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THE UNIPOLAR BRUSH CELL: A REMARKABLE NEURON FINALLY RECEIVING THE DESERVED ATTENTION

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Abstract

Unipolar brush cells (UBC) are small, glutamatergic neurons residing in the granular layer of the cerebellar cortex and the granule cell domain of the cochlear nuclear complex. Recent studies indicate that this neuronal class consists of three or more subsets characterized by distinct chemical phenotypes, as well as by intrinsic properties that may shape their synaptic responses and firing patterns. Yet, all UBCs have a unique morphology, as both the dendritic brush and the large endings of the axonal branches participate in the formation of glomeruli. Although UBCs and granule cells may share the same excitatory and inhibitory inputs, the two cell types are distinctively differentiated. Typically, whereas the granule cell has 4–5 dendrites that are innervated by different mossy fibers, and an axon that divides only once to form parallel fibers after ascending to the molecular layer, the UBC has but one short dendrite whose brush engages in synaptic contact with a single mossy fiber terminal, and an axon that branches locally in the granular layer; branches of UBC axons form a non-canonical, cortex-intrinsic category of mossy fibers synapsing with granule cells and other UBCs. This is thought to generate a feed-forward amplification of single mossy fiber afferent signals that would reach the overlying Purkinje cells via ascending granule cell axons and their parallel fibers.

In sharp contrast to other classes of cerebellar neurons, UBCs are not distributed homogeneously across cerebellar lobules, and subsets of UBCs also show different, albeit overlapping, distributions. UBCs are conspicuously rare in the expansive lateral cerebellar areas targeted by the cortico-ponto-cerebellar pathway, while they are a constant component of the vermis and the flocculonodular lobe. The presence of UBCs in cerebellar regions involved in the sensorimotor processes that regulate body, head and eye position, as well as in regions of the cochlear nucleus that process sensorimotor information suggests a key role in these critical functions; it also invites further efforts to clarify the cellular biology of the UBCs and their specific functions in the neuronal microcircuits in which they are embedded. High density of UBCs in specific regions of the cerebellar cortex is a feature largely conserved across mammals and suggests an involvement of these neurons in fundamental aspects of the input/output organization as well as in clinical manifestation of focal cerebellar disease.

The Unipolar Brush Cell: Introduction

Beneath the simple, three-layered histological structure of the cerebellar cortex that is centered upon the sole output neurons, the Purkinje cells, lies a complex map of mossy fibers originating from different sources and of climbing fibers derived from different subdivisions of the inferior olivary complex; the two afferent systems are in relation with a zonal array of chemically distinct Purkinje cells (Apps and Hawkes, 2009; Redies et al., 2010). The extraordinarily developed dendritic arbor of the Purkinje cell is composed of a proximal domain of larger branches and a distal domain of spine laden branchlets (Cesa and Strata, 2009); the arbor is orientated perpendicularly to the course of the folia, which are folds of the cortex containing the three-layered cerebellar gray matter and the white matter core. Whereas the mossy fiber inputs reach the myriad spines of the distal branchlets, climbing fiber inputs target the sparser spines of the proximal domain. Upon reaching the cortex, mossy fiber inputs greatly diverge, as they form several branches that innervate a multiplicity of granule neurons, whose axons then ascend to the molecular layer and form 5 mm long parallel fibers by T-division; the ascending granule cell axons (Mugnaini, 1972) and the parallel fibers form synaptic contacts with the distal spines of two geometrically distinct sets of Purkinje cells (Llinás, 1982; Bower, 2010). By contrast, individual climbing fibers form a limited number of branches, each of which directly innervates a single Purkinje cell arbor. Information from mossy and climbing fiber inputs reaching individual Purkinje cells and their targets in the cerebellar nuclei are likely topographically congruent (Sugihara et al., 2004, 2009). In contrast to this seemingly crystalline homogeneity of the cortical cerebellar circuit, a class of highly specialized neurons, the unipolar brush cells (UBCs) - which are inserted into the mossy fiber/granule cell/Purkinje cell/pathway- are differentially distributed in cerebellar lobules, indicating the existence of a cortex-intrinsic regional localization in the cerebellum.

Although UBCs had probably been observed already during the 1960s, the term UBC was first introduced in the early 1990s, when both somatodendritic and axonal compartments and the cell's fine structure were finally brought into focus. It is now clear that neurons previously termed pale cells, Rat-302 cells, monodendritic cells, chestnut cells and mitt cells on the basis of light and/or electron microscopic observations are part of the UBC neuron class. The Federative International Committee on Anatomical Terminology (FICAT), which is a subcommittee of the International Federation of Associations of Anatomists (IFAA), officially recognized the "unipolar brush cell" as a new cell type of the cerebellar cortex only recently (Terminologia, 2008), providing clarity to the subject. Presently, the search term "unipolar brush cell" elicits about 100 entries of articles that have appeared in PubMed-listed scientific journals since the introduction of the denomination in 1993–1995 (Harris et al., 1993; Floris et al., 1994; Mugnaini and Floris, 1994; Mugnaini et al., 1994; Rossi et al., 1995).

The adjective unipolar denotes the fact that for the most part these cells have a single dendrite and thus differ from other granular layer neurons (i.e., granule cells, Golgi cells, Lugaro cells, and the still poorly defined non-traditional large neurons; Ambrosi et al., 2007), which have more than one dendrite and are therefore described as multipolar. The term brush denotes the fact that the tip of the UBC dendrite forms a paint brush-like tuft of dendrioles. In the vast majority of cases, it is at the brushes that UBCs come in synaptic contact with afferent axon terminals within the context of a special cerebellar glomerulus, different from the light and electron microscopically characterized canonical glomerulus (Figs. 1 and 2) (Eccles et al., 1967; Fox et al., 1967; Palay and Chan-Palay, 1974; Jakab and Hámori, 1988). The brush, therefore, is the morphologically and functionally most distinctive feature of the somatodendritic compartment; it forms an extensive contact with an individual, large, excitatory mossy fiber terminal (a one-to-one relationship) and with

multiple, smaller Golgi-type axon terminals. The brush dendrites emit numerous, thin evaginations (or filopodia), which contribute to the uniqueness of the cell class. The filopodia, which are evident at both light and electron microscopic levels, emanate even from the dendritic stem and/or the cell body in some cells. Although UBC filopodia do not bear synaptic junctions, they are nevertheless involved in cell signaling.

The UBC is provided with a single axon that appears to branch locally. As UBCs were proven to be GAD- GABA- and glycine-negative and intensely positive for glutamate (Mugnaini and Floris, 1994; Nunzi and Mugnaini, 2000; Crook et al., 2006), they are considered as a class of excitatory “local circuit neurons”. Evidence obtained with multiple methods shows that UBC axons form *en passant* and terminal swellings similar to the rosettes of cerebellar mossy fiber afferents, establishing a novel and unorthodox class of cortex-intrinsic mossy fibers. Through their axons, individual UBCs innervate other UBCs, as well as hundreds of granule cells, thus amplifying the signal of a single, cortex-extrinsic mossy fiber. Thus, the powerful feed-forward excitation engendered by UBCs could focally modulate the basic cerebellar input/output organization.

In all adult mammals analyzed so far, UBCs show an uneven distribution within the granule cell domains of the hindbrain, their highest density of occurrence being in the median cerebellar cortex, part of the flocculus/paraflocculus complex, and layers 2–4 of the dorsal cochlear nucleus (Diño et al., 1999; Takács et al., 1999, 2000; Víg et al., 2005). This mode of distribution, together with studies of afferent connections and responses to vestibular stimulation, indicate that the UBCs might play a major role in the regulation of body posture, head position and eye movements (Jaarsma et al., 1996; Diño et al., 2001; Sekerkova et al., 2005; Diño et al., 2000; Kalinichenko and Okhotin, 2003; Oertel and Young, 2004; Sekerkova et al., 2005; Barmack and Yakhnitsa, 2008; Diño and Mugnaini, 2008). Electrophysiological recordings in which UBC activity is directly related to Purkinje cell responses, however, remain to be done.

Functionally, UBCs are characterized, at least in the rat, by the unmistakable long-lasting responses to synaptic excitation recorded in cerebellar slices and that ensue from glutamate entrapping in the complex glomerular structure (Rossi et al., 1995). In vivo and in vitro recordings also suggest that UBCs are capable of intrinsic firing. The complex interactions of these intrinsic properties with the peculiar synaptic responses remain to be analyzed and may shed light on the role of these neurons in the microcircuits of the cerebellum and the cochlear nucleus.

The history of the UBC represents a particular case, as the cell came to light nearly 150 years after the first description of the much larger Purkinje neuron (Purkinje, 1837). This process of discovery is still ongoing. Work over the last ten years has generated the notion that the UBC cell class, in spite of its fundamentally homogeneous morphological and developmental aspects, is composed of three or more subsets, whose phenotypes have been explored with antibodies against a variety of proteins, including secretory peptides, calcium binding proteins, neurotransmitter receptors, transporters and intracellular signaling molecules. These UBC subclasses have different chemical makeups, different intrinsic functional properties, and presumably also different synaptic connections. The non-homogeneity of the UBC population in different regions of the cerebellum and the cochlear nucleus complex will therefore complicate future attempts to associate aspects of cellular diversity of the UBCs with regional circuitries, putting new demands in the design of experiments to define their specific connections and functional role.

In the following, we will trace the pioneering steps that brought to the definition of the cell class and its sublineages and are only known to specialists. The UBC provides an example

of how original observations by different authors may first seem to differ, but successively a unitary interpretation of the data becomes possible after further research and/or methodological advances. Naturally, this review is no substitute for the original papers, which contain a critical mass of detailed information. Morphological and functional aspects of cerebellar and cochlear nucleus UBCs have been reviewed previously at different stages of the research progress (Mugnaini et al., 1997; Slater et al., 1999; Kalinichenko and Okhotin, 2005; Diño and Mugnaini, 2008).

The Early Years

While the criteria for the identification of the somatodendritic and axonal compartments of the UBCs were first laid out in 1993–1995 with a combination of the Golgi method, electron microscopy, immunocytochemistry and patch clamp recordings, the existence of these cells had been gleaned in several earlier studies, and especially the electron microscopic (Chan-Palay and Palay, 1971), autoradiographic (Altman and Bayers, 1977), and immunocytochemical investigations of Hockfield (1987) and Cozzi et al. (1989).

In fact, UBCs had been observed even earlier than the years 1971–1989, albeit they had been confused with other types of neurons. Hámori and Szentágothai (1966; see also Eccles et al., 1967) were the first to publish electron micrographs of cat cerebellar glomeruli containing dendrites that are now recognizable as UBC's brush dendrites, in view of their filopodial extensions; these dendrites formed shaft synapses with mossy fiber terminals and were mistaken for Golgi cell dendrites. Mugnaini (1970, 1972) subsequently published similar images from the cat cerebellum, repeatedly attributing them to Golgi cells; he used the term "hairy dendrites" to highlight their numerous non-synaptic evaginations. Interestingly, he pointed out –without noting the discrepancy – that true Golgi cell dendrites that run in the "non-glomerular neuropil", i.e., among clusters of granule cell bodies, had only few genuine spines and formed shaft and spine synapses with the ascending portions of granule cell axons, thus being virtually devoid of filopodia. Subsequently, Chan-Palay and Palay (1971; see also Palay and Chan-Palay, 1974) brought attention, for the first time, to neurons whose cell bodies formed a large, scalloped synaptic contact with individual mossy fiber endings. These authors even found Golgi impregnated cells whose contours resembled those in their electron microscopic images; they coined the term "*en marron* synapse" to highlight this previously unreported synaptic configuration and attributed these features to small, canonical Golgi cells. Monteiro (1986) later added the information that neurons he classified as Golgi cells in the rat cerebellum could form such chestnut-like synapses not only on the cell body but also on dendritic shafts. Furthermore, Mugnaini et al., (1980a) illustrated chestnut-like synapses on the cell bodies of presumed "small Golgi cells" in the rat cochlear nucleus, and described special glomeruli - erroneously termed "Golgi cell glomeruli", in which mitochondria rich dendrites, clearly distinct from granule cell dendrites, completely surrounded the mossy fiber terminals. Similarly shaped "mitt cells" and "chestnut cells" were later described in the cochlear nucleus of different mammals by Hutson and Morest (1996), Weedman et al. (1996) and Josephson and Morest (2003).

We now know that all of the above features (hairy dendrites, mitochondria rich dendrites, chestnut cells and *en marron* synapses, mitt cells and special glomeruli with dendrites differing from granule cell dendrites) are attributable to UBCs and not to neurons of the classical Golgi cell category. Since the classical descriptions of the cerebellar cortex by Golgi (1903), Retzius (1892), Cajal (1904) and others, the prevailing assumption became that any neuronal cell body larger than the granule cell would usually be classified as "Golgi cell", with the exception of elongated neurons underneath the Purkinje cell layer that became known as Lugaro cells (Lugaro, 1894; Fox, 1959; Mugnaini, 1972); this misconception was aided by the fact that Golgi cells vary considerably in cell body size and

dendritic branching pattern. Thus, it is perfectly understandable that the UBCs, when first observed and illustrated in electron micrographs by so many different authors, would be identified as small Golgi cells, virtually by default.

In retrospect, also authors analyzing the differential distribution of Golgi cells across lobules might have “noticed” the UBCs, as they reported that Golgi cells are most frequently encountered in the ventral vermis and the flocculus (i.a., Jakob, 1928; Brodal and Drabløs, 1963; Fox et al., 1967; Lange, 1974); in fact, the alleged higher density of neurons larger than granules in these regions is probably explainable, at least for the most part, by the presence of UBCs (Sturrock, 1990; but see section on UBC density below). Nevertheless, the issue of the lobular densities of “Golgi cells” has continued to attract the curiosity of cerebellar students until recently.

In this context, the intriguing observation by Brodal and Drabløs (1963) should also be emphasized. With remarkable discernment they indicated the presence of two types of mossy fiber terminals in the cat cerebellum: the classical type, which was present throughout the cortex, consisted of sizeable enlargements with side-projections; while the second type, which was most common in the granular layer of the nodulus and the ventral uvula, consisted of smaller and less complex enlargements. In view of more recent data (Nunzi and Mugnaini, 2000; Diño et al. 2000b), it appears now reasonable to conclude that the former are cortex-extrinsic mossy fibers, while the latter represent the cortex-intrinsic mossy-like endings of UBC axons. Thus, the Norwegian investigators may have offered one of the earliest clues, albeit an indirect one, to the existence of the UBC.

In view of its special features, it is surprising that the UBC escaped the attention of the classical neuroanatomists, including Golgi (1903), Cajal (1904), Fox (1967), and Lorente de Nó (1981), who studied the cerebellum and the cochlear nuclei with several variants of the Golgi method. Identification of the UBCs and recognition that they represent a specific cell type may have been impaired by multiple considerations, including the facts that: brushes seen in isolation from the dendritic stem may resemble artifactual incrustations; metal precipitates often obfuscate the components of vestibulocerebellar folia, which contain most of the UBCs; UBCs that lack the dendrites may resemble poorly impregnated cell bodies and UBC axons resemble extrinsic mossy fibers. In a conversation with one of us (E. M.), the late Sanford L. Palay once suggested that, in Golgi sections, UBCs might have been mistaken for Purkinje cells that had remained in a dwarfed state, having failed to reach the proper position in the cortex. Due to several specific cell markers, such as guanosine 3:5-phosphate dependent protein kinase spot35/calbindin, we now know that migrating, immature or ectopic Purkinje cells (De Camilli et al., 1984; Takahashi-Iwanaga et al., 1986; Vastagh et al., 2005a,b) are much larger in size and do not resemble in profile migrating, immature or ectopic UBCs (Morin et al., 1981; Munoz, 1990; Diño et al., 1999; Ibrahim et al., 2000; Víg et al., 2005). Another intriguing possibility may be that the UBCs, if noticed, were ignored because they did not fit with current epistemic systems. Many of the old histological archives have unfortunately been dispersed or discarded; and to our knowledge the presence of UBCs in classical Golgi collections has so far not been verified.

The rat “pale cells” of Altman and Bayer

With the introduction of cell birth-dating methods over fifty years ago, the cerebellar cortex became one of the most intensively studied brain regions for the analysis of cell lineages. It was indeed the use of the autoradiographic method that provided the first distinct clue of the existence of a new neuronal population in the cerebellar cortex. Altman and Bayer (1977, 1996) identified a developmentally unique cell class in the rat cerebellum on the basis of the “progressively delayed cumulative labeling”, ³H-thymidine protocol. Nuclei of cells undergoing their last division within the individual 2-day interval of the injections

appeared densely labeled, while labeling was more dilute if cells that had continued to divide. Altman and Bayer identified a distinct class of cerebellar cells that were produced between E17 and P2, after Purkinje cells and before granule cells; the cells were enriched in the vestibulocerebellum and measured 7–8 mm in perikaryal diameter. Limited by their observation of the cell bodies without any of the processes, Altman and Bayer were very careful in defining the nature of the cells they had discovered. They tentatively termed them “pale cells” because of the lighter appearance of their nucleus and cytoplasm and could not make definitive statements whether they were neurons or astrocytes; yet, they advanced that the pale cells were a class of Golgi type II neurons, because they had a prominent nucleolus and a restricted distribution in the cerebellar cortex. Albeit they could not provide absolute counts due to the opaque autoradiographic grains often obscuring the cell nucleus, they emphasized that the pale cells occurred at much higher densities in nodulus, flocculus, paraflocculus, ventral uvula and lingula than in other lobules. At their peak frequency, pale cells outnumbered Purkinje cells and the “specific” Golgi cells in lobular distribution.

Immunostaining with Rat-302 and granin antibodies

A number of subsequent breakthroughs were brought about, during the 1980s and early 1990s, by the introduction of antibodies that incited the study of neural cell lineages and sublineages, as they afforded immunolocalization of the synthetic enzymes for specific neurotransmitters, or turned out to be completely or predominantly cell-class specific, and even produced partial or complete immunocytochemical staining of the neuronal somatodendritic and axonal compartments.

Hockfield (1987), in her quest of antibodies specific for spinal cord neurons, was serendipitously able for the first time to image the entire somatodendritic compartment of small neurons in the cerebellar granular layer that differed both from granules and Golgi cells. Her Mab Rat-303 stained large multipolar neurons that she identified as Golgi cells, whereas Mab Rat-302, which recognized a doublet of 160 kD bands in Western blots, stained Purkinje cells throughout the cortex, and also stained small neurons in the vermis, flocculus and paraflocculus. These “Rat-302 cells” had a round cell body, ~10 µm in diameter, rarely displayed more than one dendrite “ending in a spray of appendages”, and in some cases a “locally confined” axon. Hockfield noted that the Rat-302 cells had restricted distribution and their dendrites could be longer than those of granule cells, and concluded that they may be neurons “interposed between cortical processing and the cerebellar afferent projections of the flocculus and vermal Purkinje cells”. Thus, Hockfield came a long way in defining the specific cell-class features of her Rat-302 cells, although she did not bring up their possible relation to the pale cells - whose dendritic compartment had so far not been uncovered. Interestingly, it still remains to be ascertained whether the entire population that we now define as UBCs presents Rat-302 immunoreactivity.

Antibodies against protein/s of a cytoplasmic organelle, the large dense core vesicles (LDCVs), serendipitously provided additional markers for presumed UBCs. The core of these vesicles contains a class of proteins, the granins (chromogranins or secretogranins), which are secreted by the regulated pathway and proteolytically generate bioactive peptides. Rosa et al. (1985) succeeded in producing rabbit antigranin sera of unequalled potency that lent themselves to very accurate neurocytological investigations and brain mapping. Using these antisera Cozzi et al. (1989) found intense secretogranin II (SgII, or chromogranin C) immunoreactivity in climbing fibers, in mossy fibers and also in small granular layer neurons, some of which emanated a thick short process terminating in a glomerulus. The cell body and the process were sharply marked by individual puncta and larger clumps of immunoreaction product, each of the puncta presumably representing individual LDCVs. Cozzi and coworkers pointed out that these neurons distinctly resembled “Rat 302-positive cells” by their size, shape and distribution, but they made no mention of the pale cells. Three

years later, the neuropathologist Munoz (1992) stained human cerebellar sections with a commercially available, monoclonal antibody (LK2H10) to a human chromogranin A (CgA) peptide. He identified small CgA⁺ neurons, whose single and unbranched dendrite (10–80 μ m in length and 2.5 μ m in diameter) terminated in a club-shaped expansion, approximately the same size of the cell body, which was located in a glomerulus and in some cases issued multiple stubby projections. These “monodendritic cells” were most common in the vermis and decreased laterally to become absent in the hemispheres; their vermal density was substantial, as it equaled that of Purkinje cells when measured along a linear stretch of the cortex. While he noted the similarity between human CgA⁺ neurons and Rat-302⁺ cells, he did not relate them to the pale cells or the SgII⁺ cells. In addition to human CgA, antibody LK2H10 recognized more than one band in Western blots and the nature of the cerebellar peptide could not be answered. Recent studies indicate that SgII and CgA are differentially distributed in murine cerebellar neurons (Nunzi and Mugnaini, 2009), and that SgII is specifically present in the subclass of CR⁺ UBCs. Thus the issue of SgII and CgA expression in subclasses of human UBCs is still open to investigation. The importance of granin peptides in the cerebellar cortex also remains to be discovered.

Calretinin immunostaining

Specific antibodies to cytosolic proteins with the property of binding calcium with high affinity have effectively incited many neuroanatomical and embryological studies on cerebellar neuron classes. As widely known, antibodies to calbindin, parvalbumin and PEP19 have been valuable markers for Purkinje cells, stellate/basket cells and large neurons of the granular layer, although specific markers to differentiate subclasses of the latter continue to be sought (Geurts et al., 2003; Simat et al., 2007; Galliano et al., 2010). A 29 kD, EF-hand motif protein (coded by *Calb2*; Wilson et al., 1988; Parmentier, 1990) was independently isolated by the groups of Rogers and Jacobowitz from retina and auditory nuclei (Rogers, 1989; Winsky et al., 1989a,b) and termed calretinin (CR) and protein 10, respectively. The two groups then mapped CR expression throughout the nervous system and observed that it stained the somadendritic and axonal compartments of the granule cells (Rogers, 1989; Arai et al., 1991, 1993; Floris et al., 1992; Résibois and Rogers, 1992), as well as the presumed pale cells/UBCs and other cerebellar neurons. The presumed UBCs stood up quite sharply among granule cells, which were moderately stained, but many Golgi and Lugaro cells were often distinctly CR⁺. Braak and Braak (1993) also described CR⁺ human monodendritic neurons and found that they co-localized immunoreactivity to CgA⁺ by two-color fluorescence. Successively, CR-immunoreactivity was also used to distinguish UBCs from granule cells and Golgi-like neurons in dissociated cerebellar cell cultures (Marini et al., 1997; Anelli et al., 2000). UBCs were sparse in classical granule cell cultures prepared from P7-8 rodents, but their density increased substantially in cultures prepared from late embryonic stages (E17-18 mice and E17-20 rats; Anelli and Mugnaini, 2001). Although CR-antibodies raised in different species to this day remain an excellent tool to study the chemical phenotypes of UBC sublineages (Sekerova et al., 2004; Englund et al., 2006), ultimately the CR⁺ UBCs turned out to be a relatively small subset of UBCs (see sections below: The UBC Subclasses).

The UBCs in Golgi impregnated sections

A significant forward step was the publication of three papers, in which light and electron immunocytochemistry with CR-antibodies, Golgi impregnations, and standard electron microscopy were convergently applied to the rat cerebellar granular layer (Mugnaini and Floris, 1994; Mugnaini et al., 1994; Floris et al., 1994). These papers closed most of the gaps among previous studies by revealing the UBCs in full within the context of the other tissue elements. By producing unusually large proportions of impregnated neurons, one of the Golgi method variants (Adams, 1979) utilized in the Mugnaini lab had brought into

focus the cerebellum-like microcircuit of the dorsal cochlear nucleus (Mugnaini et al. 1980a,b; Osen and Mugnaini, 1981; Wouterlood and Mugnaini, 1984; Wouterlood et al., 1984; Mugnaini 1985; Adams and Mugnaini, 1987; Mugnaini and Morgan, 1987; Mugnaini et al., 1987; Berrebi and Mugnaini, 1988; 1990; 1991; 1993), as well as the UBCs in the cerebellar cortex. Most of the UBCs neurons had a single, randomly orientated dendrite of varying length that ended with a conspicuous brush formation that embraced a mossy fiber terminal. Some of them, however, appeared at variance from the common type. i) They had a single dendritic stem that formed two branches, or even two separate (occasionally opposite poles) dendritic stems; yet, each of the two dendritic branches and individual dendritic stems terminated with a sizeable brush; or b) they were adendritic, but had a brush of dendrioles arising directly from the cell body. The axon - frequently but far from regularly - emanated from the side of the cell body opposite to the dendritic stem; in some cases it arose from the dendritic stem itself. None of these morphological variations, however, represented *per se* a sufficient base to separate the cells in different subtypes, even though each of the variations could admittedly results in functional differences. For instance, cells provided with two brushes would expectedly show higher capacitance.

More recently the issue of morphologically definable UBC subforms has been re-proposed by studies of variations in dendritic form in aquatic mammals (Kalinichenko and Pushchin, 2008) and sheep (Avarez et al., 2008), as well as of cell size (Kim et al., 2010). The reasons for such morphological variations are not apparent; the forms might originate from factors regulating gene expression during early development, they could arise epigenetically from adaptation to random variations of the cell environment or could possibly be related to aspects of the mossy fiber afferents and their functional parameters.

The results of the Golgi study - submitted in 1993 - were based on adult animals and failed to show the axon in its totality, leading to the tentative suggestion that it projected to mossy fiber source neurons outside the cerebellar cortex that would generate a positive feedback loop (Mugnaini and Floris, 1994). This conclusion was soon revised on the basis of cell fills with Lucifer Yellow obtained in the acute slice preparation (Rossi et al., 1995) that showed locally branching UBC axons with large endings resembling mossy fiber rosettes. By use of the Golgi method applied to cerebellar sections, Axelrad's lab confirmed the local branching of UBC axons (Berthié and Axelrad, 1994; by a quirk of the reviewing process this study appeared before that of Rossi et al. (1995). Biocytin cell fills and electron microscopy finally proved that the *en passant* and terminal rosettes of UBC axons targeted granule cell dendrites and UBC brushes (Diño et al., 2000b). Many of UBC rosettes appeared decidedly smaller and simpler in outline than those of extrinsic mossy fibers.

Nevertheless -due to the meandering course of the axon and the geometry of the slices - it was difficult to visualize the UBC axonal territory in its entirety. A fair number of UBC axons are still to be analyzed in full. Therefore, it remains an open question whether the axons of all the UBCs, including those situated in the white matter, ramify locally in the granular layer. Berthié and Axelrad (1994) and Diño et al. (2000) observed UBC axons that reached the white matter and after a short distance reentered the granular layer on the same or opposite side of the folium. Takács et al. (1999) mentioned unpublished experimental evidence that at least some UBCs project to the cerebellar nuclei.

Subsequent studies by Nunzi and Mugnaini (2000) in cultured cerebellar explants further showed that terminals of the mossy fiber-like UBC axons form a conspicuous proportion of all the mossy fiber terminals in the nodulus/uvula preparation. There is agreement that, in young rats and mice, many of these terminals are smaller and less convoluted than the rosettes of extrinsic mossy fibers (Berthié and Axelrad, 1994; Rossi et al., 1995; Diño et al.

2000), bringing to mind the distinction between lobular and classic mossy fiber terminals in the cat nodulus observed by Brodal and Drabløs (1963).

Golgi impregnated neurons identical to UBCs were also reported in the dorsal cochlear nucleus, thus complementing the analogy between the neuronal microcircuits receiving descending branches of the vestibular and acoustic nerves. The existence of a special cerebellum-like neuronal microcircuit in the cochlear nucleus that lacks the climbing fiber component is now widely accepted and additional studies in other laboratories have clarified several sources of its mossy fiber afferents, as discussed in the reviews of Oertel and Young (2004) and Young and Oertel (2004). Furthermore, a recent immunocytochemical study, which utilized newly discovered cell-class markers, has provided refined maps for granule cells and UBCs in the cochlear nuclear complex of the rat (Diño and Mugnaini, 2008).

The ultrastructure of UBCs

The light microscopic characterization of the UBCs was clearly instrumental in engendering their identification under the electron microscope (Mugnaini et al., 1994; Floris et al., 1994). Here we review the general cytological features that we tentatively consider common attributes of the cell class, as well as features that are may likely be attributed to one or more UBC subclasses. The existence of subclass-specific ultrastructural features, however, has yet to be verified by serial section electron microscopy in a sizeable sample.

General UBC features

The nucleus and the perikaryal cytoplasm of UBCs appear more transparent to electrons than those of Golgi cells and granule cells, in accordance with the pale cell's description of by Altman and Bayer (1977). Electron microscopy consistently shows that small Golgi cells contain stacks of cisterns of the granular endoplasmic reticulum and numerous free polyribosomal arrays, whereas the UBCs have fewer granular cisterns and free ribosomal rosettes.

The UBC brush forms a large apposition with a mossy fiber terminal that is characterized by multiple asymmetric synaptic junctions with prominent postsynaptic densities (PSDs). The sum of the synaptic junctional areas may be in the order of 20–40 μm^2 /brush (Mugnaini et al., 1997). The excitatory mossy fiber-to-UBC synaptic complex could therefore be considered as a special form of giant synapse, examples of which are the calycine endings of the anterior subdivision of the cochlear nucleus and the medial trapezoid body, and the climbing fiber termination on the proximal compartment of the Purkinje cell dendrite.

Brush dendrioles are generally provided with rich complements of ionotropic and metabotropic glutamate receptors (see section below). They are also richly endowed with mitochondria, which may supply the energy requirements for ionic pumps and participate in the glutamate-glutamine cycle, as they contain at least one form of its synthetic enzymes, the kidney-type of phosphate-activated glutaminase (PAG) (Laake et al., 1998), and at least one member of the amino-acid transporter family with high affinity for glutamine (Jenstad et al., 2009). PAG, which forms glutamate through glutamine deamidation, occurs in at least three isoforms (K-PAG, L-PAG, and GAC) having different kinetics and activation/inhibition properties; K-PAG and L-PAG are the products of two related genes, and GAC is probably an isoform of L-PAG lacking the C-terminus (Roberg et al., 2010). In brain there are mRNAs for all the three isoforms, but it is not known which of them is present in UBCs of the CR⁺ and mGluR1 α ⁺ subtypes.

In spite of their small size, UBCs contain a significant apparatus of microtubules and cytoskeletal filaments that could be important for the maintenance of cell shape and provide

a scaffold for robust somatodendritic trafficking. Harris et al. (1993) showed that UBCs are stained by antibodies to phosphate-independent neurofilament triplet proteins NF-H, NF-M and NF-L, and the additional Class IV neurofilament protein α -internexin. Their study also demonstrated that Hockfield's Mab Rat-302 recognizes a dephosphorylated form of heavy neurofilament protein NF-H. This indicated that the Rat-302 cells correspond to UBCs. Some of the neurofilament-positive UBCs formed *en marron* synapses, suggesting that also the Palays' chestnut-like cells are part of the UBC population.

In the rat, the PSDs of brush dendrioles contain an unusually dense apparatus of postsynaptic actin microfilaments, which for the most part are inserted perpendicularly into the PSD on one end, while on the other hand are anchored to components of the dendriole's cytoskeletal core that have yet to be characterized cytochemically (Diño and Mugnaini, 2000). The postsynaptic apparatus also includes a rich complement of epidermal growth factor receptor substrate 8 (EPS8), a binding/capping protein also present in the digitiform endings of the granule cell dendrites, albeit at lower density (Sekerova et al., 2007). It has been suggested that the rich actin/EPS8 complement of UBC brushes is involved in plastic rearrangements reflecting the activity of the mossy-fiber synapse (Diño and Mugnaini, 2000; Sekerkova et al., 2007).

The brush formation of a single UBC may form a type of glomerular synaptic array in which the brush represents the only dendritic element, as granule cell dendrites are completely lacking; axon terminals of the Golgi cell's axonal plexus form symmetric synaptic junctions with the brush dendrioles downstream of the excitatory mossy fiber synapse. Such glomeruli are purely UBC glomeruli, as the brush is the sole postsynaptic element. Most of the glomeruli comprising UBC dendrites, however, contain a varying number of granule cell dendrites in addition to the multiple dendrioles of a single brush. As the glomeruli generally represent the primary link between mossy fiber afferents and the microcircuit of the cerebellar cortex, their diverse nature in folia containing UBCs (*i. e.*, the classical purely granule cell glomeruli, the glomeruli containing UBC brushes only, and the mixed-glomeruli with both UBC and granule cell dendrites, has significant functional correlates. Cerebellar glomeruli are sites at which excitatory glutamate receptors and inhibitory GABA receptors act cooperatively ensuring motor coordination and adaptation (Nakanishi, 2009). Whereas in the canonical cerebellar glomerulus the input of a single excitatory axon terminal is channeled to approximately 50 granule cell dendrites (Jakab and Hátori, 1988), ensuring maximal divergence (Marr, 1969; Ito, 1984), mixed glomeruli containing UBC brushes provide much reduced divergence. Like pure UBC glomeruli, they too favor individuality of the mossy fiber inputs.

Putatively subclass-specific ultrastructural features

A sizeable proportion of UBCs contains a high density of large dense core vesicles (LDCVs). CR⁺ UBCs belong to this category, which is in accord with the finding that SgII expression is present in only one third of the UBC population (see section below). As dendro-dendritic UBC/granule cell synapses (see section below) are present in a minority of the mixed glomeruli, they too may belong to the CR⁺ UBC subset.

Furthermore, the mossy fiber-to-UBCs synapses vary in sectional lengths between two extremes, albeit overall they are larger than those of the mossy fibers-to-granule cell contacts (Mugnaini et al., 1994). This difference is also notable in rat pups (Morin et al., 1998). The extremes are so conspicuously different that they may possibly be related to different UBC subpopulations or to diversity in the afferent mossy fibers. Combined tract tracing and immunocytochemistry with cell subclass specific antibody may help resolving the issue.

Many UBCs in the rat cerebellar and cochlear nucleus contain a particular, discrete organelle, or inclusion body, consisting of ringlet subunits (Mugnaini et al., 1994; Alibardi, 2004), in mouse (Nunzi and Mugnaini, 2000; Ilijic et al., 2005), and in chinchilla (Hutson and Morest, 1996), but not in guinea pig (Alibardi, 2002). UBCs with ringlet subunits were also present in dissociated cell cultures of the mouse cerebellum (Anelli et al., 2000). The inclusion body, which is often situated near the Golgi apparatus and at the base of the dendritic stem, may be involved in somatodendritic vesicular traffic. Its absence in some perikarya and the small volume of the cytoplasm suggest that the organelle is present in the majority, but not all of the UBCs.

In a remarkable study of the cat visual cortex, Kind et al. (1997) found a similar neuronal organelle, termed “*botrysosome*” from the Greek *botrys* (meaning cluster of grapes), which was positioned between the ER and the cis-Golgi and appeared to be transitory, as it was present in kittens but not in adult cats. The organelle was immunopositive for mab Cat-307, which is specific for the phosphodiesterase phospholipase C beta 1 (PLC β 1), an enzyme known to be activated by receptor-stimulated G protein Gq/11 leading to production of DAG and IP3 and Ca²⁺ release from internal stores. Fractionation studies indicated that the ringlets of the botrysosome may be transport vesicles coated by PLC β 1, similar to previously described vesicles that transport synthesized proteins destined for additional translational modifications by the Golgi apparatus. The presence of grape bunch-like organelles both in cortical neurons and UBCs is intriguing, as subsets of the latter express the phosphodiesterase isoform PLC β 4 (Nakamura et al., 2004; Chung et al., 2009; see section below). It should be noted, however, that in cortical neurons botrysomes are restricted to the period of developmental plasticity, while in UBCs they are also found in the adult.

In sum, on the basis of all the data reviewed above it is fair to state that already by 1995 the distinctive morphological and immunocytochemical features of the UBC's somatodendritic and axonal compartments had finally formed a coherent picture that seemed to explain disparate findings obtained in different species with different methods. The cumulative evidence engendered the compelling notion that the cells in question were indeed elements of a special neuronal cell class that is distinct from both granule cells and Golgi cells and usually receives its input on the brush formation. Some of the immunocytochemical and ultrastructural data were also suggestive of the presence of subcategories of UBCs, although this notion had yet to congeal.

The UBCs as post-synaptic elements

Published morphological and electrophysiological data indicate that UBCs receive and transmit signals via chemical synapses. So far, gap junctions and/or electrical coupling among the UBCs have not been demonstrated, either morphologically or electrophysiologically. None of three connexin signals figured in the Allen Brain Atlas (Lein et al., 2007) seemed to label cells mimicking the UBCs in distribution, perhaps with the exception of the message coding for pannexin 2. This stands out in contrast to stellate, basket and Golgi cell classes of interneurons, which are known to form gap-junctions mediating electrical synapses between like cells in normal animals (reviewed by Sotelo, this volume). Connexins and pannexins are also expressed in Purkinje cells (Teubner et al. (2000; Zappalà et al., 2006).

Morphological aspects of the synaptic junctions on the somatodendritic compartment of cerebellar UBCs have been analyzed extensively in the rat cerebellum *in situ* and *in vitro*, and less systematically in long-term slice cultures of the murine nodulus. Afferent synapses of UBCs situated in the cochlear nucleus have been described in rat, guinea pig and chinchilla. The published studies agree that excitatory and inhibitory synapses are precisely

targeted in UBCs of both cerebellum and cochlear nucleus and are mostly localized to the brush formation.

Excitatory synapses

In the rat cerebellum, the mossy fiber-UBC apposition is marked by asymmetric synaptic junctions that in sections appear to be of three main kinds: a) individual or multiple synaptic junctions measuring 0.6–1.0 μm in length or larger; b) a series of synaptic junctions 0.3–0.5 μm in length; and c) a mixture of the two varieties. In the glomeruli, mossy fiber synapses on granule cell dendrites consist of series of 4–6 asymmetric synaptic junctions shorter than 0.3 μm . Longer junctions, therefore, usually indicate the presence of UBC dendrites. Most of the mossy fiber synapses on the soma of rat chestnut-like UBCs are also of the longer variety. Climbing fiber and parallel fiber synapses on Purkinje cell spines also measure less than 0.3 μm . The synaptic junctional areas are thought of as rounded or ovoidal membrane patches (Mugnaini et al., 1994, 1997), although their *en face* views or 3D reconstructions remain to be described.

Synaptic specializations similar to those of cerebellar UBCs have been demonstrated by several authors in cochlear nucleus neurons that are now considered to be part of the UBCs family, although they were classified as Golgi cells, mitt cells and chestnut cells (Mugnaini et al., 1980b; Hutson and Morest, 1996; Weedman et al., 1996; Hurd et al., 1999; Alibardi, 2002, 2003; Josephson and Morest, 2003; Zhan et al., 2006).

UBC brushes are richly endowed with ionotropic glutamate receptors (GluRs) and G-protein coupled metabotropic glutamate receptors (mGluRs). AMPA, KA and NMDA receptors were demonstrated in UBCs by immunohistochemistry and by pre-embedding immunoelectron microscopy at the PSD of mossy fiber-UBC synapses (Jaarsma et al., 1995); quantitative studies of receptors with the post-embedding method, however, remain to be done. Individual GluRs typically consist of different subunits surrounding the ion channel. The main subunits of the AMPA/KA receptors present in UBCs are GluR2 and GluR5/6/7, indicating that they are permeable to sodium, but not to calcium, and can mediate fast excitatory neurotransmission. Of the GluR5/6/7 subunits, the most probable candidate is GluR6, in view of its high message in the granular layer (Lein et al., 2007). NMDA receptors are also complexes of different subunits; the channels are calcium permeable, which supports their role in synaptic plasticity. The NR1 subunit is present in all NMDA receptors and is accompanied by one or more of the NR2 subunits, which confer to the channel both physiological and cellular specificity. While NR1 is expectedly present in UBCs, the type of their NR2 subunit is not known. Granule cells densely express NR2C (Lein et al., 2007), which would mask its likely expression in UBCs.

Activation of different mGluRs is coupled to specific intracellular signaling pathways that can modulate intracellular calcium levels and membrane excitability. In particular, activation of group I mGluR1 receptors by either glutamate or calcium can induce release from intracellular calcium stores via a phospholipase C-Ins(1, 4.5)P₃ and ryanodine-sensitive receptors pathway. Another mechanism mediated by mGluR1 receptors is the modulation of intrinsic conductances such as voltage-dependent calcium channels and non-selectively cationic TRPC channels. In contrast, signaling of group II mGluRs is linked to G-protein dependent inhibition of adenyl cyclase activity and a decrease of cAMP formation from ATP.

The subforms mGluR1 α and/or mGluR2/3 were demonstrated in cerebellar and cochlear nucleus UBCs (Grandes et al., 1994; Neki et al., 1996; Petralia et al., 1966; Wright et al., 1966; Bilak and Morest, 1998). Using isoform specific antibodies, Grandes and coworkers (P. Grandes, personal communication) did not find UBCs expressing mGluR1b and

mGluR1c, thus the only isoform of the mGluR1 receptor subtype expressed by UBCs is mGluR1 α . Moreover, Oishi et al. (1998) demonstrated that mGluR2, and not mGluR3 is the isoform expressed by UBCs. Initially the mGluRs were thought to be localized at the PSDs of the mossy fiber-UBC synapse; subsequent studies, however, showed that the mGluRs were localized to the perisynaptic membrane surrounding the PSD and extrasynaptically with an enrichment at the filopodia, and would supposedly be activated by glutamate spillover from the synapse (Jaarsma et al., 1998; Petralia et al., 2000). It was suggested that the UBC filopodia provide a large area of membrane capable of capturing a substantial amount of glutamate from the extracellular space. As considered in more detail below, mGluR α is definitely restricted to one of the UBC subsets (CR⁻ UBCs). The presence of postsynaptic mGluR2 in UBC dendrites and presynaptic mGluR2 in Golgi axon terminals (see below) within the cerebellar glomeruli complicates mGluR2 localization in the light microscope and has engendered some uncertainty concerning the extent of mGluR2 expression in the UBC population (Jaarsma et al., 1998). Quantitative, electron microscopic studies of the distribution of postsynaptic glutamate receptors on UBCs using rapid freezing/post-embedding protocols have not been done.

Inhibitory synapses

Axon terminals smaller than mossy fiber terminals and containing pleomorphic synaptic vesicles have been observed in direct contact with brush dendrites at the periphery of the glomeruli, with the shaft of the dendrite, and only occasionally with the cell body of UBCs (Floris et al. 1994). These terminals generally form small (1–2 μ m) and symmetric synaptic junctions. They are immunoreactive for GABA and glycine (Diño and Mugnaini, unpublished observations). Since Golgi cells are known to release both of these transmitters (Ottersen et al., 1988), Mugnaini et al. (1994, 1997) attributed these inhibitory-like terminals to Golgi cells.

Whereas a small number of GABAergic mossy fibers from the cerebellar nuclei have been shown to terminate in the cerebellar cortex (refs. in Angaut et al., 1996), no such fibers have so far been demonstrated to contact UBCs. Alibardi (2002) however, illustrated one case of a large GABAergic terminal in synaptic contact with an UBC in the dorsal cochlear nucleus. One may conclude, therefore that the UBCs only rarely participate in feedback loops of the cortico-nucleo-cortical type.

Research during the last 10–15 years has uncovered a considerable degree of heterogeneity in the Golgi cell population (Geutz et al., 2003; Crook et al., 2006; Simat et al., 2007). One might therefore argue that UBCs may be innervated by specific subsets of “Golgi cells”. However, Dugué et al. (2005) showed, using morphological and electrophysiological techniques, that individual Golgi cells contact both UBCs and granule cells. These authors also showed that Golgi cell terminals are in close apposition to markers of inhibitory PSDs (gephyrin) on the UBC membrane. Using paired recordings, they demonstrated, that Golgi cells make monosynaptic contacts with UBCs. Finally, they revealed, that unlike granule cells (the other target of Golgi cells), UBCs are endowed with glycine receptors, thus exhibiting mixed GABA/glycinergic IPSCs.

Dugué et al. also demonstrated, that the phenotype of inhibitory transmission at Golgi cells-UBC synapses is not homogeneous and distinguishes two subgroups of UBCs depending on the predominant inhibitory neurotransmitter (i.e. UBCs exhibiting mostly glycinergic or UBCs exhibiting predominantly GABAergic/mixed inhibition). It is thus interesting to relate these different phenotypes to the different subgroups determined by the specific expression of several markers of UBCs. This question has since been addressed in a preliminary form by Rousseau et al. (SfN 2009 abstract). These authors have shown that the different inhibitory phenotypes are strictly related to the nature of the synaptic receptors present at the

mossy fiber-UBC synapse. The group of UBCs that receive predominantly glycinergic inhibition express mGluR2 receptors and vice versa. This might be relevant in the integration of synaptic inputs in UBCs and may play a role in the selection of firing patterns by UBCs (Diana et al. 2007).

Finally, unlike other structures receiving mixed GABA/glycinergic inhibition (Jonas et al., 1998), GABAergic IPSCs at the Golgi cell-UBC synapse are actually faster than glycinergic IPSCs. Uncovering the type of GABA_A and glycine receptor subunits expressed by UBCs could explain these surprising results. This information is particularly relevant in view of the essential role of Golgi cells in motor coordination (Watanabe et al., 1998; Nakanishi, 2009).

The UBCs as presynaptic elements

In most regions of the nervous system, axons are the presynaptic elements at chemical synaptic junctions. In the cerebellum of normal mammals, this predominant principle is broken solely by UBC brushes, which have been shown to form glomerular dendro-dendritic chemical synapses. In fact, it has been thought for many years that only in pathologic conditions cerebellar neurons other than UBCs do become engaged in chemical dendro-dendritic synapses.

Chemical dendro-dendritic synapses between UBCs and granule cells

In the glomeruli in which the dendrites of UBC brushes come in contact with granule cell dendrites, they can form small dendro-dendritic chemical synapses, at which the UBC is presynaptic to the granule cell dendrite (Mugnaini et al., 1994). Presumably, this unusual property of the UBC dendrites may not extend to the entire cell population, as electron microscopic immunocytochemistry has revealed dendro-dendritic synapses in CR⁺ UBCs (Floris et al., 1994), whereas similar synapses in the mGluR1 α ⁺ UBC subclass remain to be demonstrated. The functional correlate of this dendro-dendritic connection remains to be established with paired cell recordings. Conceivably, the contact may increase the probability that the granule cell receiving the synapse fire in response to the mossy fiber input. With their presynaptic and postsynaptic dendrites, UBCs provide a further element of discordance from general cerebellar tenets and a minor example contradicting Cajal's law of neuronal dynamic polarization.

Interestingly, Hátori and Somogyi (1982) might have been the first authors to spot dendro-dendritic synapses of UBCs in rat cerebellar glomeruli. They demonstrated dendro-dendritic synapses between different types of neurons in deafferented cerebella, and plausibly attributed them to plastic rearrangement of synaptic connections following removal of the afferents. Two to 30 days after complete isolation of the cerebellar cortex of adult rats, they also found small Golgi II type neurons in the granular layer provided with junctional complexes on their dendrites and perikarya that were presynaptic to dendritic digits of granule cells. In addition, they found some granule cells with presynaptic sites on their somata and dendritic digits, "resulting in the formation of (sometimes reciprocal) dendro-dendritic synapses between granule cells or between granule cells and Golgi type II neurons". It is likely that at least some of their small neurons with presynaptic dendrites (see the mitochondria rich dendrite in their Fig. 1) were in fact UBCs.

The UBC axon and its chemical synapses

Synaptic targets of UBC axons were identified by Diño et al. (2000b) after biocytin cell fills with patch clamp microelectrodes. Like the terminals of pre-cerebellar mossy fibers, UBC axon terminals form the central element of a glomerulus, as suggested by light microscopy; in these glomeruli the granule cell dendrites and UBC dendrites are the postsynaptic targets. Synapses of the UBC axons with Golgi cell dendrites were identified only

tentatively, due to the limited preservation of ultrastructure in the acute slices. The terminals of the UBC axons differ from the rosettes of classical mossy fibers in so far as they appear smaller and less complex in shape, as also noted by Berthié and Axelrad (1994) with the Golgi method.

Another advancement was provided by the use of “organotypic” slice cultures of the isolated nodulus/uvula preparation, in which all cortex extrinsic mossy fibers degenerate in the first few days in vitro and the UBC axons remain as the sole cortex-intrinsic mossy fiber source (Nunzi and Mugnaini, 2000). These cultures indicated that mossy endings of the UBC axons form a substantial proportion of all the nodulus/uvula mossy endings. Additionally, long-term slice cultures of the isolated nodulus indicated with high probability that the UBCs are glutamatergic neurons. Indeed, by post-embedding immunocytochemistry they appeared as rich in glutamate as the granule cells, possibly eliciting glutamate responses in granule cells (Nunzi et al., 2001; but see section below).

The UBC Subclasses

It was the cerebellar immunolocalization of glutamate receptors that brought about the first clear signs of complexity in the UBC lineage, albeit the signs were a corollary of studies centered primarily on the glutamatergic mossy fiber UBC synapse (Rossi et al., 1995).

Calretinin and mGluR1 α

The substantial expression of most ionotropic and metabotropic receptors in the cerebellum demonstrated by in situ hybridization invited immunolocalization of the respective proteins in UBCs. The idea of a dichotomy in the UBC population originated from the observation that mGluR1 α UBCs appeared more numerous and more widely distributed than CR⁺ UBCs (Jaarsma et al., 1998; Diño et al., 1999). It then became clear that in the adult mouse cerebellum UBCs expressed either of two markers, CR or mGluR1 α . CR⁺ and mGluR1 α ⁺ UBCs showed overlapping, but distinct distributions in cerebellum, and UBCs colocalizing both markers were observed only rarely (Nunzi et al., 2002). Distinct CR⁺ and mGluR1 α ⁺ UBC subclasses were later observed also in two species of monkey (Mugnaini, unpublished observations), indicating that the distinctive chemical phenotypes may be conserved in evolution. In BAC transgenic mice TgG GRP mice from the GENSAT project (Doyle, 2009; and work in progress) the transgene is expressed in mGluR1 α ⁺ UBCs but not in the CR⁺ subset.

Other markers

The existence of two subclasses of UBCs was also supported by expression of the vesicular glutamate transporters VGLUT1 and VGLUT2; indeed, CR⁺ UBCs were found to be VGLUT1⁺/VGLUT2⁺, whereas the mGluR1 α ⁺ subset only expressed the VGLUT1 subform and none of the subsets expressed VGLUT3 (Nunzi et al., 2003). Furthermore, when Nunzi and Mugnaini (2009) reanalyzed the issue of granin expression in cerebellar neurons, they found that SgII is expressed solely by the CR⁺ UBC subclass.

The CR/mGluR1 α dichotomy is also maintained in UBCs of the rat cochlear nucleus, although in this center the percentage of UBCs co-expressing the two markers was slightly larger than in the cerebellum (Diño and Mugnaini, 2008). These observations seemed to fit with the BrdU finding that prenatal proliferation of UBC precursors occurred with two slightly different peaks (Sekerova et al., 2004).

Recently, the presumed dichotomy of the UBC population was put into question by new studies in mouse and one species of monkey. Chung et al. (2009) reported that cerebellar UBCs of CD1 and C57BL/6 mice consist of three subclasses of approximately equal size

(i.e., $CR^+/PLC\beta 4^-$; $PLC\beta 4^+/mGluR1\alpha^+$; $PLC\beta 4^+/CR^-/mGluR1\alpha^-$) and a much smaller population of $CR^+/PLC\beta 4^+/mGluR1^-$.

While the $CR^+/PLC\beta 4^-$ and $PLC\beta 4^+/mGluR1\alpha^+$ phenotypes may be in register with the UBC subsets expressing CR or mGluR1 α , the larger $PLC\beta 4^+/CR^-/mGluR1\alpha^-$ subset and the smaller $CR^+/PLC\beta 4^+/mGluR1\alpha^-$ would be novel UBC subclasses, and thus the dichotomy of the UBC cell class may be a gross oversimplification. As PLC $\beta 4$ is a crucial member of important intracellular signaling cascades that regulate Ca²⁺ homeostasis, protein trafficking and synaptic plasticity, its expression or lack of it may be linked to special properties of the UBCs that are yet to be recognized. Thus, diversity of PLC $\beta 4$ expression among UBCs would engender the need to reassess the distribution of UBC subsets across the cerebellar cortex and the cochlear nuclear complex and their possible conservation in selected mammalian species. Interestingly, three UBCs subclasses have been identified in the cerebellum of the monkey *Cercopithecus aethiops* on the basis of double immunostaining with antibodies to CR and neurogranin (NG), a brain-specific protein kinase C substrate involved in the regulation of calcium signaling and neuronal plasticity: CR^+/NG^- UBCs, CR^-/NG^+ UBCs, and CR^+/NG^+ UBCs (Singec et al., 2003).

In sum, the data surveyed in this section strongly support the existence of UBC subclasses that are characterized by specific patterns of protein expression metabolic pathways and signaling cascades, and invite a comprehensive gene profiling of the UBC population. Because UBCs share in common numerous morphological features, it seems reasonable to assume that they may also share a number of chemical phenotypes. This prediction is confirmed by the expression of several markers by both CR^+ and $mGluR1\alpha^+$ UBCs, namely the ionotropic glutamate receptor GluR2, metabotropic glutamate receptor mGluR2, acting binding protein EPS8, class III β -tubulin, and T-domain transcription factor Trb2/Eomes (Sekerikova et al., 2004, 2007; Englund et al., 2006; Diño and Mugnaini, 2008).

UBC Neurogenesis and Postnatal Development

In their autoradiographic studies on cerebellar development in the rat, Altman and Bayer (1977 Altman and Bayer (1997) produced evidence that the pale cells/UBCs were generated on E 19 and thereafter and suggested that they originate from the ventricular neuroepithelium. The question of the neurogenesis and postnatal development of the UBCs was later revisited in different mammals with immunocytochemical approaches.

CR-immunostaining

Abbott and Jacobowitz (1995) identified a subpial hot spot of CR-immunoreactive cells in the developing mouse cerebellar cortex at E14, which corresponds to E16/17 in the rat; this EGL-related hotspot might have been the source of UBCs and granule cells. A ventricular zone origin of UBCs, however, was re-proposed by studies of postnatal development in rat pups (Morin et al., 2001), human embryos and infants (Vig et al., 2005b), and kittens (Takács et al., 2000). These studies demonstrated migratory and maturing UBCs in cerebellar peduncle, folial white matter and granular layer, but not in the molecular layer. Accordingly, in the cerebellum of the mouse mutant reeler and scrambler, which show impaired cell migration, CR^+ and $mGluR1\alpha^+$ UBCs were displaced and reduced in number; furthermore, the maps of displaced CR^+ UBCs indicated that they migrate towards the cortex from the ventricular side, in contrast to granule cells, which descend to the granular layer from the cerebellar surface (Ilijic and Mugnaini, 2005).

Trb2+ve UBCs

Englund et al. (2006) recently investigated the main specific point of origin and the route of migration of the UBCs with a remarkable, innovative study of experimental mouse neuro-

embryology. Taking advantage of the expression of a roster of transcription factors in normal and mutant mice and in organotypic cerebellar slices, the authors demonstrated that UBCs express *Trb2* during neurogenesis and throughout adulthood, confirmed that cerebellar and cochlear nucleus UBCs are produced during the late embryonic and perinatal period, and provided the first clear evidence that UBCs originate from the rhombic lip, which is also the source of cerebellar nuclei neurons and cells destined to produce the EGL. Indeed, the rhombic lip was necessary for the production of both UBCs and EGL cells, but not of Purkinje cells and GABAergic interneurons, which derive from the ventricular zone. Moreover, the rhombic lip is likely to be the only source of UBCs, as it was the only site where *Tbr2*⁺ cells incorporate BrdU (a marker of the mitotic S-phase), pass through the M-phase, and express the processivity factor, proliferating cell antigen (PNMA). UBC neurogenesis, thus, would confirm the notion that embryonic sources of cerebellar excitatory and inhibitory neurons are compartmentalized. Notably, this conclusion contrasted with repeated reports that in adult animals several extra-cortical UBCs are aligned with the ependymal layer of the fourth ventricle and as well as the medullary velum (Mugnaini and Floris, 1994; Diño et al., 1999; Takács et al., 2000; Ilijic et al., 2005), which had suggested they originated from the ventricular neuroepithelium.

Englund et al. (2006) observed that UBCs exit the core of the rhombic lip through a narrow channel and migrate - dorsally through the immature white matter towards the cerebellar anlage and rostrally towards the brain stem - to finally reach the internal granular layer of the cortical lobules and the cochlear nucleus, respectively. Newly generated UBCs linger in the rhombic lip for one or more days before they migrate. There is a burst of UBC migration at P0.5 and most of the UBCs already reside in the granular layer of the cerebellum by P10, well ahead of the completion of granule cell neurogenesis. Migrating UBCs generally avoid the cell masses that become the cerebellar nuclei, and they never reach the EGL. Double labeling showed that early UBCs begin to express CR already at E17.5, and in the adult cerebellum nearly all CR⁺ UBCs expressed the transcription factor *Trb2* (97.3%; 148/152 CR⁺ cell counted in sections sampling one hemi-cerebellum); mGluR1 α expression could not be analyzed directly, as it is primarily localized to the late forming brushes.

The studies of Englund and coworkers produced a host of other interesting data that helped differentiate the UBC neurogenesis from that of other cerebellar neurons. UBC progenitors in the rhombic lip co-localize *Math1* and *Trb2* proteins; and UBC neurogenesis, like granule cell neurogenesis, is *Math1* dependent. Early UBCs are faintly Pax6⁺ and distinctly *Trb2*⁺, while cells in the EGL express Pax6 cell strongly but *Trb2* weakly or not at all. While both of CR⁺ and CR⁻ UBCs subclasses express *Trb2* during migration and into adulthood, granule cells are *Trb2*⁺ only transiently while they exit the external granular layer (EGL) and descend into the granular layer, after which *Trb2* expression shuts off (Englund et al. 2006; Diño and Mugnaini, 2008). Thus, although UBCs and granule cells originate from *Math1*⁺ precursors situated in the same region of the cerebellar primordium, their lineages are distinct. Whether this is due to cell lineage restricting factors, however, remains to be determined. Also, the role of *Tbr2* in UBCs is still unclear.

Lundell et al. (2009) reported an intriguing new observation in Tg-Ngn1/EGFP BAC-transgenic mice from the GENSAT project. They found that the caudal cerebellum and rhombic lip region in these mice contains a small number of migratory cells that are immunopositive not only for the glutamatergic lineage markers *Trb2* and Pax6, but also for the GABAergic lineage marker pro-neural bHLH factor neurogenin 1/EGFP (*Ngn1*). The findings suggest that a number of UBC precursors veer off established rules in the timely activation of transcriptional networks.

Although Englund and co-workers provided excellent data on UBC neurogenesis and migratory pathways, it is still insufficiently clear how gene expression programs are controlled as rhombic lip progenitors differentiate into specific cell lineages and subtypes, as well as by what mechanisms UBCs are guided to the cerebellar lobules and different areas of the cochlear nuclear complex. As the cerebellar lobules are differentially innervated from rostral and caudal spinal cord, vestibular ganglion, vestibular nuclei, pontine nuclei and numerous other brain stem nuclei, the maps of UBC subclasses and afferent fiber terminations may also hold promising clues to sublineage fate determining signals (see section on Purkinje cells/UBCs relations below).

Combined BrdU labeling and immunocytochemistry

Using BrdU labeling and immunocytochemistry with double labeling protocols, Sekerkova et al. (2004) found that rat UBCs proliferate from E14 to early postnatal stages with two peaks at E17 and E19. A subset of 30% of UBCs, primarily born in a narrow time window at E17-18, was CR⁺ and GluR2⁺. The majority of UBCs were CR⁻/GluR2⁺; they were generated at E19-21, and only a small proportion was generated around and after E22.

The pairing of BrdU labeling and immunostaining indicates that UBCs and pale cells are part of the same cell population and that the pale cells may primarily correspond to the CR⁻/GluR2⁺ subset, which presumably includes the mGluR1 α ⁺ UBCs. As reported in another study on rat pups, cerebellar CR⁺ UBCs can be demonstrated at earlier stages than mGluR1 α ⁺ UBCs (Nunzi and Mugnaini, unpublished observations). The data are compatible with the view that heterogeneity in the UBC population may originate prenatally, perhaps from the evolving actions of some of the basic helix-loop-helix (bHLH) transcription factors that regulate specification of cerebellar cell lineages (Carletti and Rossi, 2008; Zordan et al., 2008). Interestingly, pilot cell counts in the mouse cerebellum show that CR⁺/mGluR1 α ⁺ UBCs occur much more frequently in young pups than in the adult (Nunzi and Mugnaini, unpublished observations). This suggests that acquisition of CR and mGluR1 α UBC phenotypes involves epigenetic mechanisms and indicate the need for further investigations.

Morin et al. (1991) and Víg et al. (2005) showed that the UBC maturation is a slow process and relatively mature features are acquired after all the EGL have reached the internal granular layer. This is in accord with the fact that granule cell dendrites are a major target of UBC axons. Morin et al. (1991) divided the UBC maturation in 4 stages (protodendritic, filopodial brush, intermediate brush, and dendriolar brush stage) on the basis of the changing dendritic pattern of CR-immunostaining. In contrast with mature UBCs, many of the early rat UBCs had two dendrites or single branching dendrites, indicating that dendritic pruning takes place around P4 and P8. Moreover, UBC-to-mossy-fiber synapses were larger in older pups than in younger pups, while granule cell-to-mossy fiber synapses followed an opposite trend. Thus, the size of the synaptic junctions contacted by mossy endings in the glomeruli is cell type-dependent and may be related to differences in the expression of PSD components. Whether these components include subforms of the NMDA receptor and ion channels subtypes is uncertain, as the subunits of NR2 and the full array of ion channels (Monyer et al., 1994; Watanabe et al., 1994; Liu et al., 1998) expressed in UBCs have yet to be ascertained.

It is possible that ATP from unknown sources plays a role in UBC development, as P2X-immunoreactive UBCs have been demonstrated already in P3 rat pups, and P2X continues to be expressed in the majority of UBCs throughout cerebellar morphogenesis (Xiang and Burnstock, 2005).

Developmental relations between Purkinje cells and UBCs

Available immunostaining data in rodents with antisera to CR, mGluR1 α and PLC β 4 (Altman and Bayer, 1997; Jaarsma et al., 1998; Nunzi et al., 2002; Nakamura et al., 2004; Chung et al., 2009) concord that UBC subclasses show partly overlapping, but distinctly differential distributions. Sturrock (1990) provided the only dissenting opinion, as he reported approximately equal numbers of “pale cells” in the murine nodulus, spinocerebellum and pontocerebellum. His criteria for distinguishing small Golgi cells from the pale cells of Altman and Bayer, however, appear questionable in light of more recent data (Mugnaini et al., 1994; Takács et al., 1999). Along the mediolateral axis the UBCs form bands of higher and lower densities on each side of the midline. The bands are particularly evident in coronal sections of the vermal lingula, nodulus, and uvula, in which UBCs are most numerous. In vermal lobules I–VIII there is an overall trend for the UBC density to taper off laterally in the intermediate cortex; indeed, it is often stated that the cells are absent from hemispherical folia of the corpus cerebelli, although some reports have shown clusters of CR⁺ UBCs at the lateral edges of crus I and crus II.

Because mossy fibers originating from different sources distribute differentially to cerebellar lobules and categories of mossy fibers reach the cerebellar primordium while UBCs are still being generated, it had been suggested that the differential migration of subcategories of UBCs to distinct cerebellar lobules and their zonal intralobular distribution depend on attractant and repulsive interactions with the afferent fibers (Mugnaini et al., 1997).

Recently, Hawkes and coworkers have proposed the alternate idea that the topographic distribution of UBCs is guided by their interactions with chemically distinct clusters and stripes of Purkinje cells during their perinatal and postnatal development. Thus, as the early Purkinje cells orchestrate the topography of climbing fibers and mossy fibers (Sotelo and Chédotal, 2005), they would also restrict the deployment of migrating brush cells. The evidence came from mice homozygous for the *early B-factor 2 (Ebf2)* null or *scrambler (Dab1^{scm})* mutations that show severe disruption of the Purkinje cell topography. In normal mice UBCs do not express Ebf2 and scrambler in development and become primarily aligned with zebrin II stripes; whereas in the mutant mice CR⁺, mGluR1 α ⁺ and PLC β 4⁺ UBCs become homogeneously distributed, density of mGluR1 α ⁺ UBCs increases and many PLC β 4⁺ UBCs are situated ectopically in association with specific Purkinje cell clusters (Chung et al., 2009).

This notion would be in register with the ideas that the cerebellar cortex is organized according to a single, Purkinje cell-centric map of early-onset gene markers (Apps and Hawkes, 2009) or to a combinatorial molecular code that would link -in a bauplan- subsets of precerebellar and cerebellar cell clusters (Redies et al., 2010). Nevertheless, a role of mossy fiber afferents in orchestrating the perinatal UBC deployment in the cerebellar cortex remains attractive, in view of the occurrence of UBC patches in the lateral hemispheres and of local spots devoid of UBCs in the uvula (Diño et al., 1999), as well as of the differential distribution of UBC subclasses within the same parasagittal section of the nodulus (Nunzi et al., 2002). Molecules of the cadherin family could be one of the early or intermediate factors (Redies et al., 2010) involved in the finely tuned matching of mossy fiber afferents with granule cell and UBC dendrites within regions of the granular layer across folia or even in the same folium. Cadherin 11 (Lein et al., 2007) would seem a good candidate for UBCs or at least for a UBC subclass.

Comparative Studies on the Lobular Distribution of Mammalian Cerebellar UBCs

In all the mammalian species studied so far, including human, UBCs are most frequently encountered in the vermis and especially the flocculonodular lobe (Altman and Bayer, 1977; Altman and Bayer, 1997; Munoz, 1990; Braak and Braak, 1994; Yan and Garey, 1996; Diño et al., 1999; Takács et al., 1999, 2000; Víg et al., 2005). Although UBCs are swamped by granule cells in all lobules, their density is nevertheless notable; on average, rat UBC outnumber Golgi cell by a factor of 3 or more and approximately equal the Purkinje cells; in the nodulus, the ratios of UBCs/Golgi cells and UBCs/Purkinje cells increase to 7.3 and 2, respectively (Altman and Bayer, 1997).

These examples emphasize the need for quantitative analyses of UBC distribution after total labeling of the cell population and after differential labeling of the UBC subclasses. Maps of CR⁺ UBCs can readily be produced thanks to high titer antisera of wide cross-reactivities, cytosolic distribution of CR throughout the somatodendritic department, and CR's resistance to denaturation by formaldehyde fixatives. By contrast, only some of the commercially available mGluR1 α -antibodies stain the brush at high intensity, with only faint labeling on the perikaryal and dendritic plasma membrane. Immunostaining with PLC β 4 antiserum is more discreet. In larger mammals, such as non-human and human primates cell counting difficulties are compounded by the size of the cerebellum.

Comparative studies on the distribution of UBCs in individual lobules constitute an important aspect of the neurobiology of UBCs, because differential density may be linked to topographic specialization of neural functions. The uvula, particularly in mouse and rat, seem to contain a watershed-like border in its ventral division marking the limit for the dorsal distribution of CR⁺ UBCs. In opossum, mouse and rat (Jaarsma et al., 1998; Diño et al., 1999), an unusually high density of CR⁺ UBCs has been reported at flocculus/paraflocculus transition. This region merits particular emphasis, because it likely corresponds to the ventral paraflocculus of non-human primates and to the human accessory paraflocculus (Larsell and Jansen, 1972; Apps and Hawkes, 2009; Voogd et al., 2010). Correspondingly, studies in new-world and old-world monkeys show high densities of CR⁺ and mGluR1 α ⁺ UBCs both in the flocculus and ventral paraflocculus, in contrast to paucity or absence of these cells in most of the folia belonging to the dorsal paraflocculus (Nunzi et al., 2006; and manuscript in preparation). The high density of UBCs in cerebellar regions comprising the oculomotor vermis, the flocculo-nodular lobe and the dorsal cochlear nucleus is particularly interesting because clinical and/or experimental evidence indicates these regions are involved in vestibulo-ocular and optokinetic reflexes and head orientation in an auditory space frame (Oertel and Young, 2004; Horn et al., 2010; Voogd et al., 2010).

There are regions or areas of the rodent cerebellar granular layer that contain numerous mGluR1 α ⁺ UBCs but few, if any, cells of the CR⁺ subcategory. One such region is the rostral folium of the rodent lingula (vermal lobule IX), which has a rich complement of mGluR1 α ⁺ UBC, but few or none CR⁺ UBCs. Notably, the lateral part of the uvula shows a large spot deprived of both CR⁺ and mGluR1 α ⁺ UBCs; this circumscribed region is likely to be innervated by mossy fibers that are repulsive to UBCs during cerebellar development. Nunzi et al. (2002) described a differential distribution of CR⁺ and mGluR1 α ⁺ UBCs in the rostral and caudal leaflets of the nodulus; this might reflect the differential distribution of mossy fibers from different vestibular end organs.

In young animals UBCs tend to be situated at higher frequency near the white matter and in ventral portions of the folia than dorsally, and a more even distribution is often noted in older animals (Munoz, 1990; Braak and Braak, 1993; Yan and Garey, 1996; Víg et al.,

2005b). Scattered UBCs also occur ectopically in the white matter of the cerebellar peduncle and the medullary vela (Ibrahim et al., 2000). These findings likely reflect the cells' migratory pathways, as considered above.

Available immunostaining data in rodents with antisera to CR, mGluR1 α and PLC β 4 (Altman and Bayer, 1997; Jaarsma et al., 1998; Nunzi et al., 2002; Chung et al., 2009) concord that UBC subclasses show partly overlapping, but distinctly differential distributions. Along the mediolateral axis the UBCs form bands of higher and lower densities on each side of the midline. The bands are particularly evident in coronal sections of the vermal lingula, nodulus, and uvula, in which UBCs are most numerous. In vermal lobules I–VIII there is an overall trend for the UBC density to taper off laterally in the intermediate cortex; indeed, it is often stated that the cells are absent from hemispherical folia of the corpus cerebelli, although some reports have shown clusters of CR⁺ UBCs at the lateral edges of crus I and crus II. As already mentioned, a roughly similar pattern of distribution had been observed for autoradiographically labeled pale cells and Golgi impregnated UBCs before the introduction of antibodies to chemical markers. Sturrock (1990) provided the only dissenting opinion, as he reported approximately equal numbers of “pale cells” in the murine nodulus, spinocerebellum and pontocerebellum. His criteria for distinguishing small Golgi cells from the pale cells of Altman and Bayer, however, appear questionable in light of more recent data (Mugnaini et al., 1994; Takács et al., 1999).

Comparative Studies on UBCs in Mammalian Cochlear Nuclear Complex and Cerebellum-like Centers of Non-Mammalian Species

Although initially they were thought to be exclusively present in the mammalian cerebellum, more recent data indicate that UBCs are present also in non-mammalian species. The evolutionary history of the UBC lineage, therefore, represents an issue of particular interest.

Whereas a distinct cerebellum is present in virtually all vertebrates, cerebellar morphology and organization in non-mammalian species vary substantially, reflecting the development of sets of peripheral sensory receptors specialized for motion, electroreception, gravity and sound. In addition to the cerebellum, regions of the hindbrain targeted by octavolateralis sensory inputs contain cerebellum-like structures of which the dorsal cochlear nucleus of mammals is a distinct representative. These cerebellum-like structures share in common masses of granule cells, whose thin axons form a molecular layer and impinge upon larger neurons that may resemble Purkinje cells and system output neurons (Mugnaini and Maler, 1993; Montgomery et al., 1995; Bell, 2002; Oertel and Young, 2004; Bell et al., 2008; Rubio et al., 2008). As in mammals UBCs are present both in the cerebellum and the granule cell domain of the cochlear nuclear complex, this presents at least three kinds of approachable questions. 1) Are the major three subsets of UBCs (labeled by CR, mGluR1 α , and PLC β 4) present in the non-mammalian cerebellum of non-mammalian species? 2) Do UBCs also occur in cerebellum-like centers? 3) Are UBCs residing in the cochlear nucleus non-homogeneously distributed similarly to the cerebellum?

Presently, information about UBCs in the cerebellum of non-mammalian species is limited to light microscopic observations. Using CR-immunostaining and intracellular biocytin injections, Campbell et al. (2007) provided the first description of neurons in the cerebellum of the mormyrid fish *Gnathonemus petersii* that bore striking resemblance to mammalian UBCs, although the axons were not revealed. The small soma emitted a single dendrite terminating in a spray of processes. The processes formed bulbous swellings, rather than dendrioles, but this feature might be artifactual. Successively, Meek et al. (2008) provided a host of additional data showing that mormyrid UBCs are immunopositive for CR and mGluR2/3, but unexpectedly, also for calbindin. They are conspicuously non-homogeneous

in their distribution throughout the cerebellar lobes, but their afferents remain to be characterized. Whether the mormyrid cerebellum contains mGluR1 α ⁺ and PLC β 4 UBCs is still unknown.

While CR⁺ UBCs have so far not been demonstrated in the cerebellum of amphibians, reptiles and birds, Takács et al. (1999) described mGluR1 α ⁺ cerebellar UBCs in chicken and pigeon. In chicken both UBCs and PCs were significantly smaller than their mammalian counterparts, and the UBC/1PC ratio was highest in lobules I, X, and II, intermediate in VIII, IX and III, and lowest in lobules IV–VII. Overall, the density of UBCs appeared remarkable. Cerebellar CR⁺ UBCs are present in marine mammals (Kalinichenko and Pushchin (2008). Cluster analysis of the lengths of their single dendrites suggested the possibility of morphological subforms.

A recently published map of the rat cochlear nuclear complex indicated overall correspondence between the modes of distribution of granule cells and UBCs. The density of UBCs was highest in the dorsal cochlear nucleus compared to other regions of the granule cell system and the ventral nucleus (Diño and Mugnaini, 2008). Detailed UBC maps in the cochlear nuclear complex of other species are lacking, although UBCs or forms of the UBC named shub cells, mitt cells and chestnut cells, have been described by electron microscopy and/or immunocytochemistry in the cochlear nuclei of mouse, chinchilla, guinea pig and cat (Brawer et al., 1974; Hutson and Morest, 1996; Weedman et al., 1996; Morest, 1997; Hurd et al., 1999; Josephson and Morest, 2003; Alibardi, 2004, 2006; Meltzer and Ryugo, 2006). UBCs have also been identified in the cochlear nuclei of non-human primates and human, although in these species the granule cell system has become rudimentary (Spatz, 1999, 2000, 2001; Rubio et al., 2008).

In the hindbrain of most vertebrates the eighth nerve projects both to the cerebellum and to a specialized region that in part resembles the cerebellum (Mugnaini and Maler, 1993; Bell, 2002; Oertel and Young, 2004; Bell et al., 2008). As mentioned previously, mammalian cerebella and cerebellum-like brain stem regions share in common the presence of mossy fiber afferents, granule cells, UBCs, Golgi cells, cells of the Purkinje type, parallel fibers, and interneurons of the stellate/basket cell family. Similar centers have also been identified in fish, especially those that process electrosensory information and lend themselves to sophisticated functional studies (Mugnaini and Maler, 1993; Bell, 2002; Oertel and Young, 2004; Bell et al., 2008). So far, however, UBCs have not been identified in cerebellum-like structures targeted by the eighth nerve fibers.

The Fibers Targeting UBCs in Cerebellum and Cochlear Nucleus

Because UBCs are severely outnumbered by granule cells in all folia in which they occur, precise anatomical identification of the afferent fibers innervating cerebellar and cochlear nucleus UBCs requires advanced tract-tracing methods, complemented by confocal immunofluorescence and –ideally– also electron microscopic verification. Understandably, few studies have been dedicated to this subject so far. However, the copious, older literature on neural connection in the hindbrain will certainly be of help to resolve the issue of the afferentation of different UBC subclasses.

Cerebellar cortex

The distributions of mossy fiber afferents from the vestibular nerve and the vestibular nuclei (Butner-Ennever, 1999) are roughly in register with the density of cerebellar UBCs, as seen in rodents and several other species, including non-human primates (Diño et al., 1999). Choline acetyl transferase (ChAT)-immunoreactive mossy fibers originating from the rat vestibular nuclei were shown to innervate UBCs in lobules IX and X, but the chemical

phenotype of the target cells was not established (Jaarsma et al. 1998). On the other hand, CR⁺ UBCs in lobules IX and X were targeted by primary vestibular mossy fibers that had been labeled from individual vestibular end organs in the inner ear of gerbils (Diño et al., 2001). Vestibular stimulation was shown to induce c-FOS expression in nodulus UBCs of cat and Rhesus monkey (Sekerova et al., 2005).

Ando et al. (2005) found corticotropin releasing factor (CRF)-positive mossy fiber terminals in close apposition to CR⁺ UBCs in nodulus and flocculus (approximately 100/mm²). In the rolling mouse Nagoya mutant the number of these appositions was increased significantly in the nodulus, but not in the flocculus. The source of the CRF⁺ mossy fibers in the vestibulocerebellum was not determined.

UBCs in lobles VI/VII and IX/X of the mouse cerebellum are likely to be innervated by somatostatin 28-immunoreactive mossy fibers, which target specifically these lobules (Armstrong et al., 2009), although this remains to be specifically demonstrated.

In addition to synaptic stimulation by mossy fiber neurotransmitters, UBCs may also respond to the excitatory neuromodulator ATP, since 60–80% of the rat cerebellar UBCs were found to be immunopositive for CR and different subtypes of the P2X receptor (Xiang and Burnstock, 2005). However, pharmacological studies of these receptors in the UBCs are lacking.

Vig et al. (2005a) found a posteroventral region of the reeler mouse cerebellum that contains CR⁺ UBCs, but is devoid of vestibulocerebellar afferents.

Thus, the scanty information obtained so far contributes to raise several questions concerning the further definition of UBC functions in the context of the cerebellar neuronal microcircuits. Does the innervation from a specific source, for example vestibular nerve vestibular nuclei and spinal cord, end upon specific UBC subclasses? Do the synaptic targets of PLCβ⁴ UBCs differ from those of CR⁺ or mGluR1α⁺ UBCs? Do the axons of all CR⁺ or mGluR1α⁺ UBCs innervate other UBCs belonging to the same subclass, as suggested by Nunzi et al. (2002)? May the type of mossy fiber input modulate expression of cell subclass-specific proteins in UBCs?

Cochlear Nuclear Complex

Afferents innervating cochlear nucleus UBCs remain likewise to be determined, although most of the mossy fibers targeting the granule cell system have been mapped in the rat by Ryugo and coworkers (reviewed by Young and Oertel, 2004).

Interestingly, pyramidal cell axons from the A1 auditory cortex distribute specifically to the granule cell domain of the cochlear nuclear complex in mouse, rat gerbil and guinea pig (Feliciano et al., 1995; Weedman and Ryugo, 1996a,b; Budinger et al., 2000; Jacomme, 2003; Schofield and Coomes, 2005a,b; Schofield et al., 2006a,b; Meltzer and Ryugo, 2006). In the hedgehog tenrec a similar projection seems to arise from the somatosensory cortex (Wolff and Künzle, 1997). The auditory pyramidal cell axons were found at the periphery of mossy fiber-granule cell glomeruli, usually in the form of small synaptic boutons, but occasionally also larger endings, where they formed excitatory-type synapses with granule cell dendrites that would modulate the input of mossy fibers. Remarkably, according to Weedman and Ryugo (1996b) the pyramidal axons do not synapse on UBCs and chestnut cells, which are interspersed among granule cells (Diño and Mugnaini, 2008).

Electrophysiological Properties

Synaptic Properties of UBCs

Although the number of papers concerning UBCs' cellular properties has increased steadily in the past ten years, the available information concerning their electrophysiological and functional properties is still scant. Even the fundamental conclusion that UBCs are excitatory interneurons is mainly based on histochemical data and indirect electrophysiological evidence, as no recordings of the presynaptic effects of UBCs have been performed. Indeed, only a few papers exist that have studied the electrophysiological properties of UBCs, either in culture or in acute slices, and only two papers exist presenting in vivo single unit recordings from these neurons.

Concerning the function of UBCs as presynaptic cells, only few data are available and they were obtained using recordings from rat UBCs in acute slices (Diño et al. 2000) and organotypic cultures (Nunzi et al. 2001). In the first of these papers, the authors showed that the granule cell response to white matter stimulation differs in cerebellar areas enriched with UBCs compared to other areas. In the UBC-rich areas granule cells can be found that respond to white matter stimulation after a long delay (>50 ms) and with unusually long bursts lasting over 1.5s (Diño et al. 2000). Because such responses are not observed in granule cells in cerebellar lobules that do not contain (or contain only few) UBCs, it is reasonable to assume that such granule cell responses are the result of polysynaptic activation through the intrinsic mossy fibers (the UBC axons). Thus, these data support the hypothesis that UBCs provide excitatory inputs to granule cells. In another set of experiments performed in organotypic cultures at 15–25 DIV, extracellular electrodes were used to elicit synaptic responses in granule cells and UBCs. The pharmacological characterization of the postsynaptic response showed that the response to extracellular electrical stimulation in the granular layer was almost entirely blocked by selective antagonists of the AMPA and NMDA receptors in both granule cells and UBCs (Nunzi et al. 2001). Because in organotypic cultures extrinsic mossy fibers degenerate (Nunzi and Mugnaini 2000) and there are no other known glutamatergic inputs to granule cells and UBCs, the more parsimonious explanation of the data is that the observed glutamatergic excitation was mediated by the intrinsic mossy fibers formed by the UBC axons that innervate both granule cells and other UBCs. Thus several lines of evidence suggest that UBCs are glutamatergic cells. However, direct demonstration with dual recordings of the postsynaptic effect of UBC firing remains lacking, as is the study of synaptic plasticity at these synapses.

The postsynaptic properties of UBCs received some more attention, although, as we will see, even in this area many questions still remain unanswered.

The most distinctive electrophysiological feature of UBCs is undoubtedly the peculiar glutamatergic current recorded in slices from juvenile rats in response to stimulation of the mossy fiber input (Rossi et al. 1995). These authors compared the response of granule cells and UBCs; the granule cell response was a typical fast decaying current largely mediated by AMPA/KA receptors. UBCs, on the other hand, were characterized by an unmistakable dual component response. A first fast (10–90% rise time was 0.71 ± 0.05 ms) response was followed by a much slower component having a 10–90% rise time of 395 ± 76 ms and a very slow decay (3.1 ± 0.4 s). While in the majority (26/34) of the cells the slow component was almost completely eliminated by pharmacological block of NMDA channels, in some UBCs even the slow component was predominantly mediated by AMPA/KA receptors; the relative importance of this slow AMPA/KA-mediated response appeared to increase with age, as the contribution of the NMDA responses seem to decline in older animals (between 10 and 30 days, Rossi et al. 1995).

It was suggested that this slow component is the result of the peculiar ultrastructural features of the mossy fiber-UBC synapse that causes glutamate entrapment. Bath application of cyclothiazide caused the fast and slow components to merge into a single current having biexponential decay with time constants of 170 ms and 4.5 s. Thus it appears that even the duration of the slow decay component is sculpted by inactivation, supporting the idea that glutamate entrapped in the complex synaptic structure continues to bind to postsynaptic receptors. If such interpretation is correct, then the current mediating the slow component should be a steady-state current, which has been previously described (e.g. Raman and Trussel 1992). Indeed, excised patches from UBCs showed a significant steady-state current in response to bath application of glutamate (Kinney et al. 1997). An additional support to the idea that glutamate removal from the MF-UBC synapse is unusually slow and therefore shapes the postsynaptic responses comes from the effects of glutamate reuptake blockers, which prolong the synaptic current duration (Kinney et al. 1997). Thus, multiple lines of evidence support the idea that the peculiarly extended synaptic junctional area of the rat MF-UBC synapse (12–40 μm^2) determines the properties of the postsynaptic response. Because more recent data show that in other animal species (e.g. mouse) the synaptic apposition may be considerably smaller, it will be necessary to investigate whether the same type of synaptic responses are detectable in other species as well.

In conclusion, the data at hand support the notion that UBCs are activated by glutamate released by mossy fibers and may generate unusually long synaptic currents that may explain the long (>1.5 s) burst of activity recorded in granule cells in response to extracellular stimulation of the white matter (Diño et al. 2000).

The Golgi cells provide the major source of UBCs' inhibition. Recordings in acute slices from 17–21 days old Wistar rats showed two types of UBCs according to the pharmacological properties of the inhibitory currents (Dugué et al. 2005). One type of UBCs only receives glycinergic inputs, while the second type receives both GABAergic and glutamatergic inputs (although paired recordings showed that both inputs are provided by Golgi cells).

Because both the UBC and the Golgi cell population show phenotypical multiplicity, it would be desirable to assess whether subsets of these cells are linked at random or following specific cell-to-cell affinities. Furthermore, many aspects of inhibitory transmission at the Golgi cell-UBC synapse remain completely unknown, including the presence of synaptic plasticity, the possible basal activation of either GABA_A or GABA_B receptors, and the molecular composition of Gly and GABA_A receptors.

Intrinsic Properties of UBCs

Using electrophysiological recordings UBCs are promptly identifiable on the basis of their capacitance that is 4–6 times larger than the surrounding granule cells (Dugué et al. 2005; Russo et al. 2007) and input resistance that is about half that of granule cells (527±22 M Ω vs. 1057±202 M Ω , Russo et al. 2007). Another distinctive property setting UBCs apart from granule neurons is the activation of I_h in response to large hyperpolarizations (negative to –70 mV, Russo et al. 2007).

In the presence of blockers of fast synaptic transmission, three different firing phenotypes have been described in UBCs: silent-bursting, silent-tonically firing and intrinsically firing (Diana et al. 2007; Russo et al. 2007, 2008). Interestingly important differences seem to exist between the data reported in slices from 17–21 days old rats, where only 1 in 20 cells tested were spontaneously firing, and data in 26–38 days-old mice, where almost all UBCs examined showed intrinsic firing driven by the combined effects of a persistent sodium current and a cationic, TRP-like current (Russo et al. 2007). Interestingly, in a later paper,

Russo et al. (2008) also showed that intrinsic firing of UBCs is not a static property but is dynamically regulated through type 2 mGluR-receptors positively coupled to inward rectifier channels (Knoflach and Kemp 1998), most likely Girk2, whose expression is enriched in UBCs (Harashima et al. 2006). Indeed, mGluR activation was shown to completely and reversibly stop intrinsic firing; this effect was mediated by the opening of a background potassium conductance as it was accompanied by a decrease in the input resistance and it could be overridden by potassium channel blockade via bath application of barium (0.5 mM, Russo et al. 2008). Additional support to the hypothesis that UBCs are capable of intrinsic firing comes from the observation that these cells produce robust resurgent sodium currents (Afshari et al. 2004), a property ideally suited to promote fast and intrinsic firing (Raman and Bean 1997).

Further evidence supporting the capability of UBCs to fire spontaneously comes from in vivo recordings from UBCs. Simpson et al. (2005) performed juxtacellular recordings and biocytin filling of UBCs in the uvula/nodulus of anesthetized rats and found that these cells show fairly regular firing rhythm at frequency between 10 and 35 Hz, although some cells exhibiting slower and more irregular firing were found as well. These authors also recorded from cells likely to be UBCs in the flocculus of the awake rabbit (although they could not obtain staining of these cells) and found that also under these experimental conditions the cells were characterized by spontaneous regular firing (~19 Hz). In these conditions the (candidate) UBCs could be recorded also in response to head movement; both cells recorded in these conditions showed a decrease (a complete stop in one cell) of their spontaneous activity after a considerable delay (~200 ms) relative to the corresponding eye movement, further suggesting that synaptic inputs may act by modulating an ongoing spontaneous activity. Barmack and Yakhnitsa (2008) recorded from 31 UBCs in the anesthetized mouse. Similarly to Simpson and coworkers they found that UBCs fire quite regularly at around 10 Hz. As expected for a cell that receives vestibular input UBC firing was modulated by sinusoidal roll tilt in 27 of the 31 cells recorded. Thus, it appears that in most instances UBCs exhibit regular firing in vivo and in vitro, although more experiments will be required in order to understand the reasons for the discrepancy between firing phenotypes recorded in vitro in different experimental conditions.

As reported above, most UBCs [10/14 Diana et al. (2007), 10/19, Birnstiel et al. (2009)] are capable of generating action potential bursts when depolarized from a negative membrane potential (−90 mV). These bursts appear to be largely mediated by T-type calcium currents, as they are sensitive to 10 μ M mibefradil a concentration that is selective for T-type currents. T-type currents in UBCs are expressed in the soma and, more prominently, in the brush (Birnstiel et al., 2005, 2009; Diana et al. 2007), suggesting that they may also modulate the calcium influx associated with synaptic activation. Single cell PCR analysis (Diana et al. 2007) showed that the major subunit mediating these currents is the $\alpha 1G$, which was detected in 13 of 21 cells tested. Thus, T-type channels mediate the rebound firing, although the potential role of I_h in mediating these bursts remains to be addressed; a voltage “sag” in response to hyperpolarizing current pulses is found in the majority of the UBCs examined so far (100% Russo et al. 2007; 89%, Birnstiel et al. 2009).

T-type channels are not the only calcium channels expressed in UBCs. L-type channels ($\alpha 1C$ subunit) are also widely expressed in these cells; however, contrary to T-type currents, L-type currents are equally represented along the somatodendritic axis where they can be activated by individual action potentials. This activation, in turn, regulates the activity of calcium-activated maxi potassium channels (BK), which contribute to shaping the duration of action potentials. Interestingly, single cell PCR analysis showed that other Cav subunits are expressed in a smaller subset of cells ($\alpha 1A$ in 7/21, $\alpha 1E$ in 10/21 and $\alpha 1D$ in 7/21 cells tested, Diana et al. 2007). The functional effects of such differences were not

explored further, but it is tempting to suggest that they may be linked to the heterogeneous effect caused by calcium channel blockade (with 50 μ M cadmium) on firing frequency (Diana et al. 2007). Whether these differences reflect the existence of multiple cellular subtypes or simply variations within a homogeneous population also remains an open question.

It is worth stressing that the reported difference in the firing phenotype was not the only difference between the studies in juvenile rat and adult mouse; the capacitance of the UBCs was also quite different (8–15 pF in the juvenile rat versus 24 ± 1 pF in the adult mouse). Thus, these data suggest that interspecies and developmental properties may underlie the observed differences, or that two or more cellular subtypes exist and that they differ in size. Knowledge of the intrinsic firing properties is critical to understand the role played by UBCs in the context of the microcircuits of different cortical lobules. In this context it is of great importance to record UBCs in vivo, either from anesthetized or from awake animals. This is no easy task considering that UBCs are relatively small neurons surrounded by very densely packed granule cells. Further studies will be required to provide a final answer to this question that may have important implications for the function of cerebellar systems.

UBCs and Brain Disease

While numerous mutations affecting cerebellar Purkinje cells and granule cells have been described, very little is known about mutations that affect the UBCs. Braak et al. (1999) reported that UBCs, especially those situated in lobule VII, are affected in some cases of Pick's disease; they develop cytoskeletal anomalies and are recognized by antibody to abnormally hyperphosphorylated tau protein. As UBCs are known to express *Snc* message, which codes for α -synuclein (Schilling and Oberdick, 2009), it is possible they also contain the protein and might therefore be affected by *Snc* mutations.

The neuronal constitutive isoform of nitric oxide synthase (nNOS), which is absent in rodent UBCs under basal conditions, is transiently expressed in these neurons 12–24 h after unilateral labyrinthectomy in the rat, and gradually disappears after 7–10 days during vestibular compensation (Kitahara et al., 1997). Interestingly, some of the cerebellar UBCs were found to be nNOS-positive in sheep, a species that can withstand high altitude (Rodrigo et al., 2006). To our knowledge, there are no reports of nNOS or iNOS immunoreactive UBCs in human pathologic conditions.

Another UBC marker, the oncofetal antigen throphoblast glycoprotein 5T4, is expressed in adult UBCs (Sillitoe and Mugnaini, 2010) and could be involved in anaplastic phenomena. Specifically, medulloblastomas, which are the most common malignant brain tumor in children, present as heterogeneous cancer cells and are commonly thought to stem from the external granule layer precursors of granule cells in the caudal cerebellum or from the cells of the ventricular zone (Klesse and Bowers, 2010). This location might suggest that UBCs precursors could be involved in the generation of some forms of the tumor, that include medulloblastoma, medulloblastoma with extensive nodularity, anaplastic medulloblastoma, and large cell medullo-blastoma according to the WHO classification. Discoveries in this field would help establish specific prognostic factors for risk-adapted treatment recommendations (Rutkowski et al., 2010).

Involvement of the UBCs in segmental trisomy syndromes has recently been advocated on the basis of genetic studies in Down syndrome (DS) patients and in several murine models (Baxter et al., 2000; Olson et al., 2004; Harashima et al., 2006). In addition to cognitive problems, hypotonia, and dynamic motor dysfunction, trisomy 21 patients also show developmental delays in motor skills and a distinct morphological cerebellar phenotype consisting of invariant reduction of cerebellar volume (Baxter et al., 2000).

Correspondingly, dysfunctions of balance and motor coordination have been demonstrated in murine Down syndrome models, particularly *Ts65Dn* (Olson et al., 2004). The motor deficits were consistently detected with refined testing methods (Costa et al., 1999) and were accompanied by smaller cerebellar volume and reduction of granule and Purkinje cell numbers (Baxter et al., 2000). The critical region of the human chromosome 21 contains a gene encoding the G-protein coupled inward rectifier K⁺ channels GIRK2 and IRKK (Kcnj15 or Kir4.2), which are expressed in various brain regions, including the cerebellum (Kobayashi et al., 1995; Toyoda et al., 2002). Furthermore, the UBCs show a current with the major characteristics of a G protein-coupled inwardly rectifying K⁺ channel (GIRK) (Knoflach and Kemp, 1998), and express GIRK2/3 but not GIRK1 (Lein et al., 2007; Aguado et al., 2008). Then, Harashima et al. (2006) showed that GIRK2 is overexpressed in the *Ts65Dn* cerebellum, cerebral cortex, and hippocampus in comparison to diploid littermates and crossed *Ts65Dn* mice with a *Girk2* knockout mouse to produce variable *Girk2* expression levels. They found that in the cerebellar lobule X of *Ts65Dn* mouse GIRK2 expression follows gene dosage and demonstrated GIRK2 protein expression in murine UBCs by immunocytochemistry. Interestingly, they also found that the *Ts65Dn* mouse contains a higher proportion of GIRK2⁺/CR⁺UB Cs than the diploid/GIRK2^(+/+) mouse, suggesting a shifted balance between UC subclasses in trisomy.

A single amino acid substitution (glycine-->serine) in the pore-forming H5 region of the GIRK2 channel causes the murine *weaver* mutation, which is characterized by severe ataxia attributed to degeneration of granule cells and dopaminergic neurons in the substantia nigra (Patil et al., 1995). G-protein-activated inwardly rectifying K⁺ currents are significantly reduced in cerebellar granule cells from mice carrying the mutant allele (Surmeier et al., 1996). Preliminary observations indicate that some of the UBCs that survive the mutation have enlarged brushes (Nunzi and Mugnaini, unpublished observations).

Identification of disease causing gene mutations in humans engenders the production of murine models for these diseases. In turn the animal models can increase our understanding of disease mechanisms and indicate possible therapeutic targets. It is hoped, therefore, that discoveries on the comparative neurobiology of the UBCs will lead to further clarification of their function and facilitate inquiries on their likely involvement in human diseases. The presence of UBCs in cerebellar lobules involved in sensorimotor processes that regulate body, head and eye position, as well as in regions of the cochlear nucleus that process sensorimotor information invites further efforts to clarify their biology and their specific role in the neuronal microcircuits in which they are embedded. Furthermore, the high density of UBCs in specific regions of the cerebellar cortex is a feature largely conserved across mammals and suggests an involvement of these neurons in clinical manifestation of focal cerebellar disease (Holmes, 1917; Dietrichs, 2008).

Concluding Remarks

The UBCs were discovered much later than other cerebellar neurons and have recently attracted increasing attention. In fact, they are readily identified because of their characteristic morphology, which is highly conserved among mammals and shows less interspecies variability than all other cerebellar neurons except the granule cells. The UBC population, however, subsumes subsets having diverse chemical phenotypes, and perhaps also substantially diverse morphology. Expectably, cellular profiling of the cell population and its subclasses may uncover gene expression patterns of general interest for neuronal cell biology. UBC maps in the cerebellum and cochlear nucleus of different species indicate that this peculiar cell is involved in functions, such as the vestibuloocular and optokinetic reflexes, that are evolutionary adaptive. Yet, because of its peculiar morphology, the UBC is often considered an “exotic” type of cell. Its peculiarity and paucity in hemispherical folia

have conjured against inclusion of UBCs in earlier and newer attempts to elaborate a basic cerebellar computational algorithm (Ito, 2006; 2008; Dean et al., 2010; Bower, 2010).

By way of their one-to-one relation with individual mossy fiber terminals and the topographically restricted divergence of their axonal output, the UBCs would favor individual inputs and recruit pools of granule cells large enough to modulate the activity of relatively small groups of overlying Purkinje cells (Fig. 3). Therefore, although they may be ancillary to the fundamental neuronal machinery that is the basis for most computational models of the cerebellar networks, the UBCs confer a degree of specificity to signals reaching the vermal and flocculonodular cortex. Such a degree of specific cell-to-cell signaling may have adaptively engendered in evolution an increase in the precision of sensory processing (Kevetter and Perachio, 1986; Diño et al., 2001; Makland and Fritzsche, 2003; Kevetter et al., 2004; Shinder et al., 2005). In fact, a degree of signaling specificity would seem unnecessary in the hierarchically more advanced cortico-ponto-cerebellar system. As the ascending portion of granule cell axons are synaptic (Mugnaini, 1972) and are bundled together already at the level of the Purkinje cell layer, they may have a powerful effect on their target Purkinje cells (Llinás, 1982) before feed-forward inhibition takes place. This interpretation of the anatomy, therefore, suggests that focal activation of the granular layer in regions containing UBCs and granule cells should elicit the response of groups of overlying Purkinje cells with much higher probability than in regions containing granule cells only (Fig. 3). The idea should be testable *in vivo* and/or in models.

In the last ten years immunocytochemical cell class markers have brought about a better appreciation of the diverse regional density of UBCs. Genetic tools make it possible to visualize at least two of the UBC subsets *in vitro*, *ex-vivo* and *in situ*. This progress may pave the way for ingenious experimental designs that include the UBC's function in seeking explanations of the mechanisms encoding head translation, visual orientation, body posture and head position in a sound frame (Oertel and Young, 2004; Young and Oertel, 2004; Yakusheva et al., 2007; Ilg and Their, 2008; Angelaki et al., 2010; Horn et al., 2010).

Furthermore, almost all the studies addressing the electrophysiological properties of UBCs show that at least two distinct subpopulations may be identified. Some UBCs are intrinsically firing, while others show a bursting phenotype; the calcium channels subunits expressed also differ among individual cells; finally there appear to be large size (capacitance) differences among different studies. Because the histochemical studies also show the existence of at least three cell subclasses, it is intriguing to suggest that diverse chemical profiles and electrophysiological phenotypes are related. This is a very intriguing hypothesis and is just one of the many questions that need to be addressed. The differential distribution and distinct intrinsic properties among UBC subclasses suggest that the dynamics of the mossy fiber-UBC synapse may also differ, and this would be reflected on transmission of afferent input signals to granule cells and Purkinje cells. One may expect, therefore, that the study of the synaptic dynamics of both the mossy fiber-UBC and the UBC-granule cell synapse will represent fundamental steps for the understanding of regionally specialized cerebellar microcircuits as well as focal cerebellar disease.

Another interesting question concerns the potential role of the UBC brush as both pre- and post-synaptic structure, as it has been shown it contains clusters of round synaptic vesicles. How, and when are such vesicles released? What is their physiological effect?

Thus, UBCs are likely to affect the cerebellar output machinery in ways that we may ultimately need to understand in considerable detail. Far from being the trivial pursuit of "botanizing" efforts, such studies may enrich the ways in which we approach the problems of cerebellar structure and function. Moreover, as specificity among neuronal cell classes

requires the concerted action of many different genes, attention on the UBCs may also favor the discovery of genes conferring to them cell autonomous properties as well as of genetic mutations specifically affecting the cell class.

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Fig. 1.
The UBC participates in glomerular synaptic fields at its dendritic and axonal endings.

Fig. 2.

The synaptic connectivities in regions of the granular layer containing (A) or devoid (B) of UBCs are fundamentally different. In red, UBC in synaptic contact with an extrinsic mossy fiber (eMF); in orange, UBC in synaptic contact with an intrinsic mossy fiber (iMF); in green granule cells and their axons ascending to the molecular layer; black dots, inhibitory terminals of Golgi axonal plexus. Arrows indicate direction of transmission;

Fig. 3.

The excitatory network within a cerebellar region enriched with UBCs is represented schematically. An extrinsic mossy fiber (MF, black) contacts a first order UBC (1° UBC, red) within a glomerulus containing dendrites belonging to several granule cells (GC, green). The axon of the 1° UBC provides branches innervating multiple glomeruli, one of which also involves another UBC (2° UBC). For clarity, only two of these glomeruli are represented, although in reality there would be more than ten. The axon of the second order UBC also provides branches innervating granule cells and another UBC (3° UBC). Granule cell axons form bundles that provide a strong excitatory drive to individual Purkinje cells (PC, blue) by means of synapses (dots) formed by the ascending portions before they bifurcate to give rise to parallel fibers (PFs). Arrows refer to the direction of information flow along the network. Purkinje cells targeted by parallel fibers driven by UBC-independent networks are also represented.

TABLE 1

UBC MARKERS

The table proposes lists of the proteins expressed in UBCs that may represent markers for the entire population or for individual subclasses. The lists are a work in progress, and the asterisks denote proteins whose expression across the entire population are either partially undefined (NMDAR2, GLUR5) or whose sublineage restriction is still insufficiently characterized (GlyR, GABAAR, and CR/mGluR1 in PLC β 4 expressing UBCs).

A) Likely UBC population markers		
<i>Cell lineage</i>	<i>Glutamate Receptors</i>	<i>GABA/GLY Receptors</i>
Tbr2	GluR2	GlyR*
Eps8	GluR6*	GABAAR*
TPRC3*	NMDAR1	
α -synuclein*	NMDAR2*	
VGLUT1		
B) Likely UBC subclass markers		
<i>CR⁺ UBCs</i>	<i>PLCβ4⁺ UBCs</i>	<i>mGluR1a⁺ UBCs</i>
CR	PLCB4	mGluR1 α
SgII	CR/mGluR1 α *	5T4
VGLUT2		