

TOPICAL REVIEW

Discovery and rediscoveries of Golgi cells

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When Camillo Golgi invented the black reaction in 1873 and first described the fine anatomical structure of the nervous system, he described a 'big nerve cell' that later took his name, the Golgi cell of cerebellum ('Golgi'schen Zellen', Gustaf Retzius, 1892). The Golgi cell was then proposed as the prototype of type-II interneurons, which form complex connections and exert their actions exclusively within the local network. Santiago Ramón y Cajal (who received the Nobel Prize with Golgi in 1906) proceeded to a detailed description of Golgi cell morphological characteristics, but functional insight remained very limited for many years. The first rediscovery happened in the 1960s, when neurophysiological analysis *in vivo* revealed that Golgi cells are inhibitory interneurons. This finding promoted the development of two major cerebellar theories, the 'beam theory' of John Eccles and the 'motor learning theory' of David Marr, in which the Golgi cells regulate the spatial organisation and the gain of input signals to be processed and learned by the cerebellar circuit. However, the matter was not set and a series of pioneering observations using single unit recordings and electron microscopy raised new issues that could not be fully explored until the 1990s. Then, the advent of new electrophysiological and imaging techniques *in vitro* and *in vivo* demonstrated the cellular and network activities of these neurons. Now we know that Golgi cells, through complex systems of chemical and electrical synapses, effectively control the spatio-temporal organisation of cerebellar responses. The Golgi cells regulate the timing and number of spikes emitted by granule cells and coordinate their coherent activity. Moreover, the Golgi cells regulate the induction of long-term synaptic plasticity along the mossy fibre pathway. Eventually, the Golgi cells transform the granular layer of cerebellum into an adaptable spatio-temporal filter capable of performing several kinds of logical operation. After more than a century, Golgi's intuition that the Golgi cell had to generate under a new perspective complex ensemble effects at the network level has finally been demonstrated.

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Discovery of Golgi cells

On 16 February 1873 a relatively unknown physician wrote to a friend these words: 'I spend long hours at the microscope. I am delighted that I have found a new reaction to demonstrate even to the blind the structure of the interstitial stroma of the cerebral cortex. I let the silver nitrate react with the pieces of brain hardened in potassium dichromate. I have obtained magnificent results and hope to do even better.' (Mazzarello, 2010). This was the first known recording of the invention of the 'black reaction', a

real breakthrough for brain structure research. The author of this letter was Camillo Golgi (1843–1926; Fig. 1) and for this invention he won the Nobel Prize for Physiology and Medicine in 1906. At the time of this contribution, Golgi was physician in charge in a hospital for chronic patients at Abbiategrasso, 30 km from Pavia, in the north of Italy. He afterwards became professor of General Pathology and Histology at the University of Pavia. Using his extraordinary method, Golgi undertook a systematic scientific exploration of the complex nervous system

architecture, starting his ambitious scientific adventure with the study of the cerebellum.

The Golgi cell in the first description by Camillo Golgi.

In 1874 Camillo Golgi published the work 'On the fine anatomy of human cerebellum' (Golgi, 1874), in which he described the Purkinje cells' dendritic tree and the whole cerebellar cytoarchitecture. Among the various cell types, Golgi described two kinds of 'big nerve cells' in the granular layer:

... always in the granular layer, with the silver nitrate method, I verified not only the beautiful characteristics of the nerve cell that lay here (characteristics that somebody denies), but I also underlined their exact dimensions, shapes, dispositions and ramifications ...

Speaking about their shape, they are really various, but we can roughly distinguish two main groups, that is: 1. long and narrow cells irregularly fusiform, with the maximum diameter parallel to circumvolution's surface; 2. irregularly round or polygonal cells, with round corners, quite laterally flattened, similar to some others lying in the deep layers of cerebral cortex, with the maximum diameter transversally placed in respect

to the direction of granular layer, and so perpendicular to the circumvolution's surface. Both these types have a large number of prolongations (extensions) ...

More than once from a single nervous prolongation of one of these cells, I saw the formation, cause of the fine and repeated subdivisions in all directions, of a complicated filament's interlacement, going from the periphery of the granular layer in both lateral directions, widespread for more than 200 μm .

For what concerns their position, we can say that these gangliar cells are present at the same level at the periphery of the granular layer, close to Purkinje cells, in the middle of the layer, and also in the deepest parts; sometime it is possible to see some of them in the medullary rays, where the nervous fibres are still parallel or they have just started diverging.

In this description, it is probable that the first type of cell was the Lugaro cells (named after Ernesto Lugaro, 1870–1940, who provided a detailed description of them). Moreover, there are no doubts that the second cell type is the one that now bears the name of Golgi, i.e. the 'Golgi cell' (Figs 1–3). Even today, the most relevant criterion to recognise the Golgi cell is the presence of its impressive

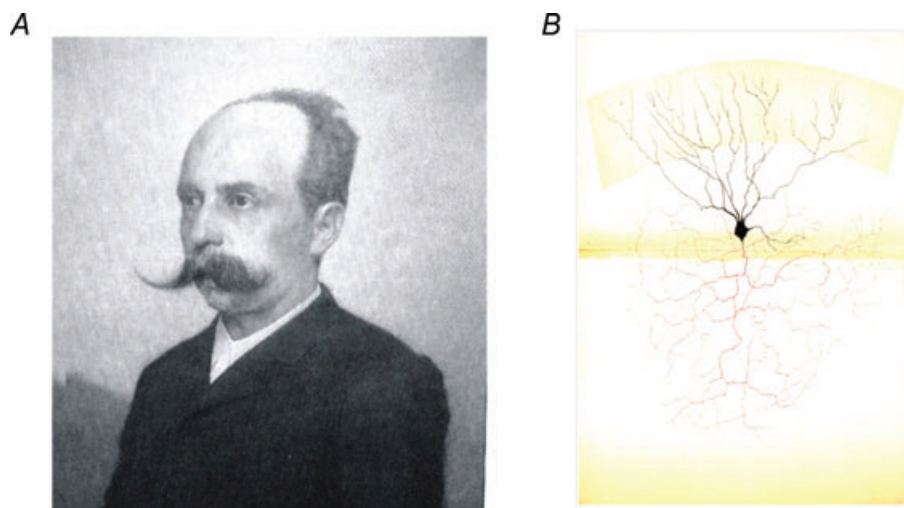


Figure 1. Camillo Golgi, the black reaction and the Golgi cell

A, Camillo Golgi, Nobel Prize winner for Physiology and Medicine (1906). Camillo Golgi (1843–1926) invented the 'black reaction', which allowed him and many other scientists to visualise the fine structure of the nervous system. B, in this picture, Golgi shows a reconstruction of a Golgi cell. Note the precise description of the basal and apical dendrites and of the large axonal plexus, the hallmark of the Golgi cell. (Gangliar cell in the (neonatal) cat cerebellar cortex; Table XIV, *Opera Omnia*, Golgi, 1903.) 'Such cells are part of the granular layer and, in the cat and rabbit, when the black reaction succeeds, can be seen in a considerable amount. ... Most ramifications of the protoplasmatic prolongations (in black) reach the superior border of the molecular layer. ... The nervous prolongation (in red), with repeated subdivisions becoming finer and finer, creates an extremely complicated interlacement of fibres. These, in the vertical plane, spread from one to the other border of the granular layer, and in the width of the granular layer mix up with the interlacements resulting from subdivisions of neighbouring cells of the same type (see Table XVII). This cell is one of the most remarkable specimens among those that are described as second type cells in the text. Regarding the cerebellum, such a cell should be compared to the one represented in Table XV (a Purkinje cell), representing one of the most remarkable specimens of the first type of cells.' Translated from *Opera Omnia* (Golgi, 1903).

axonal plexus (Dieudonné, 1998; Forti *et al.* 2006), so well highlighted by Golgi himself.

Golgi's functional hypothesis on the Golgi cell. After this first hint as to the cerebellar structure, Golgi developed a systematic investigation on the whole central nervous system, which he collected in 1885 in a comprehensive work with the title *On the Fine Anatomy of the Central Nervous System Organs* (Golgi, 1885). In the chapter of this book dedicated to the cerebellum, Golgi resumed his previous detailed morphological description and went further in considering the course of the cytoplasmatic prolongations of the various cerebellar cell types hypothesising their functional role.

It seems obvious to me to consider the cells whose prolongations go directly to form a nervous fibre, as organs with a direct influence on peripheral parts; they would likely be organs connected with motor activity. The other cells, about which I am sure to exclude a direct connection with the fibres that go from the periphery to the centre, seem to me organs connected to sensory activity, or even with automatic actions. In the filaments emanating from the nervous prolongations of this second category of cells, it is easy to recognise a central

communication way between the distinct categories of nervous elements.

Golgi noticed that Purkinje cell axons project out of the cerebellum (these fibres form indeed the sole cerebellar cortex output), so he hypothesised for these cells a motor function. He did not find a similar extra-cerebellum projection from granule cells, so he ascribed them to the sensory or 'automatic' function: modern evidence has backed up his deduction, because it is in the granular layer that mossy fibres present inputs to the cerebellar circuit, while Purkinje cells provide the output and can influence movement (e.g. see Bower, 1997). Moreover Golgi tried to give a role even to the big nervous cells: thinking about the fact that their impressive axonal plexuses do not go out of the cerebellum, he guessed that these cells (actually the Golgi cells) are connectional elements in the network. Camillo Golgi also hinted at a possible relationship of the Golgi cells with a structure ('nucleo granuloso') later named glomerulus (see below). In his Nobel Prize lecture, while classifying the first and second nerve cell types, Golgi presented a picture of the Golgi cell considering it as 'one of the most characteristic examples of the way in which the nerve process of the second type of cells

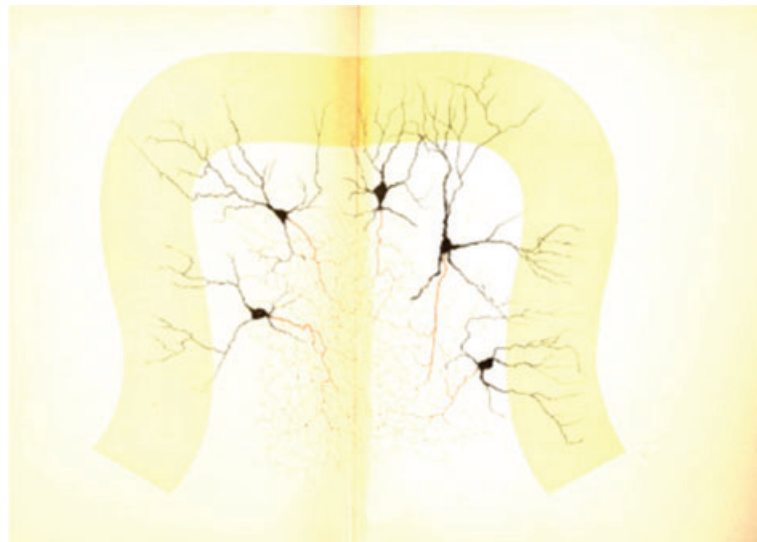


Figure 2. The spatial relationship between multiple Golgi cells

Note that no restriction is imposed to axons, which spread and overlap into the granular layer, that apical dendrites ramify in the molecular layer without specific orientation, and that basal dendrites ramify in the granular layer over a surface smaller than that occupied by the axon. In this figure, the fundamental features of the Golgi cell are captured. (Fragment of a (neonatal) cat cerebellar convolution (vertical section); Table XVII, *Opera Omnia*, Golgi, 1903). 'The drawing is specifically made to show shape, disposition, ramification laws, localisation and relationships of the large ganglionic cells of the granular layer. Protoplasmatic prolongations branch dichotomously in a very different way compared to Purkinje cells. The most distal extensions of the branches often reach the molecular layer peripheral limit. Nervous prolongations, with their fine and repeated subdivisions, form a complicated interlacement with the result that it is impossible to follow the course of single prolongations. Such interlacement does not seem to have borders either toward the inside of the granular layer or toward the molecular layer. Thus, several of these interlacings obviously mix up to form a complicated plexus.' Translated from *Opera Omnia* (Golgi, 1903).

[i.e. those with short local prolongations] behaves' (Golgi, 1967).

Golgi cells and the cerebellar network according to Santiago Ramón y Cajal. Following the introduction of the black reaction other histologists – namely the Italian Romeo Fusari, the Swiss Albrecht von Kölliker, the Belgian Arthur van Gehuchten and especially the Swede Gustaf Retzius – observed the Golgi cells of the cerebellum (Fusari, 1883; Van Gehuchten, 1891; Retzius, 1892). Retzius, in particular, published very clear and detailed drawings of these cells and described them in a paragraph

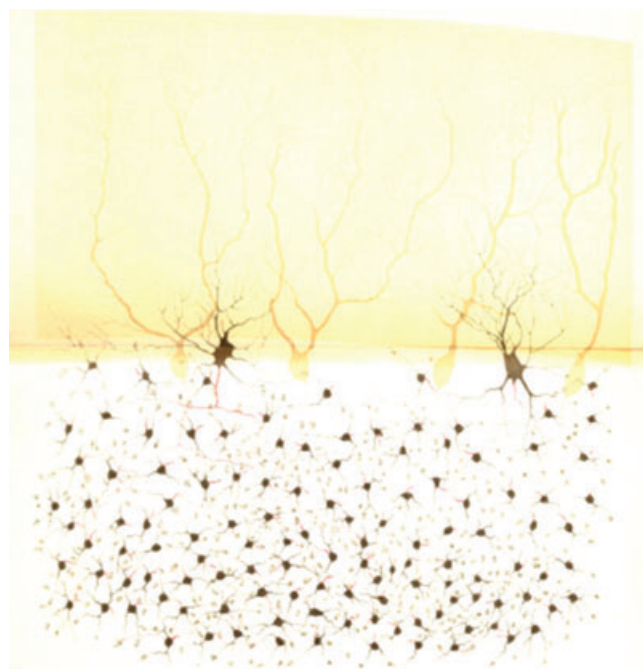


Figure 3. The relationship between Golgi cells and granule cells

Granule cells largely exceed in number the Golgi cells and are much smaller (Purkinje cells are distinctly shown in the background). Therefore a Golgi cell can innervate several granule cells lying within their axonal plexus, capturing another fundamental feature of the granular layer organisation. (Fragment of a rabbit cerebellar convolution (vertical section); Table XIX, *Opera Omnia*, Golgi, 1903). 'This drawing was specifically made to illustrate the granular layer. . . . The so-called granule cells look like nervous cells with a globose shape, really small and equipped with 3, 4, 5 or even 6 prolongations, among which just one has the features of nervous prolongation (the nervous prolongation is just outlined, red thread). Prolongations, which it seems to be correct to name protoplasmatic, even if they appear slightly different from other ganglionic cells' prolongations, end up with a small granulous mass, towards which neighbouring granule cells' prolongation often converge. In the region in which the granular layer merges into the molecular layer, two large cells are drawn. These are placed laterally and differ from Purkinje cells for the cell body shape, for the way of branching of their protoplasmatic prolongations and, overall, for the very different organisation of the nervous prolongation. . . . These two large cells are of the same type of the ones already illustrated in Tables XIV and XVII.' Translated from *Opera Omnia* (Golgi, 1903).

of a work devoted to the histology of the nervous system. Retzius in 1892 introduced the terminology 'Golgi'schen Zellen' (Golgi cells) to indicate this type of neuron. But the most extended description of the Golgi cells came with the great Spanish histologist Santiago Ramón y Cajal, who shared the Nobel Prize with Camillo Golgi in 1906.

Ramón y Cajal was one of the first and main users of the black reaction. By applying this new method, he proceeded through a systematic investigation of nervous elements. This work resulted in a fascinating and exhaustive treatment of the 'fine anatomy' of central nervous structures: the cerebellum, of course, was included. Ramón y Cajal dedicated some works (Ramón y Cajal, 1888, 1889*a,b*) and an entire chapter of his *Histology of the Nervous System of Man and Vertebrates* (first edition in Spanish 1899, 1904; French edition 1909, 1911; English edition, 1995) to the cerebellar granular layer, in which he identified four main components: granule cells (small and abundant), cytoplasmatic eosinophil islands (glomeruli), poor glial cells, and large neurons different from granule cells. Among the 'large cells' group, he identified subgroups: stellate cells (ordinary or with a long axon) that he named Golgi cells following Retzius's terminology, horizontal fusiform cells (which are quite certainly Lugaro cells), and displaced stellate cells (a heterogeneous group still today not well characterised, and identified as 'non-conventional large interneurons'; in this group we can include unipolar brush cells, synarmotic neurons, candelabrum neurons and perivascular neurons, besides Lugaro cells; Ambrosi *et al.* 2007).

Ramón y Cajal proceeded with a detailed description of Golgi cells' morphological characteristics, looking at soma, dendrites and axon, and confirming what Camillo Golgi had observed before. By considering localisation and soma, he identified them as big stellate or polygonal neurons, present everywhere in the granular layer but more abundant in the region close to Purkinje cells (compared to which they are smaller and more stellate). Golgi cells have a big nucleus, pale and eccentric, with a big spherical nucleolus; their cytoplasm is abundant and contains scarce and small Nissl bodies. Dendrites depart from the soma in any direction (determining the stellate shape) and some, as Retzius pointed out, reach the plexiform or molecular layer with spines that contact granule cells' axons. The dendritic tree is not placed on a unique plane as for Purkinje cells but, as precisely described by Golgi (Golgi, 1874, 1885, 1903) and then confirmed by Kölliker, van Gehuchten, Retzius and Ramón y Cajal (Kölliker, 1890, 1891, 1896; van Gehuchten, 1891, 1893; Retzius, 1892; Ramón y Cajal, 1888, 1889*a,b*, 1899, 1904, 1909, 1911, 1995), it is displaced, ascending and disorganised. The axon of these cells is peculiar: thick and almost similar to a dendrite, it has an initial bifurcation and a large

number of collaterals, forming a real axonal plexus, with varicose and hook-like endings contacting an enormous number of granule cells inside glomeruli. Depending on the position of the Golgi cell in the granular layer, its axon can be descending (cell close to Purkinje cells), tangential (cell close to white matter) or going in any direction (cell in the middle of the granular layer); the axon length and the arborisation degree of the axonal plexus are markers used to distinguish different subtypes of Golgi cells.

It is very interesting to focus on Ramón y Cajal's description of cerebellar glomeruli, because it is almost the same as what we are now able to produce with much more powerful histological techniques. He described the glomeruli as vacuolar dendritic cytoplasmic islands, without nuclei, underlining that they are not cells. Glomeruli, Ramón y Cajal continued, contain granule cells' dendrites, Golgi cells' axonal collaterals and mossy fibres' rosettes. In summary, the cerebellar glomerulus is a structure in which mossy fibres and Golgi cells are able to contact and influence a huge number of granule cells. Ramón y Cajal's account, extraordinarily detailed, continues with the description of the other large neurons present in the granular layer and with the comparative and developmental histology of the cerebellum. What emerges from his work is a clear interest in cerebellar neurons, particularly for the small granule cells and for the large stellate cells discovered by Camillo Golgi.

From the sixties to the nineties: first functional descriptions of Golgi cells

In the course of the first 60 years of the 20th century the Golgi cells were usually mentioned only in the anatomical handbooks, without adding new inferences about their putative physiological function. Their first rediscovery happened in the 1960s, thanks to improved histological and functional measurements leading to new hypotheses on the cerebellar circuit. The basic design of the cerebellar cortex and, inside it, of Golgi cell anatomical and functional connectivity was defined (Palay & Chan-Palay, 1974). Golgi cells were shown to receive excitatory inputs from mossy fibres and parallel fibres (the granule cell axons) and to give inhibitory outputs to granule cells into the glomeruli. Each granule cell receives three to four inhibitory synapses on different dendrites (Hámori & Somogyi, 1983; Jakab & Hámori, 1988). The Golgi cell–granule cell synapses consist of small boutons located proximally to the granule cell dendritic endings, which, in turn, receive the excitatory mossy fibre terminals. Both the mossy fibre and Golgi cell terminals, together with several tens of granule cell dendrites (see Palkovits *et al.* 1971; Ito, 1984), are included in the cerebellar glomerulus.

Observations that are not often mentioned are that the glomerulus includes Golgi cell basal dendrites (Hámori & Szentágothai, 1966) and that the climbing fibres send collaterals to the Golgi cell (Scheibel & Scheibel, 1954). Another surprising observation that was predictive of most recent discoveries on temporal coherence in the inhibitory cerebellar network (see below) was that electrotonic coupling occurred among basket cells and Golgi cells (Sotelo & Llinás, 1972).

A breakthrough in Golgi cell function happened in 1964, when R. Llinas and S. Sasaki in Sir J. C. Eccles's laboratory discovered the inhibitory nature of the Golgi cell (Eccles *et al.* 1964). This was in fact the first example of inhibitory feedback to be assigned to a particular neuronal element. Feedback inhibition of motoneurons, known as Renshaw cell inhibition (Renshaw, 1946), was originally described 25 years before the actual cell type responsible was identified anatomically (Jankowska & Lindstrom, 1971). With the subsequent elaboration of the so called 'beam theory', Eccles proposed an interpretation of Golgi cell's function (summarised in Eccles *et al.* 1967): the Golgi cell was considered to inhibit granule cells through two mechanisms, feedforward and feedback. By causing a strong inhibition in granule cells close to the core of mossy fibre activity, Golgi cells would improve the spatial discrimination of the inputs that reach the cerebellar cortex. However, this theory lacked some critical elements. Firstly, it was uniquely based on anatomy and disregarded circuit dynamics. Secondly, beam formation was uniquely attributed to lateral inhibition generated by basket stellate inhibitory innervations on Purkinje cells and had nothing to do with the Golgi cell–granule cell circuit. Thirdly, it made the assumption that Golgi cells generated a random inhibitory feedback. These are clearly three oversimplifications that have subsequently been reconsidered leading to re-evaluation of the connectivity and functional role of Golgi cells and of the entire cerebellar circuit (see below).

David Marr, one of the fathers of neurocomputation, in 1969 proposed his own theory to explain cerebellar functioning, which is known as the 'motor-learning theory'. He defined, on a theoretical basis, a computational role for Golgi cells. According to Marr, Purkinje cells are liable for the learning of motor patterns carried by mossy and climbing fibres to the granular layer. The number of patterns that can be learned at the Purkinje cell level decreases with the increase of the amount of active parallel fibres per input. It is necessary for one element of the circuit to act as regulator of the codon size, which represents the number of granule cells activated by a beam of mossy fibres. The Golgi cell is in an ideal position to play this role. Thus, Marr predicted that Golgi cells would be able to regulate granular layer excitability, and so the amount of information that can be elaborated, transmitted and learned.

Although the beam and motor learning theories were quite attractive and seemed to provide a comprehensive explanation for the whole cerebellum and for the Golgi cell inside it, the investigation of cellular dynamics and of detailed connectivity patterns moved ahead leading to new results mining the plausibility of the two theories. The contrast between the simplified Golgi cell connectivity proposed by the two theories and the morphological observations on the cerebellar glomerulus was striking. The very specific gating nature of the inhibitory feedback was investigated (Precht & Llinás, 1969) showing that the activity of a given granule cell–Purkinje cell set was more powerfully inhibited by homonymous activation of mossy fibres (in that case from the vestibular system) than from mossy fibres innervating the same granule cells from the contralateral vestibular nerve. This indicated that Golgi cell inhibition was probably glomerulus specific and not a random inhibitory feedback, as originally supposed. Moreover, as explained, potentially important observations like those on electrotonic coupling and on the fine structure of glomerular organisation were not considered. These observations, which address the real nature of the issue, have found their continuation in the

single cell recordings and detailed computational models developed in the last two decades (see below).

Golgi cells: the state of the art

A new trend in Golgi cell investigations arrived in the 1990s, when cerebellar neurons began to be recorded in acute slice preparations using the patch-clamp recording technique. Moreover, single unit Golgi cell activities were recorded *in vivo*, and the connectivity of these neurons has been revisited and their histochemical and functional properties redefined (Figs 4–8) generating the current view of Golgi cell connectivity and function. At the same time, the interest for brain dynamics has risen (Buzsáky, 2006) and the cerebellum has been subjected to intensive investigations aimed at understanding its involvement in timing and sensory expectation (Spencer *et al.* 2007) involving both the spheres of sensory motor programming and cognition (Ivry & Spencer, 2004).

Backing-up Golgi's predictions on local Golgi cell connectivity. Although the basic morphology identified

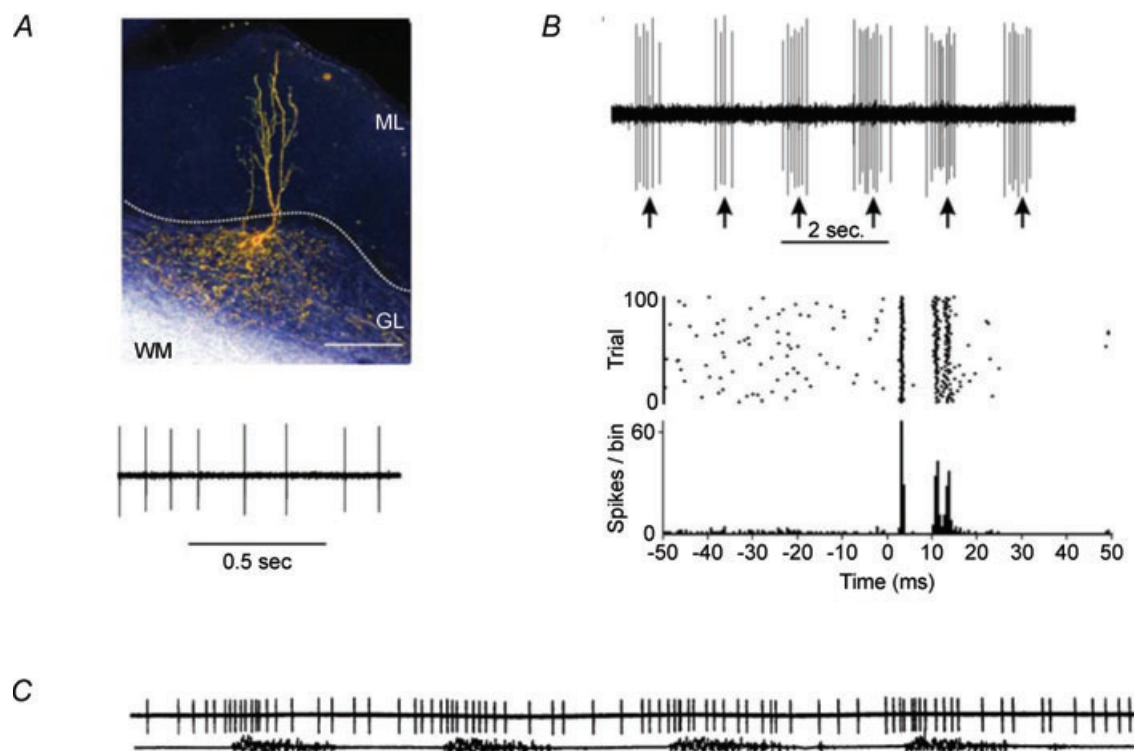


Figure 4. Golgi cell activity *in vivo*

A, the Golgi cell shows rhythmic background activity *in vivo* (from Holtzman *et al.* 2006a). B, peripheral sensory stimulation elicits bursts of activity (from Holtzman *et al.* 2006a). Each burst is usually composed of 2–3 spikes and is followed by a long-lasting inhibitory period (or silent pause in Vos *et al.* 1999). C, during locomotion, the Golgi cell is entrained into repetitive activity cycles, during which its frequency is modulated.

by Camillo Golgi is still accepted ('...irregularly round or polygonal cells, with round corners, quite laterally flattened...'; Golgi (1874)), several major advances have been made. In a quantitative description recently reported by Barmack & Yakhnitsa (2008), Golgi cells *in vivo* showed a relatively large (10–20 μm) soma. The axonal plexus extended with a parasagittal organisation, branching within the granule cell layer for $\sim 650 \mu\text{m}$ sagittally and for $\sim 180 \mu\text{m}$ medio-laterally. The dendrites showed variable morphology and often two to four dendrites emerged as thin processes from a single point in the soma and terminated with several varicosities.

The fact that Golgi cells may be heterogeneous ('...Speaking about their shape, they are really various... For what concerns their position, we can say that these ganglionic cells are present at the same level at the periphery of the granular layer, in the middle of the layer, and also in the deepest parts.' Golgi (1874)) has also been revisited. Five distinct subpopulations of Golgi cells were distinguished by Simat and colleagues (2007) based on neurochemical phenotype, and cell shape, size and location in the granular cell layer. The majority of Golgi cells are in fact both GABAergic and

glycinergic (80%), some are specifically GABAergic (15%), and some are specifically glycinergic (5%). Whereas, in general, granule cell inhibition is only GABAergic, in the vestibulo-cerebellum Golgi cells inhibit the granule cells by releasing GABA and the unipolar brush cells (UBCs) by releasing glycine. Moreover, Geurts *et al.* (2001, 2003) found various expressions of certain biochemical markers (rat-303, calretinin, mGluR2, somatostatin). The Golgi cells may thus contribute to distinct cerebellar subcircuits, although no remarkable differences in their intrinsic excitability have emerged (Forti *et al.* 2006).

It is important to note that Golgi postulated a local connectivity (Golgi, 1874), which has been subsequently redefined and analysed in detail (Eccles *et al.* 1967; Palkovits *et al.* 1971; Palay & Chan-Palay, 1974; Ito, 1984). Functional studies (Figs 4–6) have shown that Golgi cell activity can be influenced both by mossy fibres, parallel fibres and climbing fibres and by molecular layer interneurons (Eccles *et al.* 1967; Vos *et al.* 1999a; Holtzman *et al.* 2006a,b, 2009; Xu and Edgley, 2008; Barmack & Yakhnitsa, 2008; Ros *et al.* 2009). Tactile punctuate stimulation readily activates the Golgi cells generating a first rapid spike response through sensory mossy fibres; a second spike

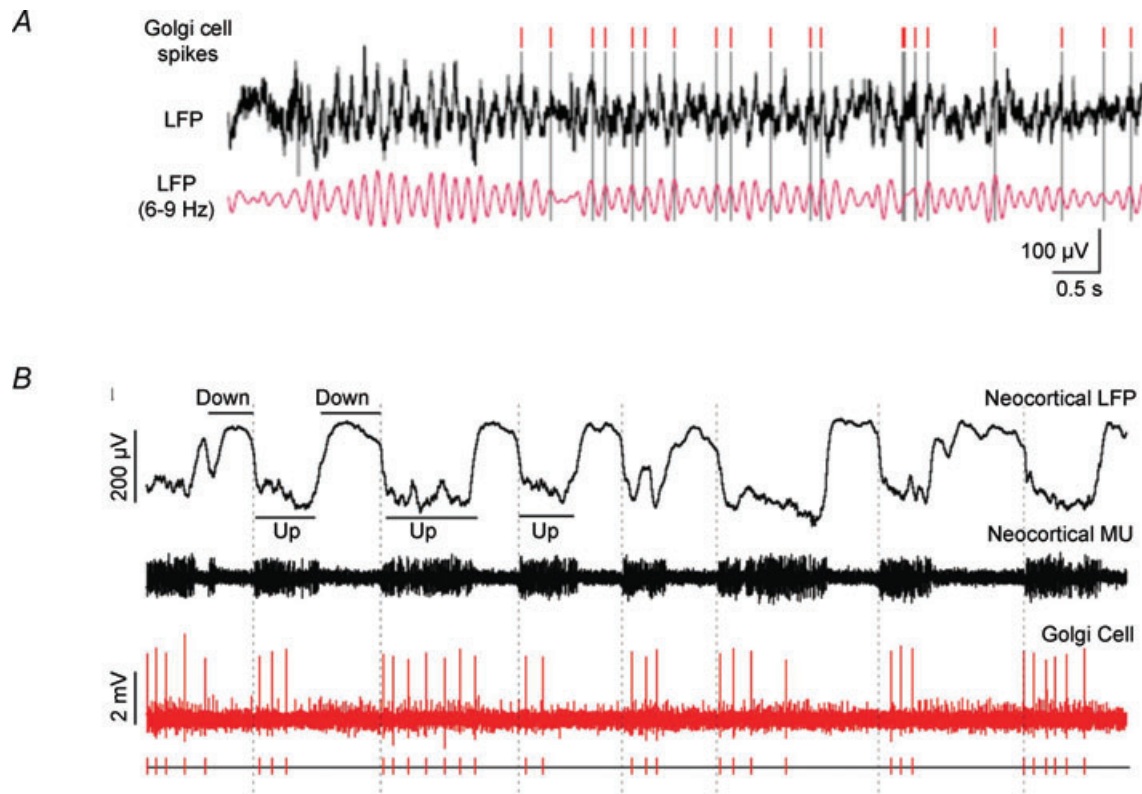


Figure 5. Golgi cell network entrainment

A, the Golgi cell spikes are in phase with the local field potential of the granular layer (Dugué *et al.* 2009). *B*, Golgi cells can show rhythmic entrainment with the UP–DOWN states characterising neocortical activity (Ros *et al.* 2009). The different behaviour in *A* and *B* may reflect different functional states or simply the fact that the trace in *A* may be part of an UP state as shown in *B*.

is then conveyed through cortico-cerebellar pathways and further spikes are possibly conveyed through the parallel fibres. However, while a physiological analysis has been performed for the granule cell → Golgi cell (Dieudonné, 1998; Bureau *et al.* 2000), stellate/basket cell → Golgi cell (Dumoulin *et al.* 2001) and Lugaro cell → Golgi cell (Dieudonné & Dumoulin, 2000) connections, the nature of connections from mossy fibres and climbing fibres to Golgi cells remains largely to be determined. Recent results indicate that the mossy fibre → Golgi cell connection is glutamatergic and rapidly and efficiently excites the Golgi cell (Kanichay & Silver, 2008), but several issues remain unanswered. Do all mossy fibre synapses originate from glomerular connections? Do granule cells form their main connections with the Golgi cell through the parallel fibres or are there also *en passant* synapses along the ascending axon? Are the afferent synapses formed by mossy and parallel fibres functionally equivalent? Concerning the climbing fibre → Golgi cell connection (Scheibel & Scheibel, 1954; Shinoda *et al.* 2000), despite climbing fibres being excitatory on Purkinje cells they have an inhibitory

effect on the Golgi cells. This can occur in at least two different ways (Xu & Edgley, 2008), (i) by activating the stellate cells, which in turn inhibit the Golgi cells, or (ii) by activating mGluR2 glutamate receptors on Golgi cell dendrites, which activate an inward rectifier current preventing depolarisation (Watanabe & Nakanishi, 2003). And what is the impact of the electrical junctions formed by Golgi cells (Sotelo & Llinás, 1972)?

Clearly much remains to be done before a complete view of Golgi cell functional connectivity can be drawn. A more detailed explanation on functional synaptic connectivity and a survey on the receptors involved at the different synapses can be found below and in Appendix 2.

How local Golgi cell connectivity may determine function.

Camillo Golgi understood that morphology and local connectivity had to reflect the function that a single nerve cell plays in the local network as well as in the economy of the whole nervous system (Golgi, 1967). We consider here six main observations connecting the structure of the Golgi cell to its function.

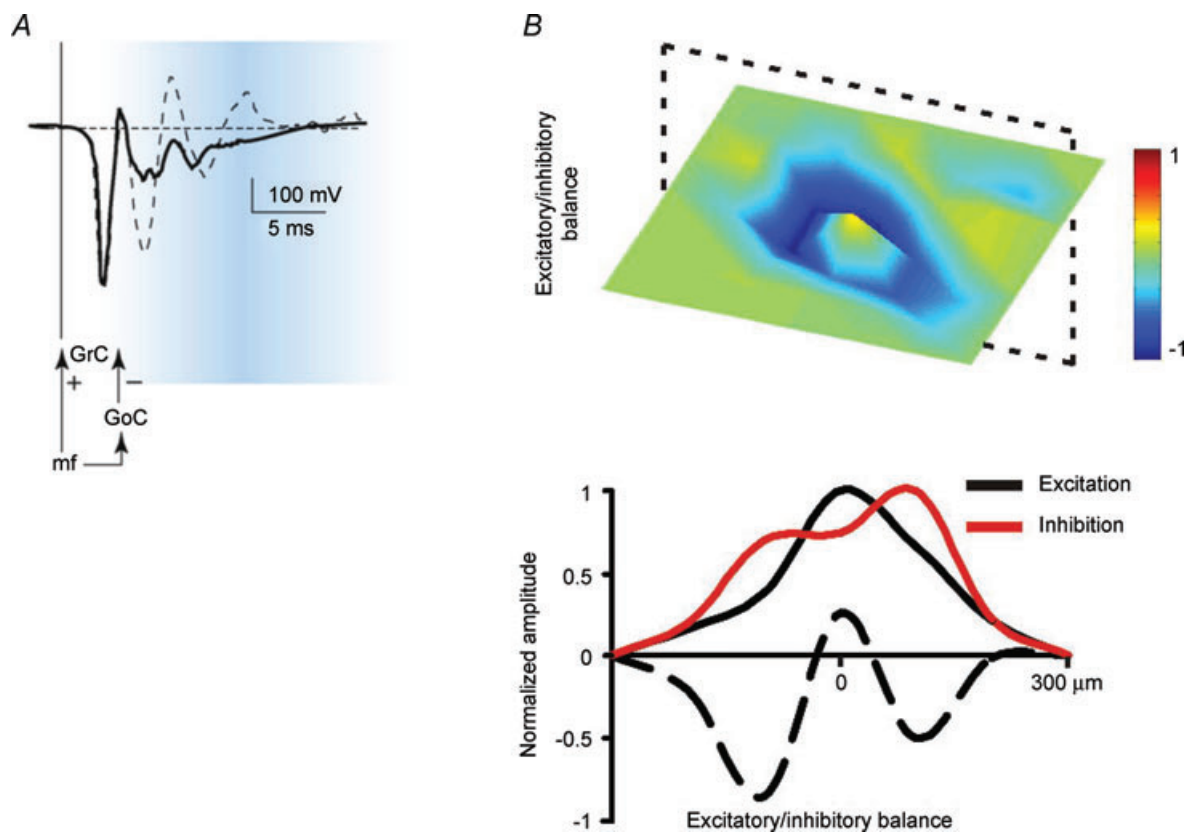


Figure 6. Control of granular layer spatio-temporal dynamics by Golgi cell inhibition

A, stimulation of a mossy fibre beam elicits local field potentials in the granular layer, which can be composed by more spikes generated in sequence by granule cells. Activation of the feed-forward inhibitory Golgi cell loop limits spike emission (time-window effect; from Mapelli & D'Angelo, 2007). *B*, stimulation of a mossy fibre beam elicits local field potentials in the granular layer, which are surrounded by lateral inhibition generated by Golgi cells (centre-surround effect; from Mapelli & D'Angelo, 2007).

As observed in Figs 1–3 and 4A (see also Dieudonné, 1998; Forti *et al.* 2006; Barmack & Yakhnitsa, 2008), the Golgi cell axonal plexus extends exclusively in the granular layer and, through thin branches, can form secondary plexuses in the same or even in neighbouring laminae (e.g. see Eccles *et al.* 1967). The broader extension of the axon than the basal dendrites provides the basis for lateral inhibition, whose functional impact has recently been demonstrated by multi-electrode array recordings and voltage-sensitive dye (VSD) imaging (Mapelli & D'Angelo, 2007; Mapelli *et al.* 2009; Fig. 6).

In some of Golgi's original drawings the axonal fields of Golgi cells overlap (e.g. see Figs 2 and 3), although in subsequent drawings the Golgi cell axonal fields appear well isolated (e.g. see Eccles *et al.* 1967; Ramón y Cajal, 1995; Ito, 1984). This overlap would be important to allow the combinatorial inhibition of granule cells and has recently been observed using fluorescence staining *in*

vivo (see Barmack & Yakhnitsa, 2008). Consistently, the analysis of Golgi cell–granule cell neurotransmission has recently shown that each granule cell receives inhibition from different Golgi cells, implying overlap of the axonal fields (Mapelli *et al.* 2009).

In Golgi's drawings, the terminal endings of Golgi cell axons generate hand-like structures matching the size and separation distance of the glomeruli (Figs 1 and 2 and Golgi, 1967), a fact even more evident in subsequent drawings by Ramón y Cajal (1889*a*). The presence of robust GABA receptor-mediated spillover responses to Golgi cell axon stimulation, which indicates diffusion of GABA onto neighbouring dendrites (Rossi & Hamann, 1998), suggests that Golgi cell terminals can inhibit clusters of granule cells sending their dendrite in the same glomerulus (Mapelli *et al.* 2009).

A feature that was already evident in Golgi's drawings and clearly described by Ramón y Cajal is that, at

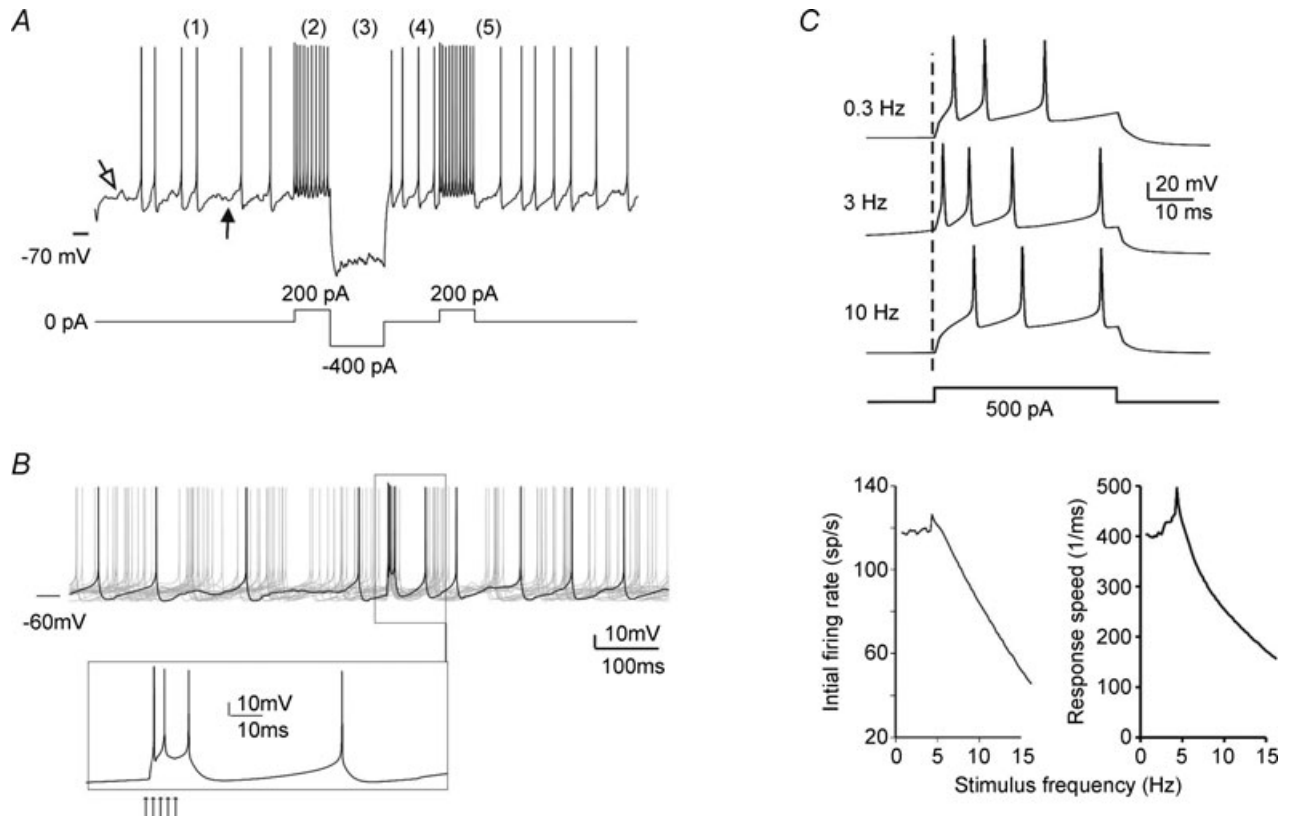


Figure 7. The electrical activity of Golgi cells

The Golgi cell shows response dynamics, which can support the cycles of activation and inactivation observed in various functional conditions (see Figs 5 and 6). *A*, the Golgi cell *in vitro* shows (1) pacemaker activity at around 7 Hz. In response to depolarisation (2), the Golgi cell shows high-frequency discharge with frequency adaptation. In response to hyperpolarisation (3), the Golgi cell shows sagging inward rectification followed by (4) rebound excitation. Bursts of activity are followed by (5) a silent pause. *B*, demonstration of the silent pause following a burst response to a mossy fibre stimulus (arrows in the inset). *C*, when stimulated with pulses repeated at different frequencies, the Golgi cell shows enhanced responses (faster and higher frequency spikes) at the resonant frequency of 6 Hz. The tracings are simulated from the models of Solinas *et al.* (2007*a,b*, 2010) and reproduce response behaviours reported in Dieudonné (1988), Forti *et al.* (2006) and Solinas *et al.* (2007*a,b*).

variance from Purkinje cells, the structure of Golgi cell dendrites is not rigorously organised in a plane but rather is more irregular and three-dimensional. Thus, Golgi cells may not be suited to detect ordered time sequences transmitted through the parallel fibres (Braitenberg *et al.* 1997). These observations combined with recent electrophysiological and modelling data support the view that Golgi cells can both precisely respond to topographically organised inputs and perform an extended spatio-temporal integration of parallel fibre information modulating their basal activity state (Vos *et al.* 2000; De Schutter & Bjaalie, 2001; De Schutter, 2002).

Another fact is that, in Golgi's and Ramón y Cajal's drawings, the axonal plexus of Golgi cells is always

shown in parasagittal sections. This implicates that the Golgi cell axon expands preferentially in the parasagittal plane, as subsequently confirmed by confocal imaging (Barmack & Yakhnitsa, 2008). The axonal organisation matches the parasagittal distribution of mossy fibre ramifications (Sultan, 2001). Moreover, the entire Golgi cells, comprising their axon and dendrites, are segregated into parasagittal compartments for zebrin-2, aldolase C, nitric oxide synthase (NOS) and other markers of granular layer neurons and Purkinje cells (Sillitoe *et al.* 2008). This observation is related to a major issue of cerebellar organisation, in which mossy fibre inputs coherently activate certain granular layer areas, certain sets of Purkinje cells and specific portions of the olivo-nuclear complex,

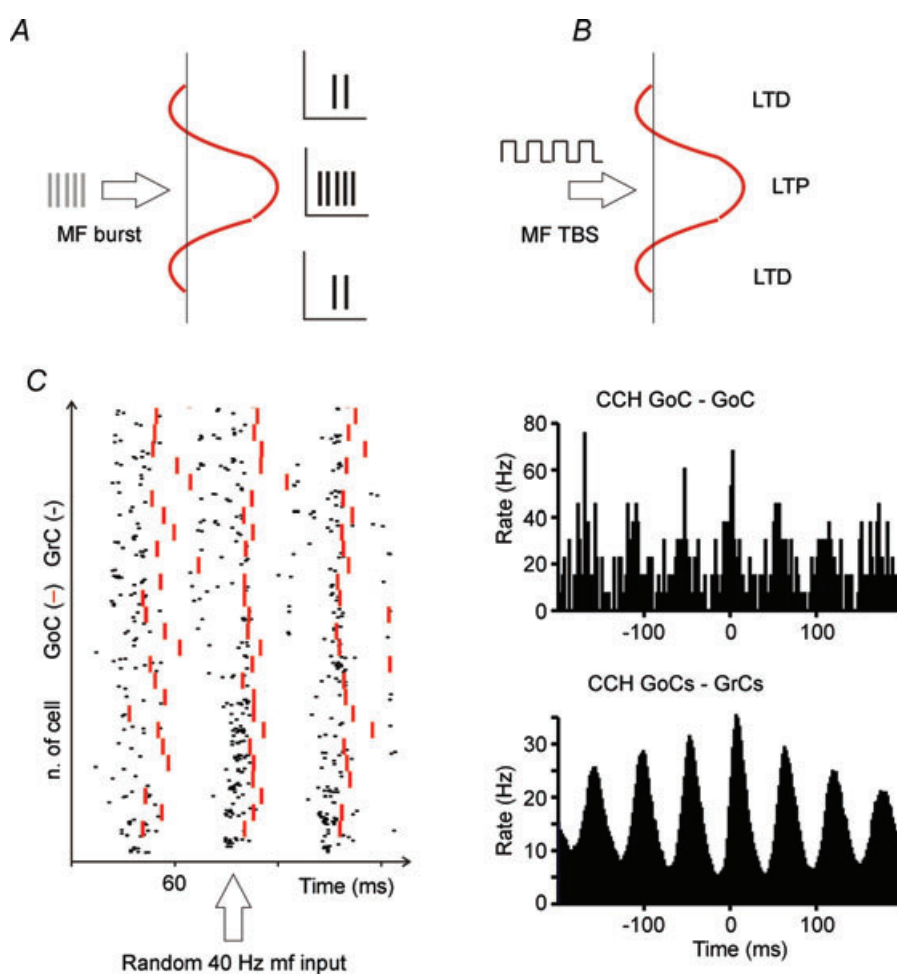


Figure 8. The physiological consequences of Golgi cell activation

Golgi cell activation has complex consequences for granular layer responses. *A*, a burst in a group of mossy fibres (MF) generates stronger and faster granule cell responses in the centre than in the surround of a granular layer field (the red profile represents the excitatory/inhibitory balance in a granular layer field activated by a group of MFs and regulated by Golgi cells; drawn after Mapelli & D'Angelo, 2007). *B*, a MF theta-burst stimulation (TBS) in a group of MFs generates more effectively LTP in the centre and LTD in the surround of the granular layer field. *C*, the raster plot shows that a diffused random stimulation of the MFs generates a coherent response of the granule cells (GrC) and of the Golgi cells (GoC). Synchronisation is due to parallel fibre feed-back inhibition. Crosscorrelograms (CCH) are shown for two GoCs and for all the granule cells and the Golgi cells in the network revealing their coherence. Data elaborated from the model of Solinas *et al.* (2010).

thus forming structural and functional modules (Brown & Bower, 2001; Voogd *et al.* 2003; Pijpers *et al.* 2006; Apps & Hawkes, 2009). Therefore, it seems that Golgi cells, through mossy fibre (and potentially climbing fibre) inputs to their dendrites, are wired within microcircuits involving specific cortico-nuclear modules, while through their parallel fibre connections they can be interconnected with multiple modules.

Finally, the presence of gap junctions among the inhibitory interneurons (stellate, basket and Golgi cells) was early observed both within and across classes (Sotelo & Llinás, 1972). The role of this inhibitory syncytium, which may facilitate the synchronisation of vast areas of the cerebellar cortex, has only been recently addressed. The functionality of gap junctions between stellate cells (Mann-Metzer & Yarom, 2000) and between Golgi cells (Dugué *et al.* 2009) has been demonstrated using double-patch recordings, while low-resistance electrical communication between molecular layer interneurons and Golgi cells remains to be demonstrated. Clearly, low-resistance electrical connections between Golgi cells have the potential, together with other mechanisms, to enhance the local coherence of cerebellar activity.

Golgi cell physiology: dynamic properties and network entrainment. A completely new view on the Golgi cell has been opened by electrophysiological recordings *in vitro* and *in vivo* (Figs 4–6).

Electrophysiological patch-clamp recordings *in vitro* (see Appendix 2) have shown that the Golgi cell is a pacemaker neuron that fires autonomously at 1–10 Hz (Dieudonné, 1998; Forti *et al.* 2006; Fig. 7A). When perturbed, it shows discharge adaptation, post-inhibitory rebound and afterhyperpolarisation (Fig. 7A–B). In addition, Golgi cells are resonant around their oscillation frequency, making them suitable to enhance responses in the theta-frequency band (Solinas *et al.* 2007a,b; Fig. 7C). Rhythmic activity is also observed *in vivo* both in awake (cat: 2 to <50 Hz, Edgley & Lidieth, 1987; monkey: 10–80 Hz, Miles *et al.* 1980) and anaesthetised animals (rat: 2–30 Hz, Schulman & Bloom, 1981; Vos *et al.* 1999a; Holtzman *et al.* 2006a,b), probably as a reflection of pacemaking modulated by synaptic activity. It has been recently proposed that electrical coupling between Golgi cells could be critical to allow the emergence of low-frequency pacemaking, at the same time synchronising oscillations in neighbouring Golgi cells (Fig. 5A; Dugué *et al.* 2009). Golgi cells show ‘loose synchrony’ over hundreds of micrometres along the coronal axis (Volny-Luraghi *et al.* 2002; Tahon *et al.* 2005), possibly reflecting synchronisation along the parallel fibre beam and feed-back inhibition onto granule cells (Vos *et al.* 1999b).

The mossy fibre inputs occur in two main modalities, namely protracted frequency-modulated discharges and short high-frequency bursts (Chadderton *et al.* 2004; Kase *et al.* 1980; Van Kan *et al.* 1993; Jörntell & Ekerot, 2006; Rancz *et al.* 2007). Accordingly, the Golgi cells present two well defined response modalities. First, Golgi cells can follow peripheral signals in a continuous fashion modulating their frequency with the intensity of the stimulus (Miles *et al.* 1980; Edgley & Lidieth, 1987). Secondly, Golgi cells respond to punctuate stimulation with a short burst of spikes. The bursts occur very rapidly (in about 10 ms upon facial stimulation) and consist of one to three well timed spikes in short sequence (Fig. 4B; Vos *et al.* 1999a; Holtzman *et al.* 2006a). The first spike corresponds to the trigeminal input (trigemino-cerebellar mossy fibres), the second spike to sensory-motor cortical input (cortico-ponto-cerebellar mossy fibres), and the third one may reflect the parallel fibre input. Following the bursts, the Golgi cell generates a long-lasting inhibitory period (Fig. 4B; Holtzman *et al.* 2006a,b, 2009; Xu and Edgley 2008) or silent pause lasting for about 100 ms, probably reflecting both intrinsic membrane properties and synaptic inputs (Fig. 7A–B; Solinas *et al.* 2007a,b).

The entrainment of Golgi cells into local and extracerebellar networks is observed in different cases (Figs 4 and 5). In states of restive attentiveness and active expectancy (Pellerin & Lamarre, 1997; Hartmann & Bower, 1998; Courtemanche *et al.* 2009), the granular layer shows rhythmic activity in the theta-frequency band. In the anaesthetised animal, Golgi cells can generate repeated bursts along with granule cells during the ‘up’ phase of the slow delta-frequency cortical oscillations (Ros *et al.* 2009). Golgi cells can contribute to tune the granular layer toward this slow frequency band through the feed-back inhibitory loop (Maex & De Schutter, 1998), their resonant properties (Figs 7 and 8; Solinas *et al.* 2007a, 2007b) and electrical synapses (Dugué *et al.* 2009).

In summary, the Golgi cell, by shifting from a rhythmic discharge to an event-drive state on behavioural demand (D’Angelo, 2008), can both entrain and be entrained in network oscillations demonstrating an intimate relationship with both local and long-range circuits (cf. Buzsaki, 2006).

Multiple inhibition mechanisms contribute to global network computation. The Golgi cells take part in granular layer processing through multiple inhibitory mechanisms as follows.

- (i) Golgi cells generate *phasic* and *tonic inhibition* (Crowley *et al.* 2009; Mapelli *et al.* 2009). This latter occurs through neurotransmitter spillover causing slow and diffused responses within the glomerulus

- (Brickley *et al.* 1996; Rossi & Hamann, 1998) and inhibiting numerous granule cells altogether.
- (ii) Golgi cells organise granular layer responses in centre-surround exploiting *lateral inhibition*. The centre-surround organisation, once occurring over partially overlapping activation fields, generates combinatorial responses (Mapelli *et al.* 2009) resembling coincidence detection and spatial pattern separation predicted by the motor learning theory (Marr, 1969; Albus, 1971).
 - (iii) Golgi cells enhance coherent oscillations in the granular layer network. Oscillations occur through *feedback inhibition* and coherence emerges from the fact that Golgi cell inhibition extends over large granule cell fields. The intrinsic Golgi cell excitable properties, including pacemaking and resonance, may tune oscillations on the theta band complementing the theta-frequency resonance of granule cells (D'Angelo *et al.* 2001; Lombardo & D'Angelo, unpublished observations).
 - (iv) Golgi cells determine a time window through which granule cell spikes are allowed to pass in response to mossy fibre bursts. This occurs through *feed-forward inhibition*, which limits the duration and intensity of granule cell excitation (Kanichay & Silver, 2008; D'Angelo & De Zeeuw, 2009).
 - (v) Golgi cells regulate the gain at the mossy fibre-granule cell relay during prolonged input bursts by exploiting *tonic inhibition*, which regulates granule input resistance and spike threshold (Mitchell & Silver, 2003).
 - (vi) Golgi cells, by regulating granule cell depolarisation through a *shunting inhibition* mechanism, control NMDA channel unblock and the induction of mossy fibre-granule cell long-term synaptic plasticity in response to repeated mossy fibre bursts (Mapelli & D'Angelo, 2007).

These properties suggest that Golgi cell inhibition can allow the granular layer to operate as an adaptable spatio-temporal filter (Dean *et al.* 2010) capable of regulating delay, controlling gain, enhancing contrast and combining multiple afferent input fields (D'Angelo & De Zeeuw, 2009). These mechanisms have been interpreted through mathematical models incorporating the main neurophysiological properties of Golgi cells (Solinas *et al.* 2010; Figs 7 and 8). And by being embedded into the granular layer, the Golgi cell operates as a hidden neuron in a multi-layered network that could provide extended computational capabilities to the whole cerebellum (Mapelli *et al.* 2009). How these mechanisms contribute to setting-up cerebellar computation as a whole remains a challenge for future research.

The discovery of Camillo Golgi: a retrospective view and future developments

One of the major issues raised by the Nobel Prize dissertations in Stockholm in 1906 concerned the complexity of brain structural organisation (Ramón y Cajal, 1967; Camillo Golgi, 1967; Appendix 1). Ramón y Cajal was undisputedly the paladin of the neuron theory according to which the nervous tissue is composed, like any other tissues, of single independent cells. On the other hand Camillo Golgi was impressed by the complexity of the brain and thought that it was not possible to account for brain performance by simple point-to-point connectivity. Based on the observations made with the black reaction, 15 years before Ramón y Cajal, he proposed a reticularist theory known as the 'diffuse nervous net'. This states that the axonal prolongations of nerve cells were fused (or intimately interlaced) in a diffuse web along which the nervous impulse propagated (Kruger, 2007; Kruger & Otis, 2007; Mazzarello, 2007, 2010). The debate between neuronists and reticularists, which was at the origin of modern neuroscience, was a fundamental clash of ideas between the 18th and 19th century. In the end, Ramón y Cajal's neuronal theory of the nervous system triumphed and it is now considered the fundamental paradigm of brain organisation. Golgi, in turn, gave a wrong answer to a real question, i.e. the difficulty of conceptualising complexity with simple point-to-point connectivity. Golgi envisioned multiform dynamical and interacting fields of activity, much as modern neural network theory is predicting even if, of course, in a different way (Rieke *et al.* 1997; Buzsáki, 2006).

The Golgi cell provides an intriguing example of this duality in that, while maintaining well defined single neuron properties, this neuron can coordinate (and be entrained into) activity of large granular layer fields. The Golgi cell makes extensive connections with the rest of the cerebellar network, receiving inputs from both the mossy fibre and the climbing fibre system (comprising granule cells, stellate cells, basket cells, inferior olivary cells, Lugaro cells) and sending outputs to the granular layer (comprising both granule cells and UBCs). Moreover, the presence of gap-junctions has been proposed to form a Golgi cell electrical syncytium (Sotelo & Llinás, 1972; Dugué *et al.* 2009), raising a puzzling analogy with the 'protoplasmic syncytium' envisaged by Golgi. Eventually, the Golgi cells may be able to coordinate the activity of large granule cell fields with extracerebellar structures (like the thalamo-cortical system) on appropriate frequency bands and to contribute to rhythmicity and to the representation of time in the cerebellum and in cortico-cerebellar loops (Ivry & Spencer, 2004). Therefore, the Golgi cell fully represents

a kind of nerve cell which revitalises, under a new light, the discussion on the relationship between single neurons and the networks they are part of.

Appendix 1. Nets versus nodes: the controversy between Golgi and Cajal

In the mid 1870s, when Golgi began his investigation, the prevailing morphological theory of the brain was that of a reticular dendritic net of 'connective vessels' extending and interlinking across the nervous tissue. Instead Camillo Golgi, supported by his observations with the black reaction, believed the axons to be interconnected in a net. However Golgi did not make any definitive statement on the precise mode of that connection: in some of his writings he tended to consider a direct fusion of axon prolongations, and in others he left open the possibility that groups of nerve cells did not form direct anastomoses but only an intimate functional web through the interlacing of the axons. Beside the exact histological intercellular connection, Golgi adhered to the physiological idea of diffuse interactions among nerve centres.

In 1886 there was a turning point in the conceptualisation of nervous system structure. The Swiss anatomist Wilhelm His theorised that the nerve cell body and its processes formed independent units. Another Swiss scholar, August Forel, reached similar conclusions in a paper published in 1887. The same year a Spanish psychiatrist, Luis Simarro Lacabra, returned to Madrid from Paris equipped with the latest literature and histological preparations, and there he was visited by Santiago Ramón y Cajal, a young professor at the University of Valencia: Cajal observed the preparations made with the black reaction and they took his breath away. He soon began an extensive investigation of the nervous system using the Golgi method and, looking at the preparations with the new neuronistic ideas in mind, he soon agreed with the Swiss hypothesis and became 'the champion of neurons'.

The emergence of 'neuronism' drove Golgi to identify even more deeply with 'reticularism', in favour of which he wrote a paper in 1891. On the other side, Cajal continued to produce evidence in favour of neuronism. This was the situation when, in 1906, the Karolinska Institute of Stockholm announced the Nobel Prize award to Golgi and Cajal. This seemed the great opportunity to end the scientific rivalries of the previous 16 years. Unfortunately, the confrontation between the two scientists became even worse after the Nobel Prize lecture, in which Golgi strongly attacked the neuronist theory. The Nobel lecture was a disaster for Golgi and, consequently, a triumph for Cajal.

Adapted from Mazzarello *et al.* (2006).

Appendix 2. The molecular level of organisation: Golgi cell channels and receptors

Far from what Golgi could imagine, the intricate connectivity and the excitable dynamics of the Golgi cell are supported by an even further complexity of ionic channels and receptors (for previous reviews see Farrant & Nusser, 2005; Geurts *et al.* 2003) providing mechanisms suitable for regulating circuit dynamics and homeostasis. Most relevant factors are the expression of specific receptor subtypes coupled to neurotransmitter spillover in the cerebellar glomerulus.

The main excitatory inputs to Golgi cells are glutamatergic, with AMPA and NMDA receptors at the mossy fibre–Golgi cell relay (Kanichay & Silver, 2006; L. Forti *et al.* unpublished observation) and AMPA, NMDA and kainate receptors at the parallel fibre–Golgi cell relay (Dieudonné, 1998; Bureau *et al.* 2000; Misra *et al.* 2000). Whether NMDA receptors contribute to regulate Golgi cell excitability or are involved in some forms of plasticity remains to be determined. The inhibitory inputs to Golgi cells are both GABAergic and glycinergic. GABAergic inputs are provided by stellate and basket cells (Dumoulin *et al.* 2001), while mixed GABAergic–glycinergic inputs are formed by the Lugaro cells (Dieudonné & Dumoulin, 2000; Dumoulin *et al.* 2001).

The main output from Golgi cells is GABAergic and inhibits the granule cells in the cerebellar glomeruli. The IPSCs consist of a fast and a slow component (Rossi *et al.* 2003) determined by differential receptor subtypes and localisation (Farrant & Nusser, 2005). Different combinations of $\alpha 1$ and $\alpha 6$ subunit-containing receptors with ancillary subunits regulate synaptic response kinetics conferring specific sensitivity to ambient GABA concentration and spillover in the glomerulus (Brickley *et al.* 1999, 2001; Tia *et al.* 1996; Nusser *et al.* 1998; Rossi & Hamann, 1998; Hadley & Amin, 2007). Thus, in addition to determining phasic inhibition, Golgi cells contribute to regulate the basal granule cell input conductance by maintaining a tonic GABA concentration level inside the glomerulus (Brickley *et al.* 1996; Chadderton *et al.* 2004). The tonic level of GABA, which is also regulated by non-vesicular release and by the rate of GABA reuptake in glial cells (Rossi *et al.* 2003) by binding high-affinity receptors (Tia *et al.* 1996), can control the gain of the mossy fibre–granule cell relay (Mitchell & Silver, 2003). Acetylcholine can increase non-vesicular GABA release from the Golgi cells contributing to set the ambient GABA level in the glomerulus and tonic inhibition of granule cells (Rossi *et al.* 2003; see below).

Although the main effects of Golgi cells on granule cells are mediated by GABA, Golgi cells also co-release glycine at their synaptic terminals (Dugué *et al.* 2005). Granule cells do not express glycine receptors, but it is attractive to

speculate that glycine plays a role in regulating activation of granule cell NMDA receptors on their glycine binding site. Conversely, both GABA and glycine receptors are expressed in UBCs, in which Golgi cell activity generates mixed GABAergic–glycinergic responses (Dugué *et al.* 2005). Interestingly, serotonin activates the Lugaro cells, thereby regulating Golgi cell inhibition (Dieudonné & Dumoulin, 2000).

Metabotropic receptors play also an important role in the Golgi cell circuit. The mGluR2 receptors on Golgi cell dendrites enhance an inward rectifier K⁺ current reducing the response following intense granule cell–Golgi cell transmission (Watanabe & Nakanishi, 2003). Metabotropic receptors also sustain cross-talk between mossy fibre and Golgi cell terminals in the glomerulus: mGluR2 receptor activation on Golgi cell presynaptic terminals inhibits GABA release (Mitchell & Silver, 2000*b*), while GABA_B receptor activation on mossy fibre terminals inhibits glutamate release (Mitchell & Silver, 2000*b*). Golgi cell functions are therefore deeply integrated with those of the cerebellar glomeruli, allowing fine tuning of their response dynamics depending on functional demand.

The mechanisms of Golgi cell electroresponsiveness have been summarised in a previous review (D'Angelo, 2008). A combination of electrophysiological, pharmacological and modelling experiments (Forti *et al.* 2006; Solinas *et al.* 2007*a,b*) has revealed the basis of pacemaking, bursting, adaptation and rebound excitation and phase reset. A set of ionic channels, including the h-current, raises membrane potential in a critical region. Here, near-threshold oscillations are generated involving a persistent Na⁺ current, the M-current and an apamine-sensitive AHP (afterhyperpolarisation) K⁺ current. The same ionic mechanism allows fast phase reset after a perturbation. In response to a stimulus, the resurgent Na⁺ current favours generation of doublets, and then adaptation is generated by the M-current and AHP current. Finally, following a hyperpolarisation, the h-current and a low-threshold Ca²⁺ current cause rebound excitation. Although the investigation of specific ionic currents in Golgi cells is far from complete, present knowledge provides a coherent mechanism through which the Golgi cell simultaneously controls pacemaking and response elicited by depolarisation and hyperpolarisation.

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