Review

Cerebellar molecular layer interneurons – computational properties and roles in learning

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In recent years there has been an increased interest in the function of inhibitory interneurons. In the cerebellum this interest has been paired with successes in obtaining recordings from these neurons *in vivo* and genetic manipulations to probe their function during behavioral tasks such as motor learning. This review focuses on a synthesis of recent findings on the computational properties that these neurons confer to the cerebellar circuitry and on their recently discovered capacity for plasticity and learning *in vivo*. Since the circuitry of the cerebellar cortex is relatively well-defined, the specificity with which the potential roles of these interneurons can be described will help to guide new avenues of research on the functions of interneurons in general.

Interneurons in the mammalian brain

Inhibitory interneurons and their functions in a behavioral context is a subject where our knowledge has long been lagging behind. Traditional methods in neuroscience have always had a strong bias toward recordings from the largest neurons, and these are typically projection neurons. Our interpretations of brain circuitry function are therefore based largely on correlations between spike firing in projection neurons and different aspects of behavior (e.g. Refs [1,2]).

However, the local interneurons which provide the projection neurons with processed inputs are likely to play an important role in defining the function and learning capabilities of brain circuitries. A full understanding of brain function therefore requires that the role of these neurons is also defined. Recent successes in obtaining high-quality intracellular recordings from inhibitory interneurons in different parts of the brain have provided important steps toward understanding learning and the functional operation of synaptically connected local microcircuits [3–6]. The different roles of inhibitory interneurons range from global inhibition [3], which helps to sharpen the population response of a local network of neurons during stimulation, to fast local feed-forward inhibition that can provide pruning of an excitatory response in the target neuron [4].

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This review summarizes and synthesizes the physiological and plastic properties of the cerebellar inhibitory interneurons of the molecular layer which provide inhibition to the Purkinje cells (PCs), the sole projection neuron of the cerebellar cortex (Figure 1). There is a wealth of *in vitro* data for these neurons, however our specific focus in this review will be on those aspects of the *in vitro* data that at present seem to have clear correlates with data obtained from *in vivo* studies. Although a number of studies have directly explored the functions of these neurons *in vivo* [7–12], intracellular *in vivo* recording similar to that commonly made in *in vitro* preparations has at present only been performed in one study [7]. This study was limited to the interneurons of one of the functional subdivisions of the cerebellum (the C3 zone) in the decerebrate cat (Figure 1). However, because the functions and computational properties that we describe in this review are of a relatively fundamental type, similar properties would be expected to be shared by interneurons in other cerebellar subdivisions and under other types of experimental preparations (at least under awake conditions), but further studies will be needed to confirm this. We have a good overall understanding of the organization and connectivity of the cerebellar circuitry, and reviewing both the *in vivo* and *in vitro* experimental data as well as the computational impact of these interneurons is therefore likely to yield important insights into the various functional roles of interneurons in general.

Cerebellar molecular layer interneurons

The inhibitory interneurons of the cerebellar molecular layer have historically been divided into two cell types – basket and stellate cells. Although there are clear morphological differences between them (Box 1), recent studies suggest that they are functionally less distinct [7,8,13]. In this review the term 'interneuron' will be used for all cerebellar molecular layer interneurons, without distinguishing between these two cell types.

Interneurons receive synaptic inputs from at least two sources: excitatory input from parallel fibers (PFs) and inhibitory input from other interneurons [14,15]. In

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Box 1. Cerebellar molecular layer interneurons

The cerebellar molecular layer contains two types of GABAergic interneurons – the stellate and basket cells. As with PC dendrites, these interneurons primarily have their dendrites and axons oriented in the sagittal plane [13] (Figures 1,4). The distinction between the two subtypes of interneurons emanates from early observations that showed that the axons of the deepest-lying interneurons formed dense pericellular nets, shaped like baskets, around the base of the PC soma [15]. These basket-forming cells were believed to be located at the bottom of the molecular layer and/or inside the PC layer. The more superficial stellate cells were believed to form inhibitory synapses with PC dendrites, but not with their somata. Later studies have suggested that the border between the two types of cells is not so distinct; a stellate cell located at mid-depth in the molecular layer can, for example, send axons that form 'baskets' [7,13].

addition, they are excited by activity in the climbing fibers (CFs) [7,9], possibly via extrasynaptic spillover from neighboring CF–PC synapses [16]. The axons of the interneurons form inhibitory synapses onto PCs and other interneurons, including Golgi cells [14,15].

Excitatory synaptic responses in cerebellar interneurons

A hallmark of cerebellar molecular layer interneurons is the presence of unitary PF excitatory postsynaptic potentials (EPSPs) with large peak amplitudes (up to 10 mV) both *in vivo* and *in vitro* [7,17]. Because interneurons at rest fire spontaneously [7,18–20], PF EPSPs typically have a strong EPSP-to-spike coupling [17]. *In vivo*, PF synapses with the largest synaptic weights would be expected to have a strong influence on the subthreshold activity [7] (Figure 2). The fact that single synaptic inputs can have such powerful effects [17] is in contrast to the situation in the cerebral neocortex, where interneurons are instead controlled by a large number of weaker synaptic inputs *in vivo* [21,22]. Interestingly, *in vitro* studies have shown that the PF synapses on cerebellar interneurons with the largest synaptic responses could contain postsynaptic AMPA-type glutamate receptors that are permeable to Ca^{2+} [23]. An intriguing possibility is therefore that the largest unitary PF synaptic responses observed *in vivo* might be partly carried by Ca^{2+}-permeable AMPA receptors. Although this remains to be shown, this possibility could imply that forthcoming high-resolution *in vivo* Ca^{2+} imaging techniques will be able to resolve activity at individual PF synapses on cerebellar interneurons [24–26].

Granule cells, which are the cells of origin of the PFs, can fire at very high frequencies both *in vivo* and *in vitro* [27–29]. Are the PF synapses able to reliably follow these frequencies? One recent study suggests that this is the case: PF synaptic boutons have been found to respond with a reliable and uniform Ca^{2+} elevation for each granule cell spike, even at 500 Hz [30]. However, these findings are at odds with previous *in vitro* studies suggesting that presynaptic modulation of PF transmission [31] and paired-pulse facilitation [32,33] are pronounced factors affecting PF presynaptic release probability as evaluated from recordings of synaptic input to interneurons. Differences in preparation could possibly play a role here: recent *in vitro* work has shown that the addition of background activity in the granule cells, a situation more similar to that found *in vivo*

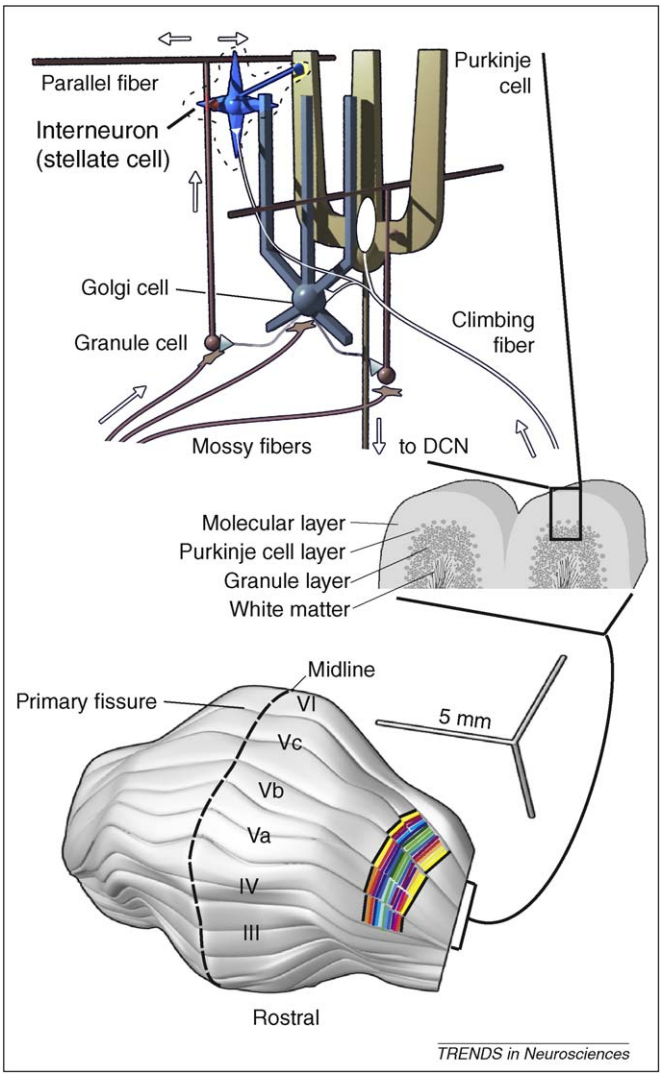

Figure 1. Schematic 3D representations of the basic cerebellar cortical circuitry and the topography of the cerebellar cortex in cat cerebellum. Above: basic circuitry illustrating the position of the cerebellar molecular layer interneurons in the cortical network. These interneurons exists as both stellate and basket cells, but are here represented only by the stellate cell (Box 1). Interneurons are excited by the mossy fiber–granule cell/parallel fiber input, and also by the climbing fiber input. The name 'molecular layer' interneurons refer to their location in the molecular layer, one of three cortical layers in the cerebellum (molecular, Purkinje, and granule cell layers; see middle panel). Below: 3D representation of parts of the anterior lobe of the cat cerebellar cortex. Roman numerals refer to the classification of the cerebellar lobules according to Larsell [98]. Each individual colored area represents one microzone (Box 2) of the forelimb area of the C3 zone [93], the borders of which are indicated with black lines (the lateral extent of the anterior lobe is somewhat truncated). Bars indicate 5 mm length in the three axes.

[28], reduces the magnitude of the presynaptic modulation of PF and inhibitory synaptic input to interneurons [34].

Reciprocal inhibition between cerebellar interneurons within a microzone

In addition to the excitatory PF synapses, interneurons also receive inhibitory inputs from neighboring interneurons [20,35]. In intracellular recordings *in vivo* it has been reported that inhibitory synaptic potentials, presumed to be predominantly from other interneurons, can only be evoked from the same peripheral input that also drives the EPSPs of the interneurons of the same microzone (Figure 1 and Box 2) [7]. In contrast to PCs, interneurons have no distinct inhibitory receptive field located outside their

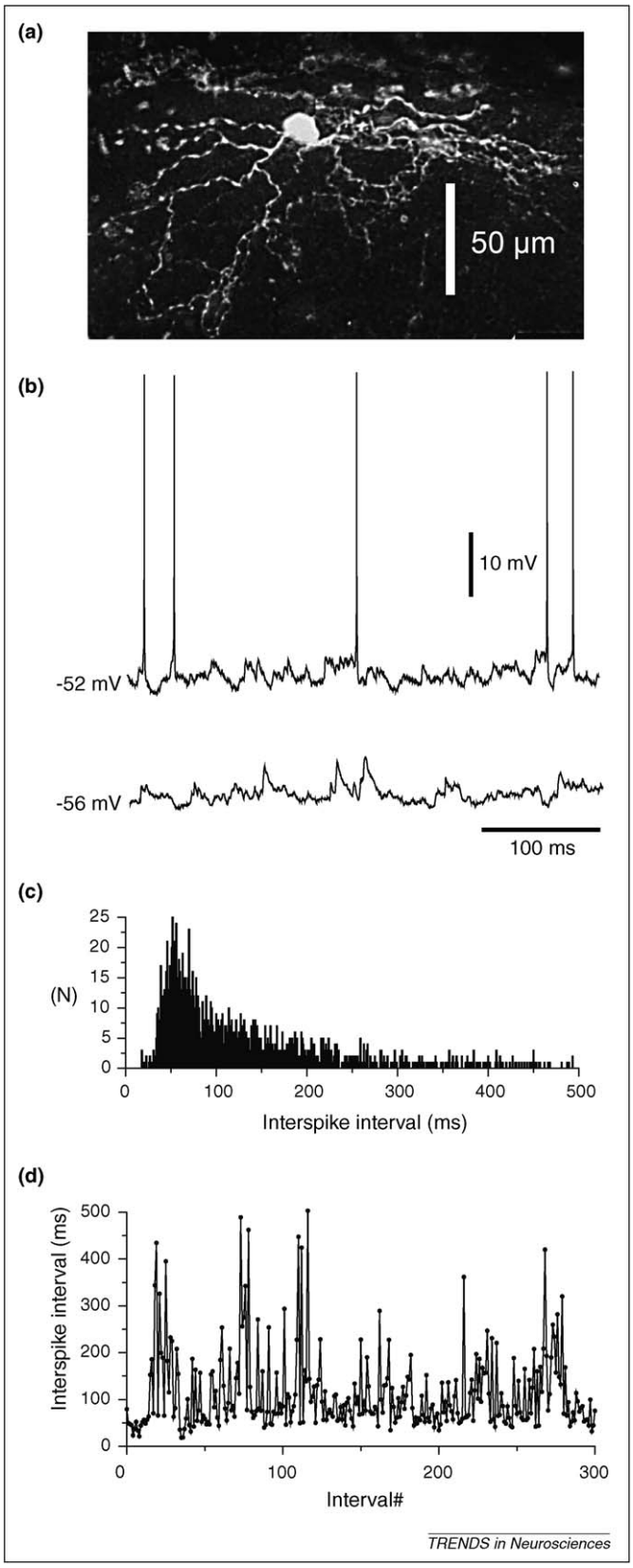

Figure 2. Irregular firing activity and synaptic inputs in interneurons recorded at rest *in vivo*. **(a)** Example of an interneuron (stellate cell) in cat cerebellar cortex. During recording, this neuron was filled with neurobiotin to visualize all of its processes. **(b)** Recordings of spontaneous activity at rest (approximately −52 mV, top, at which the neuron fired spikes off spontaneous EPSPs) and at hyperpolarized potential (−56 mV, bottom, below firing threshold) from a molecular layer (ML) interneuron recorded *in vivo* using the whole-cell electrophysiological recording technique. **(c)** Frequency histogram of interspike intervals of a ML interneuron recorded *in vivo* using the whole-cell technique under resting conditions (i.e. as in the top panel of B). The long 'tail' of the interspike intervals reflects a highly irregular spontaneous firing frequency whereas the concentration at shorter interspike intervals suggests that the interneuron has a tendency to fire in bursts. **(d)** Plot of consecutive interspike intervals recorded from the same interneuron. This plot illustrates the absence of any obvious pattern in the occurrence of long and short spike-intervals. All data in this figure are from recordings in the C3 zone of the cat cerebellum, in the decerebrate, non-anesthetized preparation [7,29].

Box 2. Cerebellar microzones and circuitry analysis

Instead of being divided into functional areas, such as the motor and primary sensory subregions of the cortex, the functional subdivisions of the cerebellum take the shape of sagittal zones [93] (Figure 1). Each sagittal zone receives climbing fibre (CF) input from a small region of the inferior olive and sends its output, via its Purkinje cells (PCs), to a restricted target region of one of the deep cerebellar or vestibular nuclei [93]. Each such nuclear region innervates a specific set of targets in the brain [94]. Zones can be further divided into microzones [95,96] (Figure 1). The definition of a microzone is a coherent band of cortex in which the CF input to the PCs is activated by essentially identical peripheral inputs [95,96]. Microzones can be extremely narrow (just a few PCs wide, corresponding to 50–150 μm) but can extend across several folia of the cerebellar cortex; they can therefore sometimes be very long (around 20 mm in the cat) [95,96]. Microzones innervate specific small groups of cells in the deep cerebellar nuclei which have a specific output function or control a small set of muscles [97]. This forms the microcomplex structure – a system of specifically connected cells in the olivo-cortico-nuclear loop [93–95]. Microcomplexes could be the smallest functional unit of the cerebellum.

Because a microzone is defined as a sagittal strip of PCs in which the CFs are driven by the same peripheral input, and CFs have been found to modify the synaptic weights of simultaneously activated PF inputs [94], it was believed that there should be a specific relationship between the PF receptive fields and CF receptive fields that might be uniform throughout a microzone. When this hypothesis was first tested it was confirmed for PCs [8–10] – the locations of their PF receptive fields were specific to (but non-overlapping with) the locations of the CF receptive fields of the microzone. However, a potentially more important finding was that the receptive fields of the interneurons were also specific to (and, in this case, overlapping with) the CF receptive field of the microzone (investigated in nearby, surrounding PCs). An important consequence of this finding is that, in PCs and interneurons with specific inhibitory receptive fields, the microzonal location of the interneurons that provide that inhibition can be identified (e.g. Refs [7–11]).

excitatory receptive field [8–10] suggesting that the interneuron-to-interneuron inhibition is primarily microzone-specific (i.e. is contained within a functionally-equivalent population of interneurons). This view is supported by studies demonstrating that the axon of an interneuron tends to run sagittally and forms synapses primarily along its proximal part [13,36] and that molecular layer inhibition is primarily distributed parasagittally, along the orientation of the microzones [37] (Figure 1). Lugaro cells [38] are potential additional sources of inhibitory synaptic inputs in interneurons [39], but they have axons that seem to cross rather than respect parasagittal borders and are therefore not likely to contribute to the parasagittally organized inhibition.

The reciprocal inhibition between interneurons within a microzone has interesting computational consequences for interneuron signaling (Figure 3A). First, with electrical activation of PF inputs, PF-EPSPs are typically accompanied by a disynaptic inhibitory postsynaptic potential (IPSP) [40], which can also be observed in *in vivo* recordings

(Figure 3B). Feed-forward inhibition can result in effective temporal truncation of the PF synaptic input, and this eliminates late spike activation on excitatory PF synaptic input and can contribute to make the spike output of the interneuron phasic and irregular (Figure 2C,D) [20,41]. Interneurons have a short membrane time-constant, fast rise and decay times in both inhibitory and excitatory synaptic inputs, a high maximal firing frequency and a very rapid spike initiation [7,17,19]. Therefore, similarly to hippocampal interneurons [42], cerebellar interneurons appear to be optimally designed to provide fast and precisely timed spike outputs to inhibit the PCs.

During responses evoked by activation of synaptic inputs from the skin, the skin areas from which EPSPs and IPSPs can be evoked overlap [7]. This indicates that during responses evoked from the periphery the local inhibitory connections between interneurons generate feed-forward inhibition (Figure 3A) [7]. Feed-forward inhibition would be expected to shorten the time-constants of evoked EPSPs. This can also be observed in subthreshold recordings of the evoked synaptic responses in interneurons *in vivo* (Figure 3D), where many evoked EPSPs have decay time-constants that are faster than those of spontaneous unitary EPSPs (Figure 3B) [7].

From experiments using electrical stimulation of PFs, feed-forward inhibition appears to be the dominating mode of operation also for the interneuronal innervation of PCs (e.g. Refs [12,15,40,43]). Nevertheless, an experimental setup using direct electrical PF stimulation can induce a bias in that the PFs that activate interneurons and the PFs that activate PCs are likely to be intermixed, and therefore electrical stimulation of a PF beam will inevitably activate both populations of PFs. In contrast to the findings from this type of setup, activation from the periphery indicates that the PF inputs to interneurons are driven from one specific skin area, whereas the PCs of the same microzone, which are innervated by these interneurons, receive their PF input from a different skin area [8–10] (Figure 4). A similar interpretation might hold for the recordings from PCs and interneurons in regions that receive vestibular inputs [11]. Therefore, it appears that during natural forms of input (i.e. inputs obtained through activation of appropriate sensors instead of by electrical stimulation of parallel fibers) the PCs and their afferent interneurons have a low chance of being activated by the same PFs. This type of circuitry organization, which can be established by various forms of plasticity (see below), is supported by recordings in the awake animal during locomotion [44] and the performance of the vestibulo-ocular reflex (VOR) [45]. During such movements, the PC simple spike (SSp) firing exhibits distinct phases of excitation and suppression [44,45], a pattern which could be partly controlled by cortical inhibition [46]. However, at the same time interneurons seem to control the overall regularity of PC SSp activity *in vivo*, and this could be compatible with a function in feed-forward inhibition [12].

Firing patterns of cerebellar interneurons

The firing patterns of interneurons recorded *in vivo* [9–11,25] are more irregular than those recorded *in vitro* [17,20,41,47,48]. This discrepancy can be explained by the presence of spontaneous synaptic inputs *in vivo*. For example, blocking $GABA_A$ receptors can increase firing regularity in cerebellar interneurons [41]. In addition, if one mimics random spontaneous, excitatory and inhibitory synaptic inputs *in vitro* with the use of dynamic clamping (a method by which currents with the same timecourse as synaptic currents are injected into the recorded neuron using a computer-based feedback model), stellate cells show increased irregularity in their spike firing patterns [49] which is qualitatively similar to that observed *in vivo* (Figure 2C,D).

The irregularity of the firing pattern of interneurons might be further enhanced by irregularities in the firing patterns of their afferents. For example, it was shown recently that CF activities, which occur spontaneously in irregular patterns [50–52], result in slow, relatively long-lasting excitation and increased spike-firing in cerebellar interneurons both *in vivo* [7,9] and *in vitro* [16]. Importantly, because neighboring CFs within the same microzone (Figure 1 and Box 2) have a tendency to fire occasionally in synchrony [53–56], this type of input could provide a mechanism by which the activities of many local interneurons within the same microzone can become relatively synchronized. This tendency could be further accentuated by the mutual inhibitory connections (see above) and the electrotonic coupling between nearby interneurons [57,58].

Because the interneurons inhibit the PCs, synchronized interneuron activity should result in temporary reductions or pauses in PC firing. The irregularities in PC firing patterns observed *in vivo* [59] could thereby at least partly depend on the patterns of interneuron activity. Indeed, in mutants in which the PCs lack functional $GABA_A$ receptors, PC firing is much more regular [12]. This phenotype might be partly due to an overall loss of background synaptic activity to PCs [20], but it is nonetheless also in line with the concept that episodic synchronization of interneuron inhibition can be induced by the CF and/or PF inputs – and that this process could be an effective way to introduce temporary, marked reductions in PC SSp firing and thereby a high degree of irregularity. If interneuronal inhibition is a main cause of the PC spike pauses, this would also explain the exaggerated pausing activity often observed under various forms of anesthesia [60] because an increase in inhibitory synaptic efficacy is a consequence of all general anesthetics that have been tested so far (discussed in Ref. [61]).

Plasticity of PF synapses on interneurons

As discussed above, the PF synapses on interneurons that contain Ca^{2+}-permeable AMPA receptors could correspond to those PF inputs that provide the largest PF EPSPs *in vivo* [7]. *In vitro* studies suggest that the Ca^{2+}-permeability of these postsynaptic AMPA receptors could be reduced by repetitive PF activation [23,62–65]. Even if the Ca^{2+}-permeable AMPA receptors are replaced with non-Ca^{2+}-permeable AMPA receptors, the net effect is a reduced EPSC, in other words long-term depression (LTD). Because most forms of plastic processes in the brain are reversible, one would expect that it should also be possible to insert Ca^{2+}-permeable receptors at novel locations in the postsynaptic membrane and thereby induce long-term potentiation

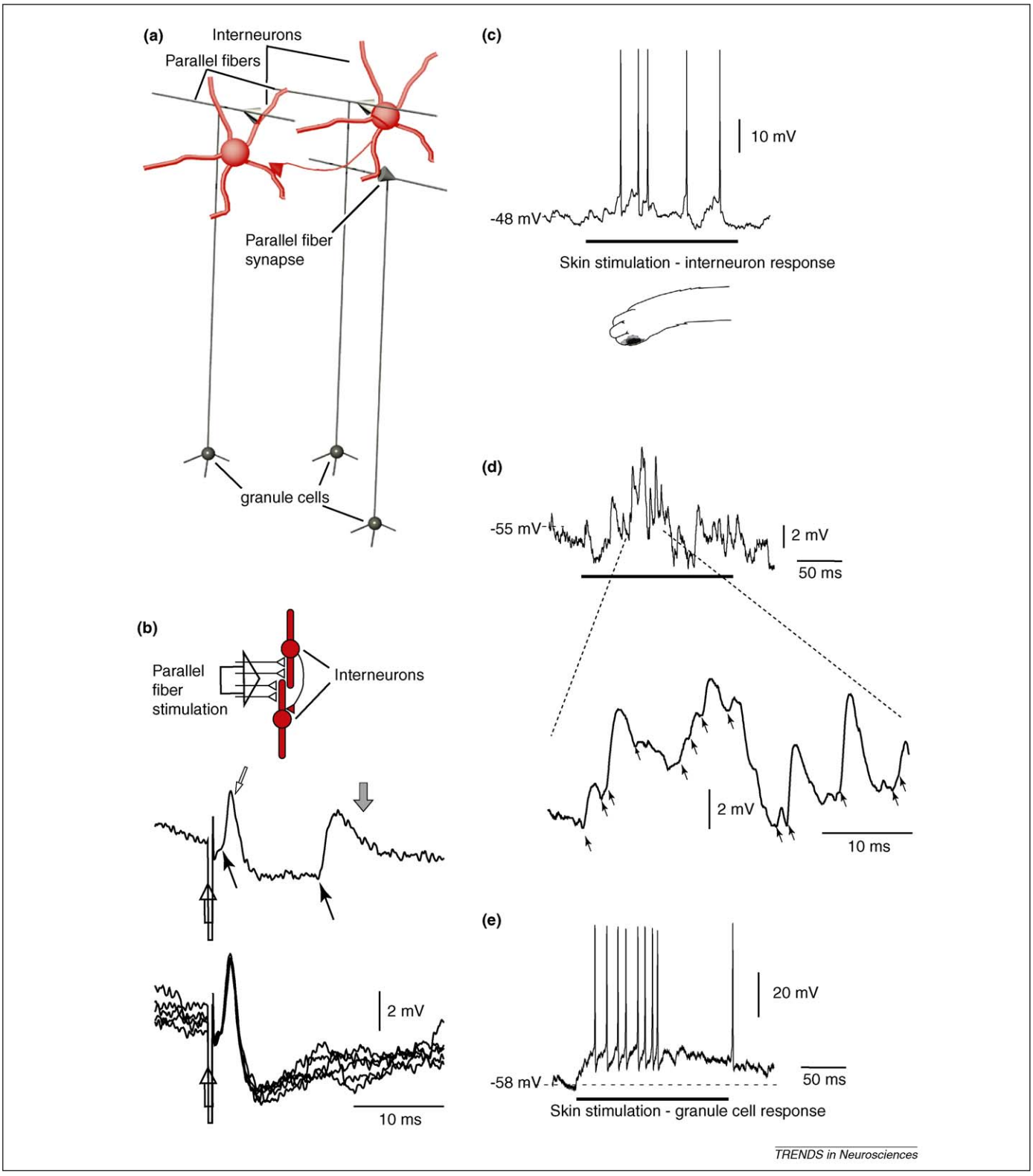

Figure 3. Potential role of feed-forward inhibition in truncating the decay time-constants of actively evoked excitatory synaptic inputs in interneurons *in vivo*. **(a)** Molecular layer interneurons (red) receive input from multiple parallel fibers (PFs) (grey) as well as from other interneurons. The excitatory PF synaptic input originates from the granule cells. **(b)** Electrical stimulation of PFs evokes both monosynaptic EPSPs and disynaptic IPSPs in the recorded interneuron when the interneuron is aligned with the stimulated PFs ('on-beam'). This can be demonstrated by comparing the very fast decay time of the evoked EPSP–IPSPs with the much slower decay time of spontaneous EPSPs (as indicated by grey arrow). Note that evoked EPSP–IPSPs *in vivo* are relatively non-variable (bottom). Small, filled arrows indicate EPSPs; the small, empty arrow indicates an IPSP; the larger empty arrows indicate the time of electrical PF stimulation. **(c)** On manual skin stimulation, the interneuron responds with an intense spike train. **(d)** The synaptic response to the same type of stimulation (recorded when the cell is hyperpolarized to prevent spiking, hence facilitating the analysis of the evoked synaptic inputs) is very intense and many synaptic events can be distinguished in the response. Most responses are excitatory (oblique arrows indicate the starting points of putative EPSPs) but appear to be truncated by IPSPs because their timecourse is shorter than the spontaneous EPSP but comparable to those evoked by PF stimulation (time and voltage calibrations in expanded sweep are equal to those in B to facilitate comparison). A high degree of parallel activation of excitatory and inhibitory synaptic inputs in interneurons is a consequence of the fact that inhibitory input primarily originates from the nearest neighbouring interneurons, which also are excited by the same (skin) input (Box 2). **(e)** An example of a granule cell response to the same type of stimulation. The intense spike discharge recorded from granule cells activated by skin

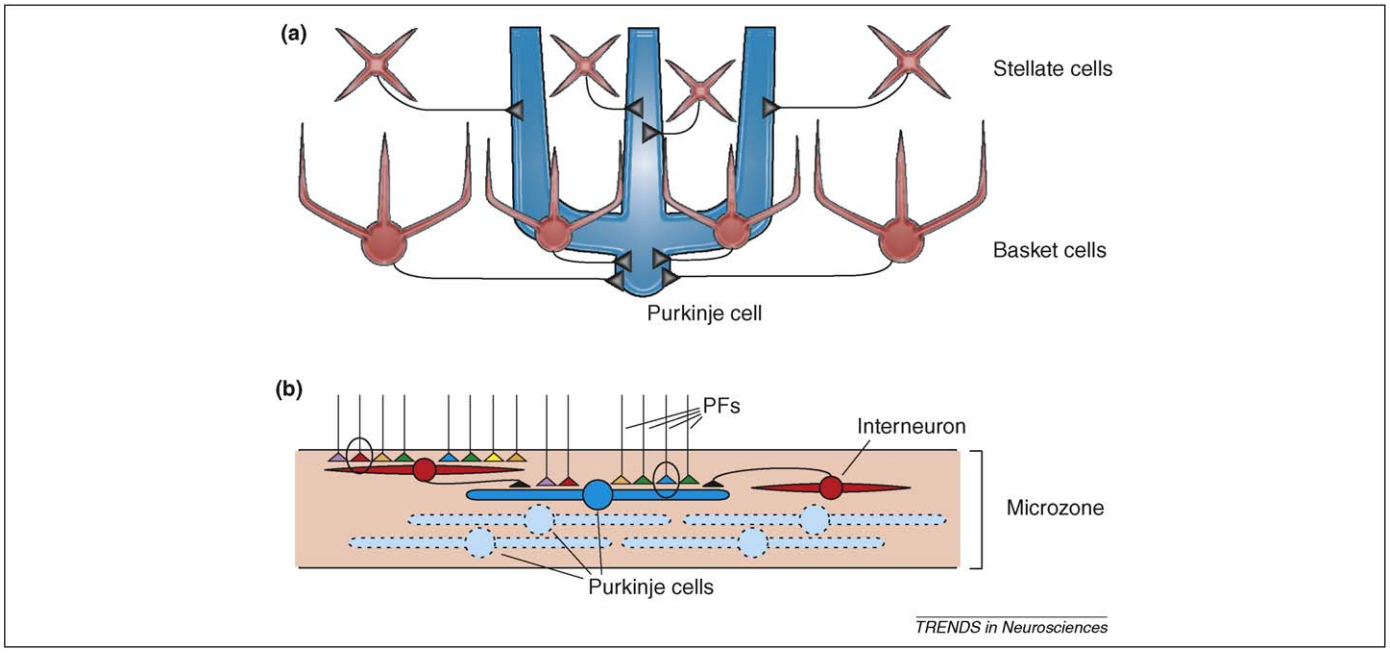

Figure 4. A model of the circuitry organization in the molecular layer of the cerebellum based on anatomical and physiological data. **(a)** Interneurons that provide inhibition to a single Purkinje cell (PC) are numerous and distributed over a relatively wide area in the cerebellum. Therefore, even though the number of parallel fibres (PF) inputs to a single interneuron is relatively low, the total number of PF inputs represented in the population of interneurons that converge on a single PC can be very large, perhaps larger than the number of PFs synapsing directly on the PC. **(b)** A model of circuitry organization in the molecular layer, based on current findings (discussed in Ref. [91]). The interneuron-to-PC and interneuron-to-interneuron inhibitory synaptic connections are parasagittally aligned within the microzone (Box 1) and therefore seem to offer little combinatorial possibilities across the microzonal organization. All the PCs of a microzone are driven by the same or functionally equivalent PF synaptic inputs, a different input from that driving the interneurons. This is illustrated by the color code: the blue coloring of the PCs indicates that they receive their primary input from the 'blue' PF input (representing one specific type of input, i.e. input from one receptive field for example); the red coloring of the interneurons indicates that they receive their primary input from the 'red' PF input (i.e. from a different receptive field than the PC).

(LTP). Indeed, it is possible to induce LTP of PF synapses onto interneurons in the C3 zone *in vivo* by applying a combination of CF and PF input [7,9]. In these studies LTP of PF input made it possible to evoke large PF EPSPs from 'novel' skin areas (i.e. specific areas that originally did not respond to any skin stimulation) [7,9]. Removal of the 'new' PF input *in vivo* can be achieved with repetitive PF activation without concomitant CF activation [7,9] in a manner similar to that observed *in vitro* [62–64].

LTP of the PF input to interneurons *in vitro* depends on intracellular Ca^{2+} and NMDA receptors [66,67]. By contrast, LTD does not depend on NMDA receptors, but requires Ca^{2+}-entry through the Ca^{2+}-permeable AMPA receptors [64]. Because NMDA receptor activation in interneurons normally requires CF input [68], CFs could play a pivotal role in regulating PF synaptic plasticity in interneurons: CF and PF activity combined would lead to LTP, whereas PF activation alone would lead to LTD. This would be in agreement with the *in vivo* findings on PF plasticity described above. Moreover, if one assumes that CFs and PFs with similar receptive fields are on average the most frequently co-activated inputs, CF-dependent LTP in interneurons can explain why the peripheral input to interneurons *in vivo* has been observed to be similar to the local CF input [7–11].

Because plasticity in the PF synapses on interneurons has been found to depend on Ca^{2+} entry [64], in future studies it would be interesting to investigate the relationship between the intracellular Ca^{2+} concentration and the induction of PF-LTP and PF-LTD in interneurons. So far, possible candidate mediators of Ca^{2+} influx in interneurons include the Ca^{2+}-permeable AMPA receptors and the NMDA receptors and conductances associated with somatic spike activation [69]. According to the 'BCM' rule, which was applied to explain the development of the orientation selectivity and ocular dominance of pyramidal cells in the visual cortex [70], named after the authors that proposed this rule (Bienenstock, Cooper and Munro), high intracellular Ca^{2+} levels lead to LTP, whereas low levels lead to LTD. Alternatively, interneurons could also follow the 'anti-BCM' mechanism, in which high Ca^{2+} leads to LTD and low levels leads to LTP, as described for PCs [71].

Plasticity of interneuron-to-PC synapses

Another form of plasticity exists at inhibitory interneuron-to-PC synapses. This form of plasticity is associative because it is induced through paired activation of the interneuron-to-PC synapses and the CF (or PC depolarization) in the PC and is expressed as a potentiation of the interneuron-to-PC IPSCs [72,73]. Interestingly, whereas some groups report that paired activation of CFs (or PC depolarization) and interneurons leads to potentiation of interneuron-to-PC synapses [72,74,75], others instead report a depression [73,76,77]. These opposing results have been suggested to depend on possible differences in the total Ca^{2+} levels obtained after the CF stimulation ([73] but see also Ref. [75]). An important issue for future studies will be to determine whether concomitant CF and interneuron

stimulation suggests that the excitatory synaptic responses recorded in interneurons **(d)** could result from the activation of only a very few granule cells – in the order of 2–8 granule cells [7]. All sample recordings in this figure are taken from the data of previous *in vivo* studies [7,29] recorded from the C3 zone of the cat cerebellum, in the decerebrate, non-anesthetized preparation.

activation leads to potentiation or depression of the interneuron-to-PC synapses *in vivo*.

However, the potential number of sources of inhibitory inputs that can be made available to the PC through this plasticity mechanism could be much less than that of the plasticity in the PF-to-interneuron synapses. This is because (i) anatomical and *in vivo* findings suggest that the interneuronal inhibition could be confined to a single microzone [7–10,13,36,37] (Figure 4), or possibly its nearest neighboring microzones [7], and (ii) *in vivo* studies show that the PF receptive fields of interneurons are equivalent to the CF receptive field, and the inhibitory receptive field, in the nearest PC [7–11] (i.e. the receptive field of the microzone). Thus, because these observations indicate that all interneurons that provide inhibition to a PC could be driven by the same PF input (Figure 4), plastic changes in specific interneuron-to-PC synapses will not change the peripheral source from which the inhibition is evoked in the PC.

Interneurons – learning effects in VOR adaptation

Whereas acute blockage of GABAergic activities in the vestibulo-cerebellum directly leads to balance problems and prominent deficits in the performance of compensatory eye movements [46,78], mice in which $GABA_A$ receptor-mediated synaptic inhibition is genetically removed from PCs show relatively mild impairments in motor performance [12]. One interpretation of this latter finding is that developmental compensation could obscure any circuitry deficits in these animals. However, the deletion of $GABA_A$ receptor function still leads to robust deficits in VOR phase reversal learning and consolidation of VOR gain adaptation [12]. Interestingly, the regularity, but not the average firing frequency, of the SSp activity of floccular PCs is affected in this mutant. Because both the firing frequency and regularity of complex spike activities are normal, it appears possible that the absence of spatiotemporal patterns of SSp activities causes the learning and consolidation deficits when molecular layer interneurons are blocked. In line with the orientation of the axons of the interneurons as well as that of their main 'plasticity teacher' (i.e. the CFs; see above), it has been hypothesized that these patterns need to be formed in some sort of coherent fashion in a sagittal strip of PCs in order to mediate a consolidation process of motor learning at vestibular nuclei neurons and/or deep cerebellar nuclei (DCN) neurons [12] (see also Ref. [79]). To investigate the level and type of coherence of SSp activity among PCs it will be necessary to perform simultaneous multiple unit recordings of PCs and their target neurons in the nuclei downstream during motor learning.

Interneurons – learning effects in classical and fear conditioning

In classical conditioning of the eyeblink reflex [80,81] a well-timed pause in PC firing is gradually acquired after training [82]. This pause could release the deep cerebellar nuclei from tonic PC inhibition and thus allow for the expression of the learned eyeblink [83], which is temporally locked to the pause [84]. However, PCs exhibit spontaneous activity that is independent of their PF input [20,85–87]. This is demonstrated by the fact that blocking excitatory input to PCs *in vivo* only results in marginal reductions in their spontaneous activity and is supported by the observation that most granule cells have no or very low spontaneous activity [27,28]. The timed reduction of the spontaneous PC activity in classical conditioning of the eyeblink response therefore requires an active and associatively learned depression of spike activity that could be explained by an acquired increase in interneuron inhibition timed to the response. Interestingly, previous investigations have suggested that inhibitory interneurons contribute to the mechanisms underlying consolidation of the learned eyeblink response [88,89]. Moreover, similar mechanisms might play a role in the association process during fear conditioning which requires a fast freeze-response. Indeed, one study has shown that the frequency of spontaneous and miniature GABAergic events onto the PC is significantly greater up to 24 h after conditioning [90]. Thus, given the properties of the molecular layer interneurons summarized in this review, they would be excellent candidates for fulfilling a role in controlling the temporal aspects of conditioned responses, during both classical and fear conditioning.

Concluding remarks

The simplicity of cerebellar circuitry provides an excellent opportunity to clarify interneuronal contributions to local and global network properties [91]. Interneuron studies in other parts of the brain have suggested that interneurons can provide either global inhibition [3], that could help to sharpen the population response of neurons, or a more localized inhibition that can prune a specific excitatory response in a targeted neuron on a rapid timescale [4]. In the cerebellum, an example of the first type of interneuron

Box 3. Outstanding questions

Two major questions remain to be answered about cerebellar molecular layer interneurons:

(i) How do these interneurons respond to the wide range of input patterns that they receive from the diverse mossy fiber and CF afferent systems in the different functional zones of the cerebellum?

(ii) How do these potentially different interneuron firing patterns affect PC spike output during behavior?

Answers to these questions require systematic investigations of the response properties of the mossy fiber and CF afferent systems, as well as the interneurons and PCs, within well-defined functional regions of the cerebellum. In addition, there are a number of more detailed questions to be answered:

(iii) Are there specific Ca^{2+} thresholds for the induction of PF-LTP and PF-LTD in cerebellar interneurons?

(iv) What processes are required for the recruitment of Ca^{2+}-permeable AMPA receptors to specific PF synapses?

(v) Can the Ca^{2+}-permeable AMPA receptors be visualized by high-resolution *in vivo* imaging techniques? If so, this could greatly facilitate the study of synaptic plasticity processes in these neurons *in vivo*.

(vi) How does the CF-dependent modification of the interneuron-to-PC inhibitory synapse work *in vivo*? Does it involve depression or potentiation, and how prominent are the effects it can induce?

(vii) What is the level and type of coherence of the SSp activity among PCs? If this coherence is present, what is the contribution of the interneurons and what are the effects on motor performance/learning? To investigate this question it will be necessary to do simultaneous multiple-unit recordings of PCs and their target neurons in downstream nuclei during motor learning.

might be the Golgi cells, because they have a widely divergent axonal arbor that provides inhibition at the input stage of the cerebellar circuitry, and they receive multiple, low-efficacy synaptic inputs [92]. The molecular layer interneurons on the other hand seem optimally configured to control cerebellar output with high spatio-temporal precision, and this could be required to optimize cerebellar learning processes. Many important questions remain to be answered (Box 3) to help extend our insights into the function and plasticity mechanisms taking place in molecular layer inhibitory interneurons of the cerebellum.

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