Molecular Layer Interneurons: Key Elements of Cerebellar Network Computation and Behavior

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Abstract—Molecular layer interneurons (MLIs) play an important role in cerebellar information processing by controlling Purkinje cell (PC) activity via inhibitory synaptic transmission. A local MLI network, constructed from both chemical and electrical synapses, is organized into spatially structured clusters that amplify feedforward and lateral inhibition to shape the temporal and spatial patterns of PC activity. Several recent in vivo studies indicate that such MLI circuits contribute not only to sensorimotor information processing, but also to precise motor coordination and cognitive processes. Here, we review current understanding of the organization of MLI circuits and their roles in the function of the mammalian cerebellum.

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Key words: molecular layer interneurons, network organization, electrical synapses, convergence, cognitive outcomes.

INTRODUCTION

As long advocated by Masao Ito, the cerebellum is a very favorable system for study of basic principles of brain function (Ito, 1984, 2006, 2008; Apps and Garwicz, 2005; D’Angelo and Casali, 2012). The relatively simple and repeating circuit organization of the cerebellum has been very valuable in revealing the relationship between circuit structure and function. Detailed understanding of the anatomical and physiological properties of the cerebellar network have made it possible to develop precise computational models that yield insights into cerebellar information processing, as well as resultant cognitive and behavioral outcomes (Medina and Mauk, 2000; Jorntell et al., 2010).

The cerebellum consists of the cerebellar cortex, which integrates and processes sensory-motor information, and the deep cerebellar nuclei (DCN) that direct this information out of the cerebellum (D’Angelo et al., 2016). The cerebellar cortex is organized into three layers: granule, Purkinje and molecular layers. Purkinje cells (PCs) are the only output neurons of the cerebellar cortex; the activity of PCs is regulated by excitatory input from parallel fibers (PFs) and climbing fibers (CFs), as well as local inhibitory input from molecular layer interneurons (MLIs; Eccles et al., 1967; Palay and Chan-Palay, 1974). Therefore, deciphering how these excitatory and inhibitory circuits collectively regulate PC activity is the key to understanding the function of the cerebellar cortex. While excitatory synaptic transmission from PFs and CFs to PCs has been extensively investigated, local inhibitory control of PCs by MLIs has received much less attention. Therefore, this review will focus on our current understanding of the function and circuit organization of MLIs in the mammalian brain. We first describe MLI types, then consider MLI synaptic connectivity and how this creates local circuits that precisely regulate PC activity. Finally, we will summarize what is currently known about the role of MLI circuits in cerebellum-mediated behaviors and conclude by posing some fundamental questions about MLI circuits that remain unanswered.

ANATOMY AND PHYSIOLOGY OF MLIs

Traditionally, MLIs have been classified into two different types – basket cells (BCs) and stellate cells (SCs) – based on their morphology and the location of their cell bodies within the molecular layer (Fig. 1; Eccles et al., 1967; Palay and Chan-Palay, 1974; Dorgans et al., 2019). In this section we compare and contrast the properties of these two types of MLI.

MLIs are derived from progenitors in the cerebellar ventricular zone (VZ) that express the transcription factor Ptf1-a. Within the first postnatal week, nascent MLIs migrate and differentiate until the end of the second postnatal week (Hoshino et al., 2005; Schilling, 2011; Sotelo, 2015; Sergaki et al., 2017). Early-born MLIs migrate earlier and tend to differentiate into BCs that settle within the inner molecular layer, whereas later MLIs migrate into the outer molecular layer, to become SCs (Schilling, 2011). Thus, the morphology of MLIs is a consequence not only of differential gene expression but also...
the timing of migration: late-maturing SCs have limited space to project their axons and dendrites after the earlier-maturing BCs have already established their more elaborate structures within the molecular layer (Schilling, 2011; Sotelo, 2015; Sergaki et al., 2017).

Both MLI types have small somata (~10 μm diameter), as well as dendrites and axons that are mainly elaborated within the sagittal plane (Fig. 1; Palay and Chan-Palay, 1974). BCs have long and relatively straight dendrites that extend upward to the pial surface with relatively few branches (Fig. 1, left). The main axon of BCs is several hundred micrometers long and runs in one direction, parallel to the PC layer, giving off multiple descending and ascending collaterals along its course (Bishop, 1993; Southan and Robertson, 1998). Thick descending collaterals form pericellular baskets around the PC soma and pinceau terminals around the PC axon initial segment, providing both the name of these MLIs as well as strong inhibitory control of PC activity (King et al., 1993; Vincent and Marty, 1996; Sultan and Bower, 1998). Several thin ascending collaterals are relatively short and are found within the inner two-thirds of the molecular layer.

In contrast, SCs are located in the outer two-thirds of the molecular layer and have multiple dendrites and axons radiating from the cell body, yielding the star-like structure that gives SCs their name (Fig. 1, right; Palay and Chan-Palay, 1974). Their dendritic arbors consist of many twisted branches and thin, beaded axonal plexus that arborize in many directions within the molecular layer, thereby providing inhibitory input to PC dendrites. SCs exhibit a gradient in their morphological features: SCs in the intermediate molecular layer tend to have long and highly varicose axon collaterals, while those in the outer molecular layer tend to have relatively short dendrites and axons (Palay and Chan-Palay, 1974). This creates a gradient of SC morphology within the molecular layer: cells in the middle of the molecular layer often have structures that are intermediate between those of canonical BCs and SCs (Sultan and Bower, 1998).

All MLIs are fast-spiking interneurons that can fire action potentials up to 300 Hz (Bao et al., 2010). They fire spontaneous action potentials in an irregular pattern, with a mean frequency of 12 Hz in brain slices (Hausser and Clark, 1997). Such irregular spiking may offer advantages for information processing by providing rapid, sensitive and linear responses to external input (van Vreeswijk and Sompolinsky, 1996). MLIs fire spontaneous action potentials even in the absence of excitatory synaptic inputs, indicating that they possess intrinsic mechanisms for generating spontaneous activity (Hausser and Clark, 1997). In vivo, MLIs fire irregularly at frequencies of 1–35 Hz, which is consistent with brain slice measurements. The irregular firing properties of MLIs are often used to identify MLIs in vivo (Ruigrok et al., 2011; Jirenhed et al., 2013). The similarity between MLI firing frequency in brain slices and in vivo suggests that, even in vivo, MLI spiking is driven by intrinsic cellular properties, rather than excitatory synaptic input (Hausser and Clark, 1997). Spontaneous activity in MLI networks plays a major role in modulating the firing pattern of their postsynaptic targets, which are both MLIs and PCs (Hausser and Clark, 1997). MLI spontaneous firing rate is increased by at least two neuromodulators: norepinephrine, which acts via β-adrenergic receptors (Kondo and Marty, 1998a), and glutamate, which activates metabotropic glutamate receptors (Karakossian and Otis, 2004; Collin et al., 2009). The physiological significance of such modulation merits further investigation.

Action potential frequency is very similar in BCs and SCs (Hausser and Clark, 1997). More generally, most electrophysiological properties of BCs and SCs are very similar (Vincent and Marty, 1996). Further, as mentioned above, there may be no sharp morphological distinction between BCs and SCs (Sultan and Bower, 1998). Therefore, a recent trend is to consider BCs and SCs as subtypes of the same interneuron and to lump them together as MLIs (Sultan and Bower, 1998; Jorntell et al., 2010; Sotelo, 2015). Nonetheless, subcategories of MLIs can be distinguished on the basis of their synaptic properties (Bao et al., 2010; Dorgans et al., 2019). Recent single-cell transcriptomics analyses that classify cerebellar neurons based on their molecular profiles (Carter et al., 2018; Rosenberg et al., 2018; Saunders et al., 2018; Peng et al., 2019; Rodrigues et al., 2019) suggest that there are more than two types of MLIs (Saunders et al., 2018; Kozareva et al., 2020). This parallels transcriptomic analyses of other brain areas that have...
revealed unexpectedly high interneuronal heterogeneity (Munoz-Manchado et al., 2018; Zeng et al., 2018).

MLI CIRCUIT ORGANIZATION: ELEMENTS, ORGANIZATION & INHIBITORY ACTIONS

Because MLIs inhibit the activity of their postsynaptic PC targets, MLI networks ultimately control the output of the cerebellar cortex. Therefore, understanding how MLI circuits are organized to control PC activity with high temporal and spatial precision is critical for understanding cerebellar information processing. This section describes the components of MLI circuits and how these circuits are organized to inhibit PCs.

a) Excitatory inputs to MLIs

**Granule cells**—Like PCs, MLIs receive excitatory inputs from granule cells (GC; Fig. 2). GC send both ascending axons and PFs into the molecular layer to directly excite both PCs and MLIs (Palay and Chan-Palay, 1974; Mittmann et al., 2005), although these excitatory synapses on MLIs have somewhat different properties than those on PCs (Eccles et al., 1967; Ito, 1984; Jirenhed et al., 2013). The PF synaptic responses of MLIs typically exhibit rapid and phasic kinetics (Fig. 3A). These responses increase in a graded fashion as stimulus intensity is increased, indicating that multiple PFs innervate each MLI (Szapiro and Barbour, 2007; Coddington et al., 2013).

PF-MLI synapses exhibit multiple forms of short-term plasticity, with a high-frequency (50–100 Hz) burst of PF activity typically causing an initial, mild synaptic facilitation followed by a pronounced synaptic depression (Bao et al., 2010; Dorgans et al., 2019). However, the relative amounts of these two forms of short-term synaptic plasticity can vary: one study indicates that PF-BC synapses exhibit more significant synaptic depression than do PF-SC synapses (Bao et al., 2010), while another study reports that four different patterns of synaptic plasticity can be identified at different PF synapses on either BC or SC (Dorgans et al., 2019). Both studies indicate that PF-MLI synaptic diversity arises from the heterogeneous expression of presynaptic proteins, such as Munc13-3 (Bao et al., 2010) and synapsin II (Dorgans et al., 2019), that regulate glutamate release from GC presynaptic terminals. Such heterogeneity of short-term plasticity at PF-MLI synapses is likely to expand the dynamic range of information coding by MLIs by controlling spiking patterns; for example, by determining the onset of action potential firing and the latency to peak action potential frequency (Dorgans et al., 2019). This heterogeneity of PF-MLI synaptic plasticity may also allow MLIs to differentially inhibit PC cell bodies and dendrites: during high-frequency GC activity, PC somata receive strong but short-lived inhibition from BCs while the PC dendritic arbor receives sustained inhibition from SCs (Bao et al., 2010). Therefore, the diverse properties of PF-MLI synapses may have fundamental significance for input-specific control of PC information processing, which in turn underlies cerebellar cognitive and motor output.

High-frequency activity at PF synaptic inputs onto MLI not only changes the amplitude of postsynaptic EPSCs, but also reduces postsynaptic calcium entry via a rapid change in AMPA receptor subunit composition (Liu and Cull-Candy, 2000). This activity-dependent change in synaptic receptor properties can also be induced by norepinephrine release associated with emotional stress, suggesting that such plasticity may play a significant role in shaping emotional learning and the resultant behavioral changes (Liu et al., 2010). Further
fine-tuning of PF-MLI synapses may be provided by many other mechanisms, such as retrograde signaling (Soler-Llavina and Sabatini, 2006; Bender et al., 2009), intracellular calcium dynamics (Collin et al., 2009), multiple neuromodulators including norepinephrine, 5-HT and GABA (Mitoma and Konishi, 1999; Howell and Pugh, 2016), and perhaps presynaptic long-term plasticity (Jorntell and Ekerot, 2002). This impressively broad spectrum of mechanisms for adjusting the efficacy of GC-MLI synapses allows MLIs to dynamically and precisely control the activity of PCs.

Electrical synapses – Electrical synapses are also an important element in constructing MLI networks. It is well established that MLI networks include substantial electrical coupling, with action potentials in one MLI producing “spikelets” that are capable of evoking action potentials in neighboring MLIs (Jorntell and Ekerot, 2002, 2003). This impressively broad spectrum of mechanisms for adjusting the efficacy of GC-MLI synapses allows MLIs to dynamically and precisely control the activity of PCs.
within the inner molecular layer (BCs) express higher levels of connexin 36 than MLIs in the outer molecular layer (SCs). Correspondingly, BCs have more extensive electrical synaptic connections with neighboring MLIs than do SCs (Alcami and Marty, 2013; Rieubland et al., 2014). It has recently become clear that electrical synaptic interactions between MLIs contribute significantly to the organization of MLI circuits by creating a specific pattern of spatiotemporally synchronized activity (Alcami and Marty, 2013; Kim et al., 2014; Rieubland et al., 2014). Thus, differences between BCs and SCs in the formation of electrical synaptic circuits suggests that SCs may generate a more spatially restricted, local inhibitory influence on PC dendrites, while BCs provide stronger, broader and more concerted inhibitory control over PC cell bodies.

b) Inhibitory input to MLIs

MLIs– MLIs mainly receive inhibitory inputs from neighboring MLIs (Fig. 2; Hausser and Clark, 1997; Kondo and Marty, 1998b; Mittmann et al., 2005; Rieubland et al., 2014). Their inhibitory control includes both phasic and tonic inhibition, which are respectively mediated by synaptic and extrasynaptic GABA_A receptors (Llano and Gerschenfeld, 1993; Hausser and Clark, 1997; Farrant and Nusser, 2005). Synaptic connections between MLIs have been most directly characterized by multiple patch-clamp recordings (Fig. 3C; Kondo and Marty, 1998b; Rieubland et al., 2014). The overall probability of detecting a pair of MLIs connected by chemical synapses is relatively high (about 20%), suggesting widespread chemical inhibitory synapses between MLIs. These synapses can be unidirectional or reciprocal and often include a combination of both chemical and electrical connections (Kondo and Marty, 1998b; Rieubland et al., 2014). While both spontaneous inhibitory postsynaptic currents (IPSCs) and EPSCs are found in MLIs, most spontaneous input is inhibitory: IPSC frequency is 18 Hz, while EPSC frequency is 2 Hz. Most spontaneous IPSCs result from action-potential evoked GABA release (Hausser and Clark, 1997; Kondo and Marty, 1998a, 1998b). Miniature IPSCs also occur in MLIs and are similar to spontaneous IPSCs, with both being quite variable in amplitude. It has been proposed that MLI synapses contain only a few release sites that activate a large number of postsynaptic GABA_A receptors, with the variability in IPSC amplitude arising from heterogeneity in the amount of GABA released (Llano and Gerschenfeld, 1993; Auger and Marty, 1997). Evoked IPSCs exhibit a relatively high failure rate (~0.6), variable amplitudes and short latencies (within 1–2 ms; Fig. 3C; Kondo and Marty, 1998a, 1998b).

Rough estimates of MLI inhibitory synaptic convergence (Hausser and Clark, 1997; Rieubland et al., 2014) indicate that one or two presynaptic MLIs provide chemical synaptic inputs to a postsynaptic MLI. Unpublished results from our optogenetic mapping of MLI-MLI circuits are consistent with this conclusion (Fig. 3D). The spatial organization of circuits was visualized by the photo-stimulation of ChR2-positive presynaptic MLIs by small spots of blue light, while the IPSC responses of postsynaptic MLI were simultaneously measured by whole-cell patch clamp recording (Kim et al., 2014). The presence of two discrete clusters of inhibitory input indicates that at least two presynaptic MLI innervate the postsynaptic MLI (Fig. 3D).

The phasic inhibitory input from presynaptic MLIs can precisely control spike timing in postsynaptic MLIs (Mittmann et al., 2005) and can limit the inhibitory effects of MLIs upon PCs during high-frequency activation of excitatory GC synapses (Kondo and Marty, 1998b; Chen et al., 2017). Finely tuned GC inputs, orchestrated by diverse synaptic plasticity mechanisms (see above) will also influence the activity of MLI-MLI inhibitory circuits, thereby contributing to precise control of PC activity.

Individual MLI also form synaptic contacts with themselves, called autapses (Pouzat and Marty, 1998). Morphological evidence supports the existence of autaptic connections between fast-spiking interneurons in many brain areas, including cortex, striatum and cerebellum (Llano et al., 1997; Bacci et al., 2003). Autaptic responses elicited by somatic stimulation can be detected in about 20% of MLIs; the characteristics of autaptic responses include a short latency, a wide range of amplitudes, including variable quantal sizes, and a relatively high failure rate (~80%) caused by a low release probability (Pouzat and Marty, 1998, 1999). Autaptic currents are generated by activation of GABA_A receptors and depend on voltage-dependent calcium influx, indicating that autaptic transmission is generally similar to conventional synaptic transmission (Pouzat and Marty, 1998, 1999). Autaptic signals in somatodendritic regions of MLIs may serve to modulate the firing dynamics of MLIs (Guo et al., 2016). This autaptic current is distinct from a delayed response mediated by GABA_A autoreceptors found at MLI axonal terminals. Autoreceptor-mediated responses are produced by GABA released from the same presynaptic terminal; these are excitatory and are prominent only until the second postnatal week (Pouzat and Marty, 1999; Trigo et al., 2007).

PCs– Structural observations have long suggested the possibility of feedback input from PCs to MLIs (Hamori and Szentagothai, 1968; Larramendi and Lemkey-Johnston, 1970; Chan-Palay, 1971; O’Donoghue et al., 1989). Recent physiological studies have established the presence of functional inhibitory inputs from PCs to MLIs (Kim and Augustine, 2016; Witter et al., 2016; Halverson et al., 2020). Optogenetic circuit mapping (Wang et al., 2007) and paired whole-cell recordings in cerebellar slices from transgenic mice selectively expressing channelrhodopsin-2 in PCs (Asrican et al., 2013) reveal that a subset of MLIs (~16%), located in the inner third of the molecular layer, receive strong, monosynaptic and non-reciprocal inhibitory input from PCs (Kim and Augustine, 2016). This feedback circuit between PCs and MLIs may play an important role in cerebellar information processing by synchronizing neighboring PCs via disinhibition (Witter et al., 2016; Halverson et al., 2020).

Lugaro cells– Lugaro cells are fusiform interneurons whose cell bodies are located within the granule cell layer, just below PCs. Lugaro cell axons enter the
molecular layer and form multiple symmetric synaptic junctions with the somata and proximal dendrites of MLIs (Laine and Axelrad, 1998; Castejón, 2003; Schilling et al., 2008; Prestori et al., 2019). However, the functional properties of these synapses are entirely unknown. Lugaro cells are excited by serotonin and therefore may modulate the activity of cerebellar interneurons, including MLIs and Golgi cells (Dieudonne, 2001). The organization and function of Lugaro cell synapses and circuits require further investigation.

In summary, MLI receive a number of excitatory and inhibitory synaptic inputs (Fig. 4). MLIs must integrate these inputs and pass the integrated signal on to PCs. By serving as the sole output of the cerebellar cortex, PCs represent a key control point for cerebellar information processing. The main function of MLIs is to provide this control via their spatiotemporally precise inhibitory inputs to PCs.

c) Putting it together: spatial organization of MLI networks

MLIs outnumber PCs by approximately ten to one, suggesting a high degree of convergence of MLI inputs onto PCs (Korbo et al., 1993). MLIs use their chemical and electrical synaptic connections to form spatially organized network clusters (Kim et al., 2014; Rieubland et al., 2014). These clusters can regulate PC activity – from somatic to distal dendrite levels – with highly temporal and spatial precision.

Multiple whole-cell patch-clamp recordings and optogenetic mapping techniques have revealed the connectivity rules and circuit motifs of MLIs. MLI networks exhibit a high degree of clustering that occurs by overlapping chemical and electrical connections (Kim et al., 2014; Rieubland et al., 2014). Chemical synapses among MLIs within clusters tend to show feedforward connectivity rather than a loop motif, consistent with previous observations in other neural networks (Kampa et al., 2006; Jarrell et al., 2012; Rieubland et al., 2014). This feedforward connectivity imparts a top-down directionality within the molecular layer, indicating that chemical inhibitory inputs tend to be preferentially directed from the pial surface downward; that is, from the outer SCs to inner BCs (Rieubland et al., 2014; Arlt and Hausser, 2020). Such structural motifs may create diverse MLI responses to CF input to the cerebellar cortex, which represent the sum of CF-MLI excitation and CF-MLI-MLI feedforward inhibition (Arlt and Hausser, 2020). Engagement of these various MLI microcircuits during sensory processing can disinhibit PCs activated by the same CF input, while delivering local inhibition to PC dendritic fields. This can provide intricate regulation within the soma-dendritic compartments of individual PCs (Rowan et al., 2018; Arlt and Hausser, 2020). MLI-mediated inhibition causes graded suppression of CF-evoked dendritic calcium signals without affecting the activity of PC somata. This can induce a wide range of behavioral learning outcomes by modulating the synaptic plasticity that occurs on PC dendrites (Rowan et al., 2018). Therefore, deciphering the spatial organization of MLI networks is essential for understanding how MLIs control the frequency and timing of PC activity for cerebellar information processing.

Both the chemical and electrical synaptic networks of MLIs are confined to the sagittal plane, reflecting the planar morphology of MLI dendrites and axons within this plane (Palay and Chan-Palay, 1974; Kim et al., 2014; Rieubland et al., 2014). The spatial range of chemical and electrical connections between MLI is approximately 150 μm in the sagittal plane, compared to 30 μm for electrical synapses and 50 μm chemical synapses along the transverse axis (Rieubland et al., 2014). The gradient of MLI morphological properties along the vertical axis of the molecular layer, mentioned above (Sultan and Bower, 1998), may also influence MLI connectivity and shape MLI network dynamics (Trousdale et al., 2012). Chemical and electrical synapses within MLI networks exert complementary roles in enhancing spatiotemporal synchrony by forming specific, non-random connections (Galarreta and Hestrin, 1999; Kopell and Ermentrout, 2004; Rieubland et al., 2014). Collectively, highly interconnected clusters of chemical and electrical synaptic networks between MLIs generate specific microcircuit structures among MLI populations that can precisely and efficiently control PC activity during cerebellar information processing.

Although electrophysiological recordings can provide detailed information about MLI synaptic function, this technique provides only a hint of the rich spatial organization of MLI microcircuits (Rieubland et al., 2014). Application of an optimized high-speed optogenetic mapping technique (Petreanu et al., 2007; Wang et al., 2007; Kim et al., 2014) has revealed a more complete picture of the spatial organization of local inhibitory MLI circuits (Fig. 5). To selectively photostimulate MLIs, transgenic mice were generated that express channelrhodopsin-2 (ChR2; Zhao et al., 2011) exclusively in MLIs within the cerebellum (Heiney et al., 2014; Kim...
Fig. 5. Optogenetic mapping of MLI circuit organization. (A) Focal photostimulation of a presynaptic basket cell (BC) expressing ChR2. Left: area (red) where a scanned laser spot elicits action potentials in a BC. Numbers indicate the laser spot positions corresponding to the photoresponses (yellow) shown at right, while bar below traces indicate time of light stimulation (4 ms). Middle: Image of the BC photostimulated in the left panel. Arrows indicate terminal basket structures, which identify this MLI as a BC. Right: Merger of left and middle panels, illustrating the locations (“optical footprint”) where the laser spot evoked evoked BC action potentials. Photostimulation only evoked action potentials when the laser spot was near the soma and proximal dendrites of the BC. (B) Mapping of MLI-PC inhibitory circuit. Left: positions where laser spot evoked IPSCs in a PC. Yellow traces below are the examples of light-evoked IPSCs, while bar above traces indicates time of photostimulation. Pseudocolor scale (see right panel) indicates amplitude of IPSC evoked at each laser location. Middle: Image of dye-filled PC (red), superimposed on a bright field image of a cerebellar slide. Abbreviations: ML, molecular layer; PCL, PC layer; GCL, granule cell layer. Right: merger of left and middle panels, illustrating relationship between IPSC input map and postsynaptic PC structure. (C) Relationship between the areas of MLI-PC input fields (black) and MLI optical footprints (red). The median (dashed lines) of the input area is about 6 times larger than the median of MLI footprints, indicating convergence of multiple MLI inputs upon a PC. (D) Diagram of the spatial organization of MLI (green) circuits providing inhibition to PCs (pink). Electrically coupled clusters of MLIs in the sagittal plane contribute to convergence of MLIs on to PCs. From Kim et al. (2014).
et al., 2014). Small spots of blue laser light (Wang et al., 2007; Schoenenberger et al., 2008; Kim et al., 2014) scanned in sagittal cerebellar slices focally stimulated presynaptic MLIs (Fig. 5A), while the resulting postsynaptic responses were measured in PCs (Fig. 5B, left). Correlating laser light spot position with the amplitude of the light-evoked IPSCs in PCs revealed the spatial organization of local MLI inhibitory circuits (Fig. 5B). MLI-PC input fields typically contain multiple discrete clusters within an area ~300 μm wide. These areas include the PC dendrite – revealing the location of presumed SCs that provide feedforward inhibition to PCs – and an area that extends in one direction along the sagittal axis beyond the PC, presumably reflecting BCs that produce lateral inhibition of PCs (Fig. 3B, right). By comparing the area of the MLI input field to the area where photostimulation evokes action potentials in MLIs (optical footprint; Fig. 5A), we have estimated that at least seven MLIs converge onto a PC (optical footprint; Fig. 5A) and functional estimates of 6–10 MLIs converging on a PC (Palay and Chan-Palay, 1974; Vincent et al., 1992) and two discrete presynaptic MLIs (Fig. 5A), while the resulting postsynaptic responses were measured in PCs (Fig. 5B, right). This mapping approach has also revealed the spatial organization of electrical synaptic connections among MLIs and has quantified the relative contributions of electrical and chemical synapses to MLI convergence (Kim et al., 2014). Disruption of the electrical synapses between MLIs significantly reduces the area of inhibitory input field of PCs, indicating that electrical synaptic coupling has a significant role in convergence of MLIs onto PCs. Each cluster of electrically coupled MLI contains 3–4 cells on average and is significantly disrupted by treatment of gap junction blockers. The conclusion that MLI clusters are heavily interconnected via electrical synapses is consistent with electrophysiological data suggesting that each MLI is likely to form electrical synapses with up to 4 neighboring MLIs (Alcami and Marty, 2013). Disrupting MLI network coupling, by blocking electrical synapses, reduces MLI convergence from seven to two MLIs and often fragments the structure of input fields into two smaller parts. This indicates the presence of at least two discrete presynaptic MLIs that form direct synaptic connections with a PC. Electrical coupled networks of MLIs are highly biased and occur predominantly in the sagittal plane, parallel to the PC layer (Fig. 5D; Kim et al., 2014; Rieubland et al., 2014). Therefore, electrical coupling between MLIs plays an important role in sculpting spatially clustered networks of MLIs and thereby enhancing the convergence of MLIs onto PCs.

In local inhibitory circuits in several brain regions, electrical synapses contribute to temporal integration by synchronizing action potential firing among interneurons (Beierlein et al., 2000; Galarreta and Hestrin, 2001; Long et al., 2004). The results shown in Fig. 5 indicate that electrical synapses also spatially coordinate MLIs. Spatial and temporal integration by both electrical and chemical synapses can also be dynamically controlled by neurotransmitters, neuromodulators and other signals, such as membrane potential and intracellular calcium (Hatton, 1998; McCracken and Roberts, 2006). This provides additional degrees of freedom for network integration. In summary, MLI circuits formed by both chemical and electrical synapses create networks of MLIs that are both spatially and temporally synchronized. These effects create feedforward and lateral inhibition of PCs, effects that are amplified by MLI convergence.

d) MLI network control of PCs

Excitatory input from PFs directly activates both PCs and MLIs; the latter yields feedforward inhibition of PCs activated by the same PFs (Fig. 6A; Eccles et al., 1967; Ito, 1984; Mittmann et al., 2005; Santamaria et al., 2007; Bower, 2010). This feedforward inhibition occurs within approximately 1 ms after PF excitation of PCs, resulting in a sharp reduction in the time window for integration of PF synaptic excitation of PCs and thereby enhancing the precision of spike timing and firing rate in PCs (Mittmann et al., 2005). Several lines of evidence have established that MLI inhibition of PCs is important for precise motor coordination and consolidation of cerebellar motor learning in vivo (see next section and Wulff et al., 2009; Heiney et al., 2014; Yamazaki et al., 2015; Jelitai et al., 2016; Prestori et al., 2019).

MLI axons, particularly the axons of BC, run in the sagittal plane and provide lateral inhibition to PC somata up to 250–350 μm away (Fig. 6A; Palay and Chan-Palay, 1974). In contrast, PFs are oriented in the coronal plane, orthogonal to the MLI axons and their electrically coupled partners. Thus, BCs – and, to a lesser extent, SCs – do not share the same granule cell inputs with their postsynaptic PCs. Because of their differing locations, when PFs activate postsynaptic MLIs and PCs within their path, activated BCs create a sharp boundary by inhibiting the firing of neighboring PCs (Fig. 6B; Palay and Chan-Palay, 1974; Gao et al., 2006; Dizon and Khodakhah, 2011). Electrical coupling within MLI clusters helps establish the spatial range of this lateral inhibition (Cohen and Yarom, 2000; Dunbar et al., 2004; Mittmann et al., 2005; Kim et al., 2014) by spatially synchronizing MLIs and integrating a wide range of granule cell inputs (Bao et al., 2010). Further, the lack of electrical coupling between MLIs in the coronal plane permits discontinuous inhibition of PCs along a parallel fiber beam (Bower, 2010), which has been observed in response to sensory stimuli (Gao et al., 2006). The MLI inhibitory field that controls PC responses to afferent inputs (Cohen and Yarom, 2000) is similarly compartmentalized into sagittal stripes whose boundaries may correspond to zebrin II compartments (Jorntell and Ekerot, 2002; Ekerot and Jorntell, 2003; Consalez and Hawkes, 2012; Valera et al., 2016). Recent work indicates that such compartmentalization arises from MLI inhibitory circuits obeying zebrin II band boundaries (S. Tsuda, A. Nair and G.J. Augustine, unpublished data). Further investigation is necessary to determine whether these inhibitory units for controlling the activity patterns of functional clusters of PCs (i.e. microzones) contribute to local circuit computations.

BC axon collaterals wrap around the soma and axonal initial segment of PCs to create pinceau (Palay and
Although pinceau contain some conventional GABAergic inhibitory synapses, they also participate in unique ephaptic interactions produced by the electrical fields that surround PC axon initial segments when BC axons are active (Iwakura et al., 2012; Blot and Barbour, 2014; Kole et al., 2015). Such interactions produce an almost instantaneous inhibition of PCs that limits PC firing prior to the onset of chemical inhibitory transmission (Blot and Barbour, 2014). Collectively, the organization of MLI networks yields spatiotemporally highly specific inhibitory control of PC activity in response to PF input, thereby precisely controlling the output of the cerebellar cortex.

The strength of both excitatory (PF and CF) and inhibitory (MLI) synaptic inputs to MLIs and PCs is regulated by retrograde release of endocannabinoids by MLIs and PCs (Yoshida et al., 2002; Diana et al., 2002; Safo et al., 2006; Beierlein and Regehr, 2006). Brief depolarization of PCs or high-frequency PF activity causes release of endocannabinoids that transiently suppress excitatory and inhibitory inputs to PCs for several seconds, via activation of presynaptic CB1 receptors (Safo et al., 2006). Similar to PCs, MLIs also can retrogradely control PF synaptic inputs by releasing endocannabinoids in response to either postsynaptic depolarization or synaptic activity, thereby reducing feedforward inhibition of PCs (Beierlein and Regehr, 2006). This retrograde synaptic modulation of PC synaptic strength may contribute to dynamic regulation of synaptic plasticity and cerebellar function (Safo et al., 2006).

### BEHAVIORAL ROLE OF MLI CIRCUITS

The convergence of highly organized MLI inputs onto PCs can suppress overall PC excitation and/or alter PC firing patterns by providing strong inhibition to PC somata and by inhibiting local electrical and chemical signaling within PC dendrites. These inhibitory influences may exert crucial control over cerebellar output related to motor coordination and learning (Medina, 2011; Ito, 2012). Impairment of these circuits could also underlie cerebellar-related cognitive and behavioral deficits (Wulff et al., 2009; ten Brinke et al., 2015). In this section, we consider how these MLI circuits contribute to behavior.

Previous in vivo studies have established that MLI activity is enhanced by sensory stimulation (Jorntell and Ekerot, 2003) and during motor output (Ozden et al., 2012; ten Brinke et al., 2015; Jelitai et al., 2016; Chen et al., 2017). To understand how MLI circuits are engaged during motor behavior, the spatiotemporal patterns of MLI activity has been monitored in vivo. These experiments employed Ca²⁺ imaging based on genetically-encoded Ca²⁺ sensors targeted to MLIs (Astorga et al., 2017; Gaffield and Christie, 2017). During rhythmic oromotor behaviors, such as licking, widespread and robust increases in MLI activity occur within the posterior part of Crus II (Astorga et al., 2017; Gaffield and Christie, 2017). MLI activity is highly correlated with the rate of licking, rather than lick position or sensory feedback, and is arranged along the coronal axis. This indicates that MLI activation in Crus II mainly arises from excitatory PF inputs (Astorga et al., 2017). In contrast, another rhythmic oromotor behavior, bruxing, also increases MLI activity in Crus II but without a coronally organized pattern. This indicates that even though both licking and bruxing involve jaw movements, different spatiotemporal patterns of MLI activity are recruited within the same lobule (Astorga et al., 2017). Even during licking, the kinetics of MLI activity can vary according to motivational state, such as whether an animal is licking to check for the presence of water or to consume water (Astorga et al., 2017). While licking to consume water evoked MLI activity with significant delays, during exploratory licking episodes – to check for the presence of water – there is no significant...
delay before MLI activity increases. These results indicate that MLI circuits are actively involved in processing information for control of continuous movement and that distinct patterns of MLI activity are involved in specific motor behaviors. The recruitment of different cerebrocerebellar circuits may underlie these different spatiotemporal programs of MLI activity during motor coordination. Robust bouts of activity during continuous movement have been detected in a broad population of MLIs, including both BCs and SCs, suggesting that MLIs in Crus II tend to be broadly tuned for encoding licking rate (Gaffield and Christie, 2017). Chemogenetic suppression of MLI output interrupts the precise coordination of movement and results in a reduced lick rate and less organized lick movements (Gaffield and Christie, 2017). Furthermore, suppression of MLI activity in trained mice reverses the hastened lick rate produced by training, suggesting that MLIs are not only important for controlling continuous movements but also are involved in optimizing experience-dependent motor output (Gaffield and Christie, 2017).

Temporally precise optogenetic manipulation of MLI activity has been used to understand how MLI circuits contribute to regulation of motor output. Selective photostimulation of MLIs, in transgenic mice expressing ChR2 exclusively in MLIs, directly tested the hypothesis that MLI-mediated suppression of PC activity generates motor commands (Heiney et al., 2014). Photostimulation of MLIs causes transient suppression of PC activity and elicits eyelid closure, as well as various orofacial movements. Graded inhibition of PC firing proportionally disinhibits postsynaptic neurons in the deep cerebellar nuclei (DCN) that mediate fine control of movement kinematics, including the amplitude, speed and timing of eyelid movements. These results support the “disinhibition hypothesis” of cerebellar function (Albus, 1971; Itô, 1984, 2001) that postulates suppression of PC activity by MLI inhibitory inputs generates motor output and does so by disinhibiting downstream DCN neurons.

Also consistent with this hypothesis is the observation that silencing MLIs via the light-activated proton pump, archaerhodopsin, produces the opposite effects on motor behavior (Jelitai et al., 2016). During voluntary self-paced locomotion, reducing MLI inhibitory input increases the rate and regularity of PC simple spikes and impairs locomotion. Alterations in the balance of excitatory inputs from GCs and inhibitory inputs from MLIs in PC dendrites causes bidirectional changes in simple spike firing and modulates motor output (Jelitai et al., 2016). While enhancing granule cell activity generates a sustained depolarization of PCs during locomotion, variable MLI firing rates correlate with movement changes and provide a counterbalancing feedforward inhibition to PCs (Jelitai et al., 2016). This indicates that modulating the excitation-inhibition balance of PC dendrites creates a diverse range of motor outcomes that depend upon PC activity patterns. Taken together, it is clear that MLI circuits have a major role both in integrating sensorimotor information for ongoing movements and also in commanding motor output by regulating PC activity (Medina, 2011; Provilee et al., 2014).

Additionally, several studies have shown that precise control of PC activity patterns by MLI inhibition is important for cerebellar learning (Wulff et al., 2009; ten Brinke et al., 2015; Rowan et al., 2018). Transgenic mice with GABA_A receptor mutations that selectively impair PC inhibitory synaptic inputs have compromised consolidation of vestibulo-cerebellar motor learning and partial impairment of eyelid conditioning, a cerebellum-dependent form of associative learning. This suggests that MLI circuits are involved in learning-related plasticity (Wulff et al., 2009; ten Brinke et al., 2015). Local chemogenetic suppression of MLI activity in areas involved in eyelid blink (Heiney et al., 2014; Giovannucci et al., 2017) impairs eyelid conditioning (Badura et al., 2018). Optogenetic activation of MLIs also modulates CF-dependent motor adaptation and vestibulo-cerebellar learning by suppressing CF-evoked calcium signaling in PC dendrites (Rowan et al., 2018).

Although the cerebellum has been traditionally considered to be a motor control structure, accumulating evidence indicates that the cerebellum also contributes to cognitive and emotional processing: cerebellar lesions or developmental abnormalities may cause long-term cognitive and/or social impairment such as autism and schizophrenia (Limperopoulos et al., 2007; Passot et al., 2012; Tsai et al., 2012; Reeber et al., 2013; Snow et al., 2014; Peter et al., 2016; Carta et al., 2019). To investigate the role of MLI circuits in non-motor functions, chemogenetic suppression of MLI output has been used to reversibly alter MLI activity in the posterior cerebellar lobules of adult and juvenile mice (Badura et al., 2018). Suppressing MLI activity causes various behavioral alterations that depend on the cerebellar lobule targeted: lobule VI MLI circuits are important for reversal learning, lobule VII circuits for novelty-seeking, and Crus I & II circuits for social preference (Badura et al., 2018). These diverse functional outcomes may arise from the fact that different lobules of the posterior cerebellum connect (via the DCN) to different forebrain regions that yield lobule-specific behavioral consequences (Strick et al., 2009; Wang et al., 2014; Sokolov et al., 2017; Badura et al., 2018). Therefore, MLI-PC circuits may be essential both for precise motor control and for proper cognitive and emotional processing. Suppression of MLI activity in juveniles produces long-lasting alterations in cognitive behavior that persist in adults (Badura et al., 2018), suggesting that the connectivity and activity of MLI circuits have significant impact on the development of cognitive function. Thus, a failure to maintain a proper balance between PC excitation and inhibition in cerebellar networks during development may impair proper maturation of cognitive processing.

CONCLUSIONS

As our survey has amply documented, recent work has substantially advanced our understanding of the organization and function of MLI inhibitory circuits, as well as the roles of these circuits in cerebellar information processing. Nonetheless, numerous fundamental questions about MLI and their circuits
remain unanswered. We offer here a brief list of some of the more obvious questions – at three different levels of organization – to highlight our knowledge gaps and to hopefully guide future research.

1) Circuit functional heterogeneity – Although it is clear that different lobules and compartments within the cerebellum subserve different functions, it also is clear that the structural organization of MLI circuits is quite homogeneous throughout the entire cerebellar cortex. One important question to answer is how MLI circuits – and their multiple converging excitatory and inhibitory inputs – are differentiated to perform specific computations during diverse cerebellar behavioral and cognitive processes. While part of the answer to this question lies in the extensive feedforward and feedback connections between the cerebellum and other brain areas – including the cortex, basal ganglia, thalamus, hippocampus, and brain stem (D’Angelo et al., 2016; Caligiore et al., 2017) – it is likely that local MLI circuitry also is specialized for lobule-specific computations in ways that we do not yet understand.

2) Spatial organization of MLI circuits – One of the most striking features of the cerebellar cortex is its highly organized 3-dimentional structure: to repeat just a few of the examples mentioned above, PC dendrites are oriented entirely in the sagittal plane, while PF axons run entirely in the coronal plane and BC axons are sagittally oriented, while electrical synapses between BCs are also excluded from the coronal plane. Behind this intricate and elegant architecture must be some fundamental design principles that we do not yet grasp. Recent advances in optogenetic circuit mapping permit visualization of the spatial and functional organization of the MLI networks that provide inhibitory control of PCs (Kim et al., 2014). This approach should be useful in studying other components of MLI networks in the future and allow us to understand how these networks integrate sensory-motor information and shape the output of the cerebellar cortex. Such advances in understanding the spatial dimensions of MLI network structure will not only reveal the logic of cerebellar circuit function, but should also provide insights into the rules governing circuit organization and information processing in other brain areas.

3) Integration of MLI input within PC compartments – Despite detailed knowledge of the cellular components of MLI circuits, we still have only an incomplete understanding of the logic behind the compartmentalized control of PC activity arising from MLIs selectively targeting PC somata or dendrites (Rowan et al., 2018). Clearly it is important to understand, both at the level of individual PCs as well as at the level of cerebellar cortical output, why MLIs differentially target these PC compartments.

We are confident that the recent surge of interest in MLI circuits will allow these and many other questions to be answered in the foreseeable future. While it is lamentable that Masao Ito is no longer with us to guide such efforts, it is certain that we will continue to be inspired by his enormous contributions to the cerebellum.

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