; Marshel et al., 2010; Rancz et al., 2011). Using the rabies virus tracing approach, communication networks between the cerebral cortex, basal ganglia, and cerebellum have been resolved (Kelly and Strick, 2003; Bostan et al., 2010; Coffman et al., 2011; Suzuki et al., 2012). More recently, Ruigrok and colleagues also used viral tracing to show that cerebrocerebellar connectivity respects cerebellar zonal organization (Suzuki et al., 2012). Combining viral tracing with transgenic targeting of recombinant viruses (Weible et al., 2010) will allow for unparalleled resolution of circuit topography in the olivocerebellar pathway.

In the past, lesioning, electrical stimulation, and chemical activation/deactivation have unveiled essential functions of the cerebellum and inferior olive (Llinas et al., 1975; McCormick and Thompson, 1984; Bradley et al., 1991; O'Hearn et al., 1993; O'Hearn and Molliver, 1993; Willson et al., 2007; Pijpers et al., 2008; Strick et al., 2009; Horn et al., 2010; Cerminara and Apps, 2011). However, these manipulations are limited by the lack of cell type specificity and/or the by the tissue damage that occurs. Optogenetics methods offer an ideal solution to these shortcomings as they provide an avenue for targeting induced

neural activity to specific cells *in vivo*, without damaging the circuit (Deisseroth et al., 2006; Zhang et al., 2006; Hira et al., 2009; Tsubota et al., 2011). These light-activated ion channels, which include channelrhodopsin-2 (ChR2) and halorhodopsin (eNpHR), have fast temporal kinetics to efficiently activate or inhibit the firing of action potentials (Boyden et al., 2005; Zhang et al., 2006; Adamantidis et al., 2007; Arenkiel et al., 2007; Abbott et al., 2009). Importantly, by using cell type specific promoters one can drive the expression of these light-responsive proteins in selective neuronal populations (e.g., using the *L7/Pcp2* Purkinje cell specific promoter; Oberdick et al., 1990). Indeed, a recent study used *L7/Pcp2-Cre* mice to target ChR2 and eNpHR expression to examine the role of Purkinje cells in controlling cardiovascular function (Tsubota et al., 2011). It will now be interesting to develop optogenetic methods for manipulating neuronal activity within specific inferior olivary nuclei in order to determine the contribution of olivocerebellar zones to motor and nonmotor functions *in vivo*.

SUMMARY

It is well established that the cerebellum is divided into a complex map of functional zones. Much progress has been made in delineating the zonal topography between the inferior olivary nucleus, cerebellar cortex, and the cerebellar nuclei. However, there are several important questions that remain unanswered. For example: (1) Are the olivocerebellar cells that project to each cerebellar zone born at different times and/or are they derived from different genetic lineages? (2) What are the molecular mechanisms that guide olivocerebellar projections into zonal compartments? and (3) What behaviors are encoded into each zone? In future studies, it will be interesting to combine modern anatomical tracing techniques with high-resolution imaging, sophisticated genetic approaches and electrophysiology to answer such questions.

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