Unraveling the cerebellar cortex: cytology and cellular physiology of large-sized interneurons in the granular layer

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Neuronal network behaviors emerge from complex interactions between excitatory relay cells, principal cells and inhibitory interneurons. Therefore, characterizing homogeneous cell types and their properties is an essential step towards understanding information processing in the brain. The cerebellar cortex is generally described as a repetitive circuit composed of only five cell types. However, recent studies have revealed an unexpected diversity in the morphological, neurochemical and electrophysiological properties of the large-sized granular layer interneurons. These data are reviewed here with an emphasis on the synaptic interactions of the different cell types within the cerebellar cortex. The existence of a complex network of excitatory and inhibitory interneurons controlling the spatial and temporal pattern of granule cell firing is documented, providing insights into the cellular and synaptic processes underlying oscillations and synchronization in the cerebellar cortex.

Introduction

The cerebellar cortex is generally described as a repetitive circuit composed of only five cell types: Purkinje cells, granule cells, Golgi cells and the molecular layer interneurons, stellate and basket cells. Because of this presumed simplicity, it has been used as a model to study various aspects of neuronal transmission. Recent experimental data, however, indicate that the cerebellar cortex, though highly organized, represents a fairly complex structure as schematically summarized in Figure 1.

In order to understand information processing in the cerebellum, an accurate description of the fundamental cortical circuitry and the complex interactions between excitatory relay cells, principal cells and inhibitory interneurons is essential. Most cerebellar theories gave little consideration to the function of the granular layer, and focussed instead on the output side of the cortical circuitry: the Purkinje cells.[1] The granular layer, however, harbors 98% of the cerebellar neurons, most of which are small-sized granule cells,[2,3] and receives the mossy fiber system, which is numerically the most important input to the cerebellum.[4,5] Larger cells within

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the granular layer are usually referred to as Golgi cells. However, large granular layer interneurons represent a heterogeneous population, and recent studies have revealed an unexpected diversity in the morphological, neurochemical and electrophysiological properties of these interneurons. These data are reviewed here with an emphasis on the synaptic interactions of the different cell types within the cerebellar cortex. We will show that all these interneurons, which have been classified into unipolar brush cells, Golgi cells and Lugaro cells (Figure 2A), control directly or indirectly the mossy fiber to granule cell relay, and may shape the spatial and temporal firing patterns of different granule cell populations converging onto common Purkinje cells.

Despite their abundance, we will not discuss granule cell properties in detail. In short, these excitatory cells receive an excitatory mossy fiber input which activates both AMPA and NMDA receptors[6] and are inhibited by Golgi cells (see further). The mossy fiber to granule cell synapse demonstrates many forms of plasticity. Short-term heterosynaptic interactions exist with the GABA system (see further under Golgi cells). Long-term plasticity is present as a presynaptic form of long-term potentiation[7,8] that can be induced by high frequency stimulation (see Ref. 9 for recent review). This stimulation protocol induces at the same time changes in the spike threshold of the activated granule cell.[10] Granule cells in turn relay the mossy fiber input onto Purkinje cells, a process which demonstrates plasticity as well.[9] Finally, although granule cells express the calcium-buffering protein calretinin[11] their content is much smaller than that of Lugaro or unipolar brush cells (see further).
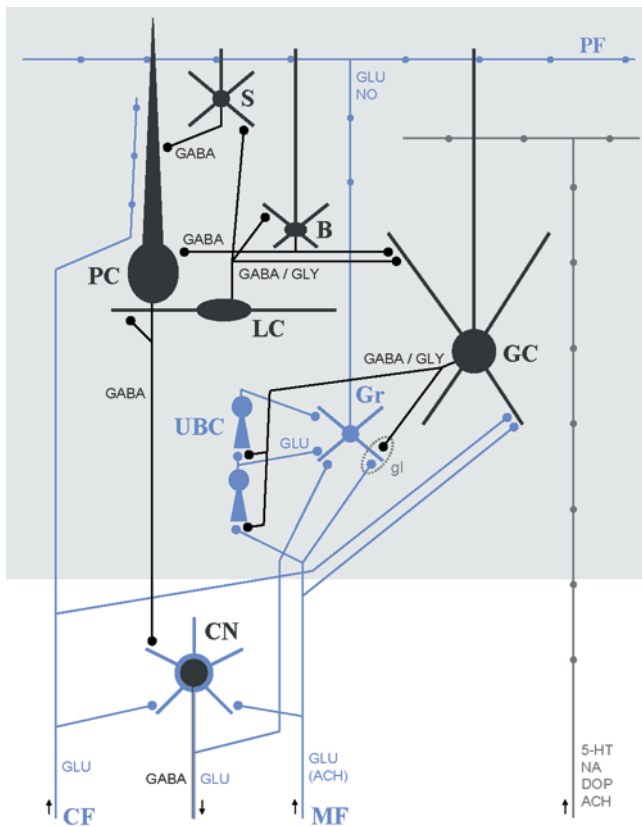
Figure 1

Schematic overview of cerebellar neurotransmission. The cerebellar cortex is indicated by the shaded area. Inhibitory neurons and synapses are in *black*. Excitatory neurons and synapses are in *blue*. Modulatory projections are in *gray*. Arrows indicate the orientation of projections. The small encircled area represents the glomerulus. The granular layer receives two types of extrinsic mossy fibers: those of extracerebellar origin representing the main cerebellar input, and nucleocortical projections from neurons in the cerebellar nuclei. Granule cells relay the mossy fiber input onto Purkinje cells, the sole output of the cerebellar cortex. Additionally, the axons of UBCs form a system of cortex-intrinsic mossy fibers controlling the mossy fiber to granule cell relay in synergy with the inhibitory actions of Golgi cells and Lugaro cells. Abbreviations: ACH, acetylcholine; B, basket cell; CF, climbing fiber; CN, cerebellar nuclei; DOP, dopamine; GC, Golgi cell; gl, glomerulus; GLU, glutamate; GLY, glycine; Gr, granule cell; LC, Lugaro cell; MF, mossy fiber; NA, noradrenaline; NO, nitric oxide; PC, Purkinje cell; PF, parallel fiber; S, stellate cell; UBC, unipolar brush cell; 5-HT, serotonin.

Unipolar Brush Cells

About 25 years ago, a new type of granular layer interneuron, now called the unipolar brush cell (UBC), was described.[12] It was characterized by the size of its cell body, twice as big as granule cells, and by its pale appearance in Nissl stain, hence the denomination 'pale cell'. The morphology of this new cell type was first described by S Hockfield, following the production of a specific monoclonal antibody (Rat-302).[13] UBCs, then called 'monodendritic cells', also showed immunoreactivity for chromogranin A and calretinin.[14,15] They are intermediate in size between granule cells and Golgi cells, and usually carry only one short dendritic trunk that terminates in a paint brush-like bush of dendrioles[16] (Figure 2C). Due to their size and location, UBCs are frequently mistaken for medium-sized Golgi cells.

The UBC dendritic brush forms an extensive synaptic junction with a single mossy fiber featuring multiple presynaptic release sites opposed to a continuous postsynaptic density,[17] making the mossy fiber-UBC synapse one of the largest in the vertebrate central nervous system.[16] This synapse may have been mistaken in electron microscopy for the synapse *en marron*,[18] which was described as the articulation found between the expanded mossy fiber axon and a Golgi cell soma.[3] The unique ultrastructure of the mossy fiber-UBC synapse ensures the entrapment of glutamate released by a mossy fiber terminal within the giant synaptic cleft, and hence the prolonged activation of ionotropic glutamate receptors located on the postsynaptic UBC.[19] The resulting long-lasting epsp evokes, in response to a single presynaptic mossy fiber stimulus, a train of action potentials that lasts tens of milliseconds.[19]

UBCs are glutamatergic.[20] The UBC axon ramifies locally within the granular layer and gives rise to 1–3 branches that typically end in knobby terminals resembling mossy fiber rosettes surrounded by dendrites of granule cells and/or other UBCs.[21–22] In this way, the axons of UBCs generate an extensive system of cortex-intrinsic mossy fibers constituting a form of distributed excitation onto granule cells and other UBCs.[20] As a consequence, the synaptic excitation of UBCs by mossy fiber input can drive a large population of granule cells.[21] This powerful feed-forward amplification system of the UBC network is associated with vestibular afferents,[23] and is particularly dense in the vestibulocerebellum as well as in the cochlear nucleus.[16] The function of UBC is modulated by inhibitory inputs formed on UBC dendrioles by presumed Golgi cell axonal boutons.[17] This inhibitory control is discussed in the following section.

Golgi Cells

Golgi cells were first described by Camillo Golgi.[24] They are a population of irregularly rounded or polygonal interneurons that can be almost as large as Purkinje cells. Their cell bodies are found dispersed throughout the granular layer and emit numerous radiating dendrites[3] (Figure 2B). Golgi cell apical dendrites ascend towards the molecular layer, where they ramify profusely and are contacted by the axons of granule cells.[2,25] In addition, several basolateral dendrites extend in the granular layer, where they are contacted by mossy fibers.[3,26] Whether Golgi cells receive also a climbing fiber input[3,27] is still a matter of debate.

The Golgi cell axon ramifies inside the granular layer, giving rise to an elaborate axonal plexus[3,25] that contacts thousands of granule cells[28] as well as UBCs.[17] Golgi
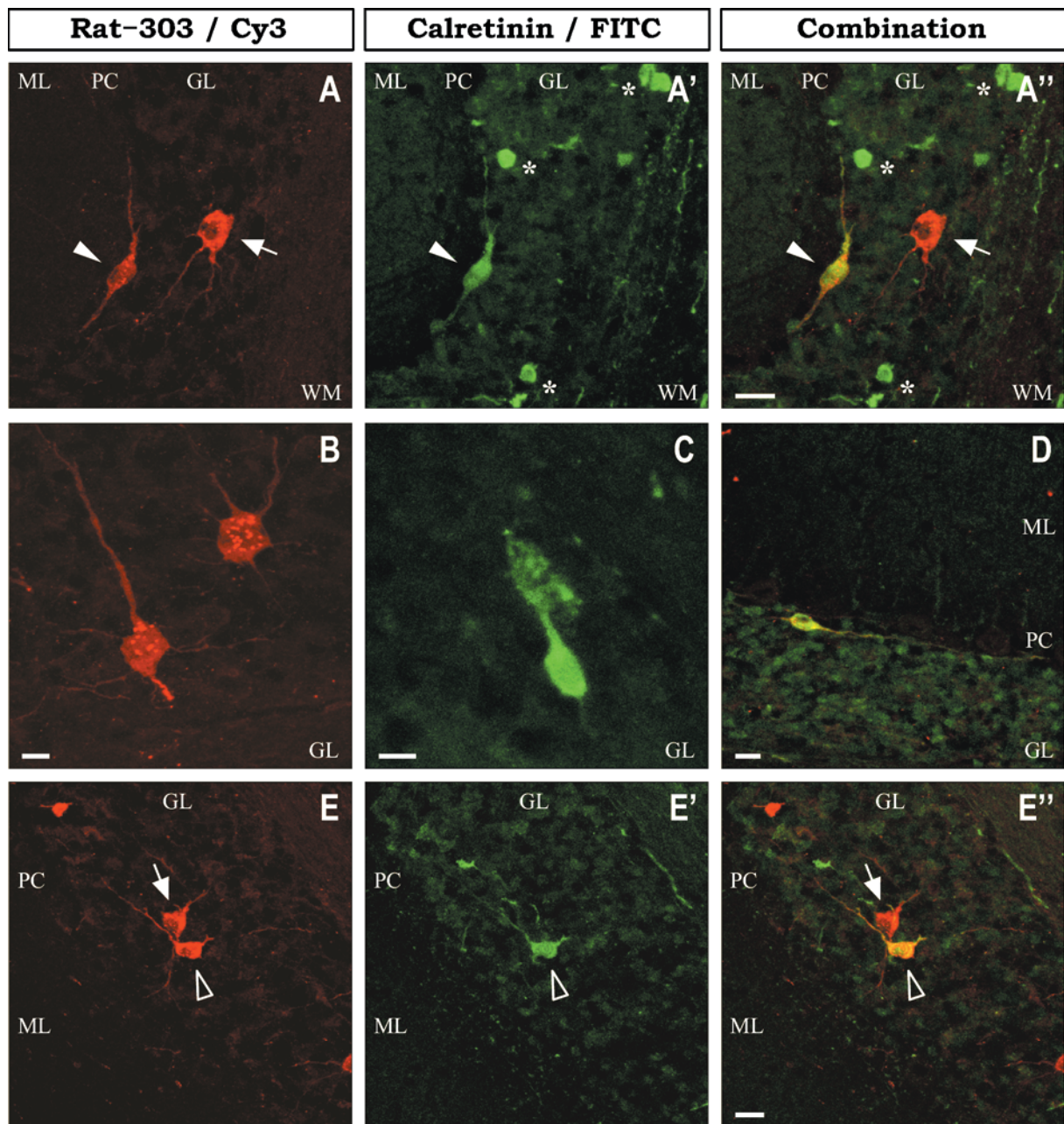
Figure 2

Large interneurons in the granular layer of the rat cerebellum, revealed by double immunofluorescence histochemistry against the unidentified cytoplasmic antigen Rat-303 (*red* Cy3-fluorescence) and calretinin (*green* FITC-fluorescence). A third column of images depicted on the right reveals co-localization (*yellow*) of both neurochemical markers by combining respective single-labeled images. (A) High magnification overview of the granular layer showing three distinct types of large interneurons: a Rat-303-positive Golgi cell (arrow), CRT-positive UBCs (asterisks) and a Rat-303/CRT-positive Lugaro cell (arrowhead). (B) Rat-303-positive Golgi cells have a large, rounded or polygonal cell body with radiating dendritic arborizations extending in all directions. (C) CRT-positive UBCs exhibit a single, characteristic paint brush-like dendritic tree. (D) Rat-303/CRT-positive Lugaro cells are located just underneath the Purkinje cell layer. Their spindle-shaped soma emits from opposite poles two thick dendrites in the sagittal plane, parallel to the Purkinje cell layer. (E) Rat-303-positive Golgi cell (arrow) and Rat-303/CRT-positive large interneuron (open arrowhead) with quite similar morphologies, lying closely together in the middle of the granular layer. Abbreviations: GL, granular layer; ML, molecular layer; PC, Purkinje cell layer; WM, white matter. Scale bars = 20 μm (A, D, E) and 10 μm (B, C). Adapted from Ref. 57.

cells are inhibitory[2,29] and use both GABA and glycine as their neurotransmitters.[29–30] However, the postsynaptic target cells do not always express receptors to both neurotransmitters. Whereas the ipscs recorded from UBCs are mediated by both GABA$_A$ and glycine receptors,[31] the ipscs recorded from granule cells are mediated purely by GABA$_A$ receptors.[32] Moreover, granule cells express extrasynaptic high-affinity GABA$_A$
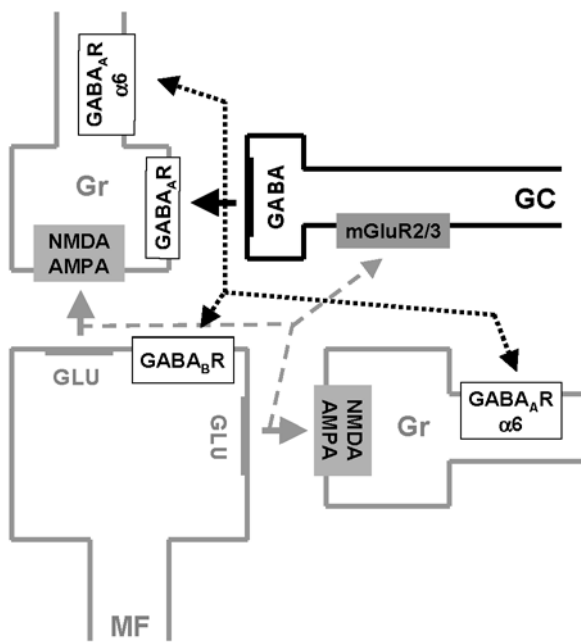
Figure 3

Synaptic and spillover-mediated transmission in the cerebellar glomerulus. Excitatory neurons and projections are in *gray*. Inhibitory neurons and projections are in *black*. Solid arrows indicate synaptic transmission; dashed arrows indicate spillover-mediated transmission. Presynaptic release sites are indicated by thickening of the membrane. Upon activation, mossy fiber terminals release glutamate that, in turn, activates postsynaptic NMDA and AMPA receptors located on granule cell dendrites. Granule cells also receive an inhibitory input from Golgi cells: GABA released from Golgi cell terminals acts through postsynaptic GABA$_A$ receptors. In addition, GABA diffuses outside of the Golgi cell-granule cell synaptic cleft and activates high-affinity α$_6$ subunit-containing GABA$_A$ receptors located extrasynaptically on dendrites of both directly connected and neighboring granule cells. Spillover of GABA also modulates mossy fiber input through GABA$_B$ receptors located on mossy fiber terminals. Furthermore, diffusion of glutamate outside of the mossy fiber-granule cell synaptic cleft inhibits GABA release from Golgi cells through the activation of mGluR2/3 receptors located at the Golgi cell axon. Finally, diffusion of glutamate may also improve transmission efficiency at neighboring mossy fiber-granule cell connections by activating synaptic AMPA receptors. Abbreviations: GC, Golgi cell; GLU, glutamate; Gr, granule cell; MF, mossy fiber.

receptors containing the α$_6$ subunit.[33] These receptors are activated by diffusion of GABA outside of the synaptic cleft, and their activation is 10-fold more persistent than that of the low-affinity intrasynaptic receptors.[34] GABA spillover activates also high-affinity receptors on neighboring but not directly connected granule cell dendrites[34] In this way, GABA spillover contributes to the regulation of granule cell excitability, both by providing an inhibitory input that operates on a prolonged time scale compared to conventional Golgi cell-granule cell ipsps, and by increasing the number of Golgi cells inhibiting a given granule cell.[34]

Spillover-mediated neuronal transmission, which is

determined by transmitter diffusion,[35] receptor affinity[34] and efficacy of transmitter uptake,[36] has been shown to affect several aspects of Golgi cell function. This is due to the fact that Golgi cell axons, granule cell dendrites and mossy fiber rosettes articulate synaptically inside glomerular structures that are ensheathed in glial processes.[37] Spillover of GABA, released from Golgi cell axonal terminals, also modulates mossy fiber afferent input to the cerebellar cortex through GABA$_B$ receptors located on mossy fiber rosettes.[38] These GABA$_B$ receptors are activated when GABA release is increased by stimulating Golgi cell input, resulting in a reduced glutamate release from the mossy fibers. Furthermore, glutamate release is more profoundly inhibited by GABA$_B$ receptors when mossy fiber firing rate is low.[38] In the other direction, spillover of glutamate released from mossy fiber rosettes inhibits GABA release from Golgi cells through the activation of type II metabotropic glutamate receptors (mGluR2/3) located at the Golgi cell axon.[39,40] Both mechanisms are likely to boost the efficacy of mossy fibers firing at higher rates. These spillover effects, schematically summarized in Figure 3, clearly indicate the complex nature of Golgi cell neurotransmission and the subtle control exerted by Golgi cells on granule cell excitability. Recently, spillover of glutamate has also been suggested to improve transmission efficiency at neighboring mossy fiber-granule cell connections by both reducing the variability and increasing the amplitude and duration of AMPA receptor epscs.[41]

The mossy fiber-Golgi cell-granule cell and parallel fiber-Golgi cell-granule cell disynaptic loops constitute, respectively, feedforward and feedback inhibitory circuits.[42,43] This connectivity led to the widely accepted theory that Golgi cells perform a gain control function[2,44,45] which is assumed to set the threshold for granule cell firing, keeping the excitation of local granule cells by mossy fibers within operational bounds. However, as described above, recent experimental and modeling studies have indicated other functions for the Golgi cell inhibition. The central role of spillover-mediated inhibition for the control of granule cell excitability was established in transgenic mice. Suppressing α$_6$-mediated spillover inhibition by homologous recombination techniques lead to the compensatory over-expression of a voltage-independent potassium conductance acting as a leak conductance and resulting in a decreased excitability.[46] Furthermore, the physiological importance of extrasynaptic inhibition was demonstrated by pharmacological block which enhanced granule cell and Purkinje cell responses to mossy fiber stimulations.[47] Because extrasynaptic inhibition occurs at the level of the glomerulus, it may allow for suppression of single mossy fiber inputs.[48]

Golgi cells fire vigorously in response to stimulation,[26] causing a pronounced phasic inhibition of granule cells in addition to the slower spillover inhibition. Golgi cells can be effectively stimulated both through the feedforward mossy fiber pathway and feedback parallel fiber pathway,
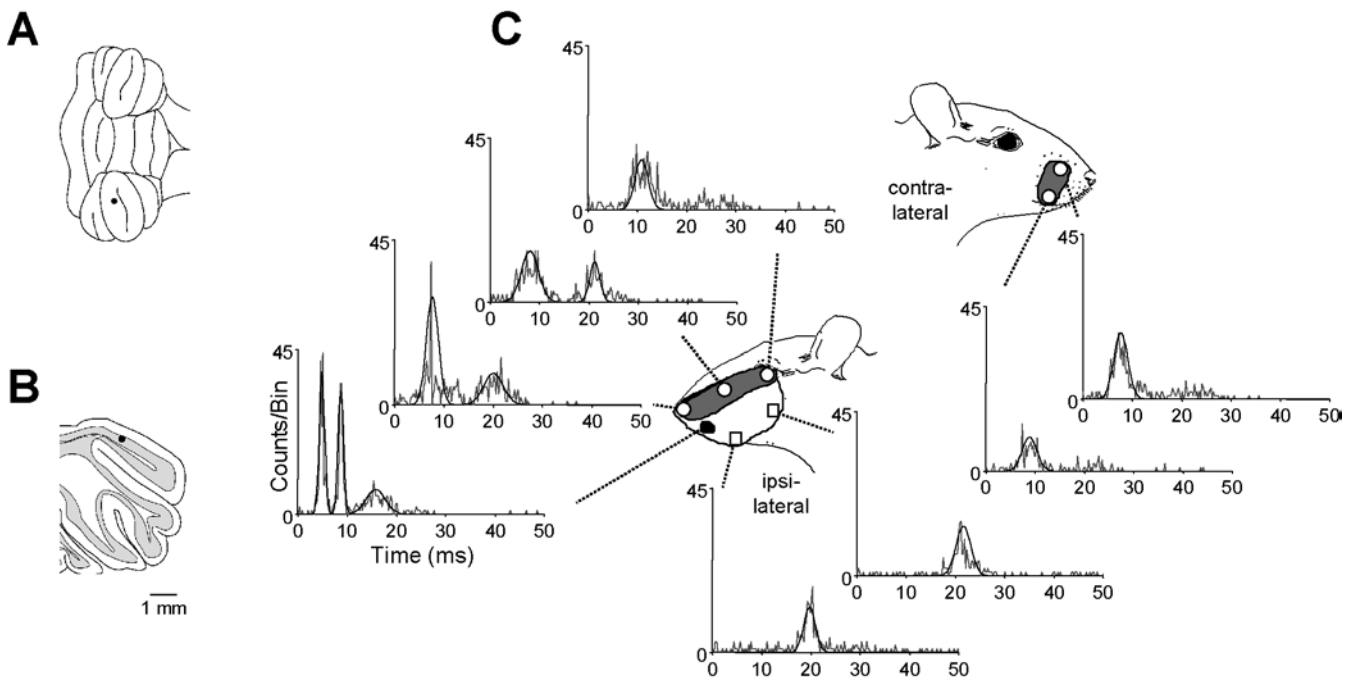
Figure 4

Large receptive fields and multiple response patterns of Golgi cells resulting from converging parallel and mossy fiber inputs. (A) Schematic representation of the extracellular single-unit recording site in Crus IIa of the rat cerebellar cortex. (B) Same on a transverse section, with the position of the recording site inside the granular layer. (C) Golgi cell responses evoked by tactile stimulation. The same stimulus (1 mm probe, 1 Hz) was applied to eight different locations in the rat face. High resolution post stimulus time histograms (PSTHs) based on stimulation of each of the locations (200 trials each) including the gaussian fit for each of the peaks are shown. The exact location from which each of the response profiles were evoked is indicated on graphical representations of the rat's face, contra- and ipsilateral to the recording site. The relation between the receptive field size and spike timing is illustrated. PSTH profiles with an early double peak are evoked from a restricted facial region (black area), while those showing an early single peak cover large parts of the rat's face on both sides (shaded area). The largest receptive fields are observed for responses that include a late component (white area). The early component of the Golgi cell response reflects trigemino-cerebellar input, whereas the late component reflects cortico-pontine input. Early double peaks are caused by direct mossy fiber input onto Golgi cells and have therefore a very short latency and can only be evoked from a restricted area. Other responses are parallel fiber mediated and comprise a very large and bilateral receptive field. Adapted from Ref. 49.

leading to large receptive fields and multiple response patterns[26,49] (Figure 4). Modeling studies have predicted that the feedback pathway can synchronize both Golgi cells and granule cells along the parallel fiber beam when the granular layer network is activated by mossy fiber input.[50] In other words, phasic Golgi cell inhibition will exert a strong effect on the timing of spikes in post-synaptic granule cells. Synchronous Golgi cell activity causes coherent spiking of local groups of granule cells leading to complex temporal patterns of multiple synchronized waves of spikes along the parallel fiber beam.[1] Partial experimental support for this temporal structure of parallel fiber activity caused by Golgi cells was obtained in anesthetized rats. It was demonstrated that Golgi cells along the parallel fiber beam fire loosely synchronized, while those not receiving common parallel fiber activity do not[51,52] (Figure 5). Moreover, as predicted, synchrony increases with network activity both during spontaneous firing[52] and during stimulus evoked responses.[53]

Finally, it must be noted that Golgi cell responses are also shaped by inhibitory inputs and intrinsic properties which have not been studied in great detail. Golgi cells show a strong after hyperpolarization,[25] which probably causes the long period of suppressed activity that follows the spiking response after stimulation.[26,53] The extra-synaptic inhibition may compensate for the lack of phasic inhibition during this absence of Golgi cells spiking. Golgi cells receive inhibitory projections from stellate/basket cells.[2,54] These synapses are GABAergic and will provide feedforward inhibition upon activation of the parallel fibers.[29] Therefore, the time scale for parallel fibers epsps summation in Golgi cells is probably short. Golgi cells receive another inhibitory input from a different type of interneuron, the Lugaro cell.[54] The nature of this input and its relevance for Golgi cell control is discussed in the following section.

Lugaro Cells

Besides Golgi cells, Camillo Golgi described a second type of large interneurons in the granular layer.[24] It was originally characterized by a fusiform soma lying directly
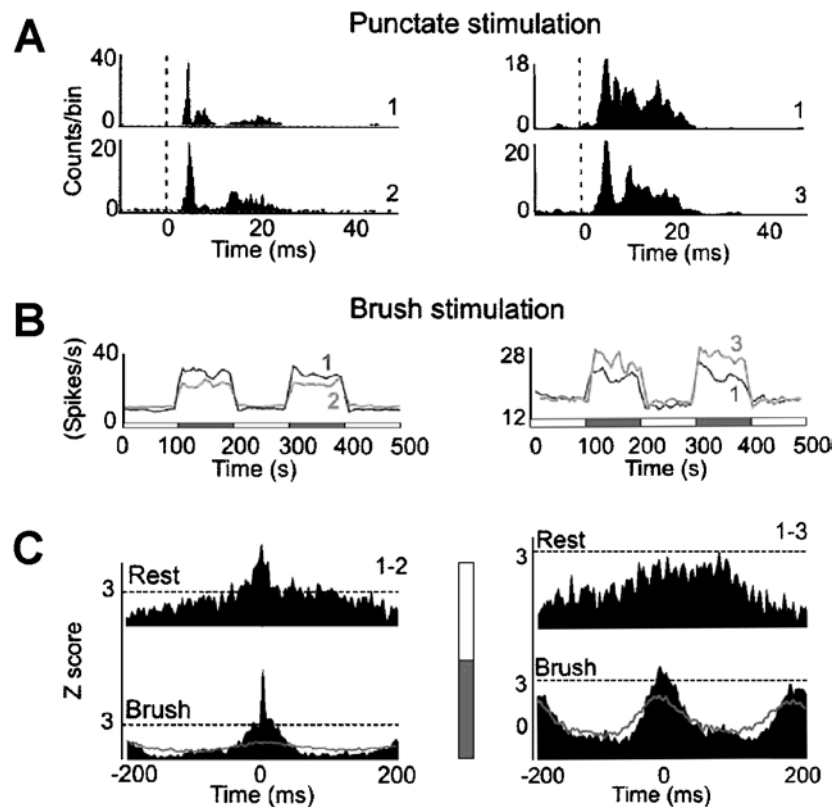
Figure 5

Responses of two pairs of Golgi cells to punctate and brush stimulation. Golgi cells aligned along the transverse axis of a folium fire synchronized (pair 1–2), both during spontaneous activity and during evoked responses. Conversely, Golgi cells positioned along the parasagittal axis (no common parallel fiber input; pair 1–3) do not develop precise coherent firing. (A) Golgi cell responses to punctate stimulation (1 mm probe, 1 Hz): PSTHs counting the number of spikes over 200 trials. Golgi cells respond with both an early and a late excitatory component followed by a silent period. (B) Golgi cell responses to brush stimulation, used to provide a continuous activation. The stimulus was carefully centered at the locus of punctate stimulation and consisted of a peripheral stimulation of a larger facial area using a manual brush. Stimulation was presented in blocks of 100 s (ON period) alternated with 100-s blocks without stimulation (OFF period), indicated by gray and white blocks, respectively. The mean Golgi cell activity increases during brush stimulation. (C) Cross-correlation histograms (CCH; bin width 1 ms) during OFF (Rest) and ON (Brush) periods expressed as Z score. Dashed lines indicate the significance level, Z = 3. Simultaneously recorded Golgi cells aligned along the coronal axis of a folium (pair 1–2) show high levels of synchronization at rest. Sagittally oriented pairs of Golgi cells (pair 1–3) lack synchrony at rest (Z < 3). Brush stimulation leads to an increased synchronization of coronal oriented pairs of Golgi cells (lower left panel). Although sagittal pairs of Golgi cells respond to brush and punctate paradigms, they never develop precise coherent firing (lower right panel): brush stimulation results in a very wide central peak which coincide with the shuffled CCH (thin gray line). Adapted from Ref. 53.

beneath the Purkinje cell layer, giving off dendrites from its opposite poles (Figure 2D). These dendrites ran parallel to the Purkinje cell layer, and occasionally extended in the granular layer. A more complete description of this fusiform cell was given by Ernesto Lugaro,[55] and was therefore named after him. Because they share some morphological characteristics with other large granular layer interneurons, Lugaro cells have been frequently classified as Golgi cells.[56] As a consequence, Lugaro cells have often been omitted from the description of neuronal cell types of the cerebellar cortex.[2,29] However, its classification as a distinct cell type was firmly established by the production of a specific antibody,[56] and has been confirmed using other immunohistochemical markers.[57]

Insight into the functional role of Lugaro cells in the corticocerebellar network has been gained recently. Lugaro cells are inhibitory interneurons that possess two types of axonal plexi.[58] The first plexus is parasagittally oriented and consists of a thin varicose axon originating from the cell body. Although the axon may run for some distance in the granular layer and even in the white matter, it always ascends back to the molecular layer, where it gives rise to a profuse plexus in the vicinity of its parent cell body.[58] The second axonal plexus is restricted to the molecular layer and consists of thick, partly myelinated transversal fibers running parallel to the parallel fibers,[58,59] and may have been mistaken for myelinated parallel fibers.[60] These fibers represent, with the parallel fibers, the only pathway for transverse
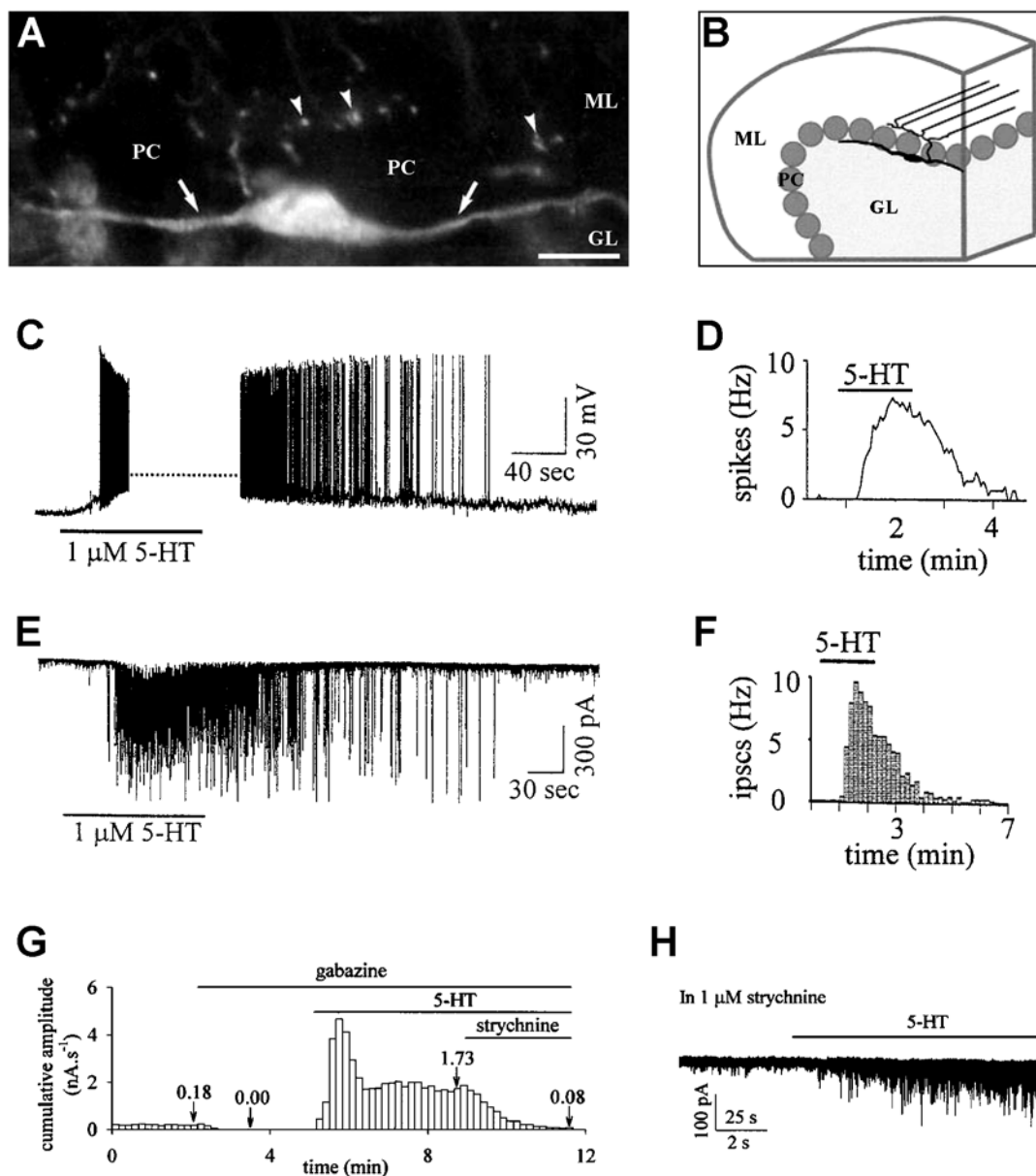
Figure 6

Serotonin (5-HT) induces a mixed GABAergic and glycinergic inhibition at Lugaro cell–Golgi cell connections. (A) High magnification of a calretinin-labeled Lugaro cell located between two immunonegative Purkinje cells in the sagittal plane. Two dendrites (arrows) originate from opposite poles of the fusiform cell body. Above the Purkinje cell bodies, immunoreactive spots (arrowheads) are observed corresponding to transversally cut Lugaro cell axonal fibers. Scale bar = 10 μm. (B) Schematic drawing of a cerebellar lobule cut in the sagittal plane, showing the morphological configuration of a Lugaro cell. Four axon collaterals are depicted in the transverse orientation, which is the orientation of the parallel fibers. Abbreviations in A and B: GL, granular layer; ML, molecular layer; PC, Purkinje cell. (C) Current-clamp recordings of a Lugaro cell in rat cerebellar slices demonstrate that the spontaneously inactive Lugaro cell is reversibly excited by serotonin. (D) Summary of the effect of serotonin on the spike firing frequency of a Lugaro cell. (E) The serotonin-induced activation of Lugaro cells evokes large ipscs in Golgi cells, which dominate the spontaneous activity evoked by stellate/basket cells. (F) Summary of the effect of serotonin on the frequency of large-amplitude ipscs recorded from a Golgi cell. (G) Summary of a series of experiments recorded from Golgi cells, illustrating that (1) gabazine, a GABA$_A$ receptor inhibitor, completely blocks spontaneous ipscs but not serotonin-evoked ipscs, and (2) strychnine blocks serotonin-induced ipscs in the presence of gabazine. (H) Reciprocally, serotonin evokes GABAergic ipscs in Golgi cells in the presence of strychnine. Consequently, besides a pure GABAergic inhibition from stellate/basket cells, Golgi cells receive a mixed GABAergic and glycinergic inhibition from Lugaro cells. Adapted from Refs 54, 59, 66.

information flow in the cerebellar cortex[59] (Figure 6A–B). Lugaro cells contact exclusively other inhibitory interneurons: with the parasagittal axon preferentially contacting stellate/basket cells[61] and the transverse fibers contacting Golgi cells.[54] The Lugaro cell-Golgi cell projection was the first connection in the cerebellum in

which functional co-transmission by GABA and glycine had been demonstrated[54,62] (Figure 6C–H). Here, again, glycine receptor expression is target-specific as they are absent at Lugaro cell contacts to stellate/basket cells.

The synaptic inputs onto Lugaro cells remain less documented. Purkinje cell collaterals, forming a beaded plexus just beneath the Purkinje cell layer,[63] are the only presynaptic elements identified to contact Lugaro cell somata and proximal dendrites.[64] These contacts may account for the pericellular nests made by Purkinje cell recurrent collaterals around some granular layer interneurons described by Cajal.[65] Although the ascending axon of granule cells might contact Lugaro cells, no glutamatergic inputs to Lugaro cells have been identified so far. As a first approximation, Lugaro cells will exert a feedback inhibitory control on Purkinje cells[61] through a trisynaptic inhibitory circuit, but its function may be way more complex. Given the peculiar transverse organization of their axons, Lugaro cells might play a role in the synchronization of Golgi cells found along the same parallel fiber beam,[59] and in particular it may organize the widespread oscillations recorded from the granular layer in the awake animal at rest. In contrast to all other cerebellar cells, the main excitatory input to Lugaro cells is performed by serotonergic modulation acting through volume transmission[59] (Figure 6C–H). Consequently, it has been proposed that the Lugaro cell is a serotonin-driven intracortical switch involved in the processing of mossy fiber information.[66]

Other Subclasses of Interneurons in the Granular Layer?

The existence of the three above-described classes of interneurons is now firmly established, but additional complexity has been reported in the granular layer circuitry. Initially, Ramón y Cajal identified four types of Golgi cells based on the extent and location of their axonal plexus.[65] A more prevalent view divides Golgi cells into small and large Golgi cells.[2,3] Recent morphological analysis does not support this division, but Golgi cells in the vermis appeared on average larger than Golgi cells in the hemispheres.[57] Besides morphological heterogeneity, neurochemical variation within the Golgi cell population has been described. Only 90% of the Golgi cells are found to express the metabotropic glutamate receptor mGluR2.[57,67] The remaining 10% is reported to express the metabotropic glutamate receptor mGluR5,[67] although this finding has been disputed.[25,68] The majority of Golgi cells display both GABA and glycine-like immunoreactivity[30] as well as immunoreactivity for glutamate decarboxylase[69] and the plasma membrane glycine transporter GLYT2.[70] However, a number of Golgi cells appear to be immunoreactive for glycine or GABA only.[71] In some species, including humans, a subpopulation of Golgi cells has also been shown to express choline acetyltransferase,[72,73] a marker for cholinergic neurons. Whether the above-described heterogeneity points towards the existence of functionally different populations of Golgi cells still needs to be resolved. In addition, a population of large neuronal cells within the granular layer has been shown to express Golgi cell morphology (regarding their somatodendritic appearance) but Lugaro cell neurochemistry[56,57] (Figure 2E). Detailed morphological analysis has recently revealed that these cells project their axon into the molecular layer, where it displays an arborization pattern reminiscent of that of Lugaro cells.[74] Furthermore, their cell body and dendrites are contacted by Purkinje cell collaterals,[74] a feature characteristic of Lugaro cells. Consequently, these cells represent a class of neuronal cells in the granular layer that does not fit the current classification. To designate these cells, and to differentiate them from Golgi cells and classical bipolar Lugaro cells, we propose the term 'multipolar Lugaro cell'. Further research will be needed to establish their exact role in the cerebellar circuitry. Finally, a recent study also indicates the existence of two distinct subpopulations of UBCs, which might be induced by differential innervation.[75] The functional importance of this specialization is presently unknown but suggests additional levels of organization in the UBC amplification network.

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