Cerebellum (2015) 14:475–483
DOI 10.1007/s12311-015-0661-0

CEREBELLAR CLASSICS XI

E. Mugnaini and A. Floris, The Unipolar Brush Cell: a Neglected Neuron of the Mammalian Cerebellar Cortex, J Comp Neurol, 339:174–180, 1994: Elucidating a Cell of the Cerebellar Cortex that Largely Evaded Detection

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Published online: 21 March 2015

Introduction

> "No matter how advanced the techniques developed to study the cerebellum, they will succeed in painting the true picture of cerebellar function only if they are based on the facts of cerebellar anatomy." Robert S. Dow, MD, PhD, 1972 [1]

A poster presented at the annual meeting of the Society for Neuroscience in 1983 (later publishing in JCN) detailed the arrangement of direct projections from the hypothalamus to the cerebellar cortex and from the cerebellar nuclei to the hypothalamus. A number of colleagues queried the authors "If these connections have always been there, why have others not seen them?" The authors responded that the cerebellum people discard the hypothalamus, and the hypothalamus people discard the cerebellum; they do not look for the unexpected. Such is the case for the unipolar brush cell, a neglected and unexpected neuron in the cerebellar cortex.

Many highly regarded scientists have examined the structure of the cerebellar cortex using a wide range of methods and techniques for many years and have made numerous important observations. What Mugnaini and Floris named the *unipolar brush cell*, existed in these early studies. However, it remained for their critical analysis, dogged scientific pursuit, and insight to recognize this cell as a unique structural and functional entity; they looked for, and they anticipated, the unexpected. This initial report and those that followed from Mugnaini's laboratory, has resulted in the *unipolar brush cell* being well recognized in the scientific literature and appearing in neuroscience textbooks. Most importantly, this term has been accepted by the International Federation of Associations of Anatomists (IFAA) and appears in Terminologia Histologica as an officially recognized histologic cell type ([2], p. 96). A further validation of the widespread acceptance of the unipolar brush cell is seen by its inclusion in a number of chapters of the Handbook of the Cerebellum and Cerebellar Disorders [3].

In this early paper, Mugnaini and Floris used mice, rats, cats, and rhesus monkeys. This approach nullified a potential criticism of novel discoveries in the modern era, this being that new observations may have eluded detection because they are species specific. The *unipolar brush cell* (UBC) is common to all vertebrates examined to date including human.

Mugnaini and Floris [4] described the general characteristics of the UBC in this report. First, the UBC at 9–12 μm is slightly larger than granule cells, has short distinctive dendritic appendages, and an axon that may arise from the cell body, dendritic trunk, or distal dendrites. Second, UBEs are most commonly found in the flocculonodular lobe, ventral uvula, ventral paraflocculus, lingual, and less so in portions of the vermis; these cerebellar cortical regions are generally considered to be largely co-extensive with the vestibulocerebellum as they receive afferents from and send efferents to the vestibular nuclei. Third, UBCs have synaptic relations with each

The Commentary article of Cerebellar Classic XI is available at http://dx.doi.org/10.1007/s12311-015-0660-1.

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other and with granule cells, Golgi cells, and mossy fiber rosettes. Fourth, based on their distribution and synaptic interactions, Mugnaini and Floris [4] suggested that the UBCs were intimately involved in pathways and loops that modulate/control various aspects of eye movement.

A number of earlier studies, using several methods, had reported data suggestive of a cerebellar cell type that was not strictly consistent with the features of granule, Golgi, and Purkinje cells ([5–8]; and others). Altman and Bayer [5] called the presumptive UBC a "pale cell". Mugnaini and his colleagues confirmed these fragmented early reports and conducted a large series of studies to characterize the UBC morphologically and functionally. They described the UBCs synaptic relationships, locations within the lobules and folia of the cerebellum, immunocytochemistry, important electron microscopic features, and neurotransmitters. These studies have been corroborated by subsequent investigators and have opened up opportunities for future studies that may focus on the function of UBCs, particularly in the arena of disorders of eye movement. Most of this extensive research effort to elucidate the UBC is summarized by Diño et al. [9] and Mugnani et al. [10].

The original paper of Mugnaini and Floris is reprinted here for the enjoyment of the readership of The Cerebellum as part of this Special Issue honoring the scientific accomplishments and contributions of Professor Enrico Mugnaini. It is anticipated that our readers will enjoy reacquainting themselves with this important paper and reading the Commentary that follows, see [11].

Acknowledgments We express our sincere appreciation to Mr. Charles Runyan, Biomedical Illustration, The University of Mississippi Medical Center, for his scanning and subsequent preparation of the original paper and to Wiley for permission to reprint the original paper from The Journal of Comparative Neurology. We are indebted to Dr. Maria Diño, Dr. Gabriella Sekerková, and Dr. Marco Martina for their thoughtful preparation, on behalf of Professor Mugnaini, of the Commentary for this Cerebellar Classic.

References

1. Dow RS. From the Foreword, The Comparative Anatomy and Histology of the Cerebellum, The Human Cerebellum, Cerebellar Connections, and Cerebellar Cortex, by O. Larsell and J.Jansen, The University of Minnesota Press, Minneapolis, MN, 1972. (Interestingly enough, Professor Mugnaini had an extensive chapter on the electron microscopy of the cerebellar cortex in this volume).
2. Terminologia Histologica. International terms for human cytology and histology. Philadelphia: Lippincott Williams & Wilkins; 2008. p. 1–207.
3. Manto M, Gruol DL, Schmahmann JD, Koibuchi N, Rossi F. Handbook of the Cerebellum and Cerebellar Disorders, Volumes 1, 2, 3, and 4. New York: Springer; 2013.
4. Mugnaini E, Floris A. The unipolar brush cell: a neglected neuron of the mammalian cerebellar cortex. J Comp Neurol. 1994;339:174–80.
5. Altman J, Bayer SA. Time of origin and distribution of a new cell type in the rat cerebellar cortex. Exp Brain Res. 1977;29:265–74.
6. Hockfield S. A Mab to a unique cerebellar neuron generated by immunosuppression and rapid immunization. Science. 1987;237: 67–70.
7. Munoz DD. Monodendritic neurons: A cell type of the human cerebellar cortex identified by chromogranin A-like immunoreactivity. Brain Res. 1990;528:335–8.
8. Braak E, Braak H. The new monodendritic neuronal type within the adult human cerebellar granule cell layer shows calretinin immunoreactivity. Neurosci Letts. 1993;154:199–202.
9. Diño MR, Nunzi MG, Anelli R, Mugnaini E. Unipolar brush cells of the vestibulocerebellum: afferents and targets, Chapter 10. IN: Gerrits NM , Ruigrok TJH, De Zeeuw CI, editors. Cerebellar Modules, Molecules, Morphology and Function, Progress in Brain Research, New York: Elsevier. 2000;124:123–37.
10. Mugnaini E, Sekerková G, Martina M. The unipolar brush cell: a remarkable neuron finally receiving deserved attention. Brain Res Rev. 2011;66:220–45.
11. Diño MR, Sekerková G, Martina M. Commentary on E. Mugnaini and A. Floris, The Unipolar Brush Cell: A Neglected Neuron of the Mammalian Cerebellar Cortex. J Comp Neurol, 339:174–180, 1994. Cerebellum 2015. doi 10.1007/s12311-015-0660-1 (*in this issue*)

THE JOURNAL OF COMPARATIVE NEUROLOGY 339:174–180 (1994)

The Unipolar Brush Cell: A Neglected Neuron of the Mammalian Cerebellar Cortex

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ABSTRACT

We describe with a variant of the Golgi method a new type of neuron that is prominently represented in the granular layer of the mammalian vestibulocerebellum but is presently neglected in all major accounts on the cerebellum. These neurons, here termed unipolar brush cells, are intermediate in size between granule cells and Golgi cells. They typically have a thin and presumably myelinated axon, and a single and stubby dendrite whose tip forms a tightly packed group of branchlets resembling a paintbrush. The branchlets often intertwine with the digitiform claws of granule cell dendrites and are occasionally approached by Golgi cell dendrites, indicating that the unipolar brush cells may share the input of the other granular layer neurons. Branchlets of neighboring unipolar brush cells converging into the same neuropil island also occur. The brush-like tip of the unipolar cell engulfs one or two mossy fiber rosettes to form an extensive synapse that appears to close recurrent loops involving the vestibular nuclei. Positive feedback in these loops could help to explain several motor responses and drive mechanisms of extended duration that are controlled by the ventral cerebellum.

Key words: vestibulocerebellum, Golgi method, Rat-302, pale cell, Golgi cell

The cerebellar cortex has been extensively studied for more than 100 years, and it is commonly assumed that its histological and cytological structure has already been clarified, needing refinement only with respect to chemical makeup and microscale connectivity (Cajal, '11; Eccles et al., '67; Mugnaini, '72; Palay and Chan-Palay, '74; Ito, '84). Nevertheless, a few publications based on autoradiographic, histologic, and immunohistochemical data have reported the presence in the cerebellar cortex of special small cells predominantly distributed in the vestibulocerebellum (Altman and Bayer, '77; Hockfield, '87; Cozzi et al., '89; Munoz, '90; Sturrock, '90; Floris et al., '92; Résibois and Rogers, '92). Similar cells have also been reported in a note on the human cerebellum that appeared while the present account was in the process of publication (Braak and Braak, '93). The precise morphology of these cells has, however, remained elusive: They have never been recognized with the "reazione nera" introduced by Golgi (1873) that has classically been instrumental in defining neuronal typology. We reasoned that, if a neglected cerebellar neuron exists, it may have escaped attention because the Golgi method impregnates only a small percentage of the neurons present in any given brain region. To circumvent the sampling problem inherent in this method, we utilized a variant of the Golgi/Del Rio-Hortega procedure (Sotelo and Palay, '68; Adams, '79; Blackstad et al., '84), which affords a much higher rate of impregnation than the classical rapid Golgi protocol.

MATERIALS AND METHODS

This study was based on adult animals only. All animals were housed and handled according to approved guidelines. From a large sample of Golgi-impregnated specimens, two C57BL/6J mice, two Sprague-Dawley rats, two domestic cats, and three adult rhesus monkeys showing optimal impregnation in the cerebellum were chosen for detailed investigation.

Under deep sodium pentobarbital anesthesia (35–60 mg/kg, i.p.), animals were perfused through the ascending aorta with saline, followed by a buffered aldehyde fixative and then by a mordant consisting of 6% potassium dichromate, 6% chloral hydrate, and 4% formaldehyde. After postfixation in the same mordant for 3 days, blocks of the hindbrain were treated with 0.75% silver nitrate for 3

Accepted September 8, 1993.

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additional days (Sotelo and Palay, '68; Adams, '79), embedded in a soft Epon mixture, and sectioned with a heated steel knife (V.L. Friedrich Jr., personal communication) as described elsewhere (Blackstad et al., '84).

RESULTS

The observations reported below were remarkably similar across species. Figure 1a, taken from the ventral uvula, a vermal folium belonging to the vestibulocerebellum, shows the degree of neuronal impregnation obtained with the protocol we have utilized. Moreover, Figure 1 illustrates not only the three types of granular layer neurons known from classical studies, a Purkinje cell and two astrocytes, but also a type of neuron, here termed unipolar brush cell (UBC; arrow at extreme left in Fig. 1) uncovered by this method for the first time. To appreciate the novelty of this cell type, a comparison with other granular layer neurons is in order. The smallest cells (7–8 μm in diameter) are the granule neurons, which have round cell bodies, two to four thin and smooth primary dendrites that terminate in digitiform claws at a short distance from the parent cell body, and a thin varicose axon that ascends towards the molecular layer, where it bifurcates in opposite directions, giving rise to the parallel fibers. The large cell (20 μm in diameter, at extreme right in Fig. 1) is the classical, multipolar Golgi neuron, which has an ovoidal cell body and several branching dendrites provided with a moderate number of spinous appendages; the axon, which arborizes profusely in the granular layer, forming a varicose plexus that innervates the periphery of the glomerular synaptic fields characteristic of this lamina, was not impregnated in this preparation. In the center in Figure 1 is the fusiform cell of Lugaro (Lugaro, 1894; Fox, '59, Palay and Chan-Palay, '74), with dendrites stretched out along the boundary of granular and Purkinje cell layers.

The unipolar brush cell

Other UBCs from the flocculus, the nodulus, the uvula, and other vermal folia are shown in Figures 1b–j, 2, and 3a–d. These neurons have rounded or ovoidal cell bodies (9–12 μm in diameter) that are intermediate in size between granule and Golgi cells. Within a given folium, the UBCs occur at all levels of the granular layer (Fig. 1c), sometimes immediately beneath the Purkinje cell layer and also in the folial white matter (Fig. 1c,f). These neurons emit a thin axon, 0.3–0.5 μm in diameter, and commonly give rise to a single, short and stubby dendrite, 2–3 μm in diameter, which usually divides only at the tip, where it forms a tightly packed group of branchlets covered by thin, spine-like appendages, resembling a paintbrush (Fig. 1b). Many of these cells also carry thin appendages on the cell body and the stem dendrite (Figs. 1e, 2d). The length of the dendritic shaft ranges from a few micrometers to 50 μm, and usually measures only 10–30 μm, although it is not uncommon to see a brush tip formed at a bulbous enlargement very close to the parent cell body (Fig. 1h–j). The dendritic shaft is straight or curved and points with nearly equal frequencies towards the molecular layer, the white matter, or lateral regions of the granular layer (Fig. 1d), indicating that it does not show any preferred orientation. The field occupied by the brush tip is rounded, ovoid, boxy, or, more often, cap-shaped and measures 10–30 μm in average diameter, forming a neuropil island, which may encompass no more than one or two glomerular synaptic fields (Mugnaini, '72). Among more than 1,000 well-impregnated UBCs, we encountered several bizarre forms (Fig. 2a–f), which recurred in all species with little, if any, interspecies variation. The brush formation remains the hallmark for every one of these cells. Although we cannot exclude the existence of UBC subclasses, we assume that these unusual cell forms are determined by local conditions during development and do not alter significantly the main synaptic properties of this neuronal type.

The axon of the UBC usually arises from the cell body (Fig. 1b), but it may also emanate from the dendritic trunk (Fig. 1g) or one of the tip's branchlets. The axon tapers soon after leaving the parent cell and loses its impregnation at a distance of 100–300 μm from the point of emergence. This feature suggests that it becomes myelinated, because the myelin sheath is known usually to prevent metallic impregnation. The axons of over 40 UBCs were followed until they entered the folial white matter. Although we often saw UBCs with ascending axons, these processes usually curved back upon entering the Purkinje cell layer and ceased to be impregnated, even in sections where the thinner, but well impregnated, axons of neighboring granule cells were followed all the way to their branch points in the molecular layer. Nevertheless, because we observed only impregnated axonal stumps, we cannot exclude that UBCs participate somehow in the cortical circuitry.

Relations to other neurons

We saw the brush tips of two neurons articulating with each other (Figs. 1c, 2g). Interdigitations of the dendritic tips of UBCs and granule cells were frequently observed (Fig. 3c). Golgi cell dendrites also entered the brush tips of UBCs (Fig. 3a,b). Mossy fiber rosettes tightly intertwined with the brush tips formed glomerular arrays nearly impervious to light (Fig. 3d), while they remained clearly resolvable when they interdigitated with the simpler digitiform claws of the granule cell dendrites (Fig. 3e). These observations indicate that UBCs share the mossy fiber input with granule and Golgi cell dendrites. An electron microscopic analysis presented separately (Mugnaini and Floris, '94) showed that the cell body and the dendritic stem of the UBC receive few synaptic contacts, while each branchlet forms an enormous synaptic junction with a portion of the mossy rosette. Thus we concluded that the brush tips of the UBCs are dendritic devices developed to maximize synaptic apposition to mossy fiber terminals. Because the brush formation is very peculiar, is shared even by the most bizarre forms, and represents a special synaptic apparatus, we determined that it should be part of the descriptive name of these cells.

Distribution of the unipolar brush cells

The UBCs were present at high densities in the nodulus (lobulus X; Fig. 1c), ventral uvula (lobulus IXc), and flocculus; at moderate densities in the ventral paraflocculus and lingula (lobulus I); and less frequently in other vermal folia and were virtually absent from the cerebellar hemispheres. The sites where UBCs occur at high densities largely overlap the cortical terminal regions of the vestibular afferents, including the cholinergic mossy fibers originating from the vestibular nuclei (Ojima et al. '89; Barmack et al., '92a,b). In the nodulus, ventral uvula, and flocculus, 4–10 well-impregnated UBCs could be seen within 1 mm^2 of the granular layer. Although the Golgi method usually does not lend itself to a precise evaluation of the frequency of

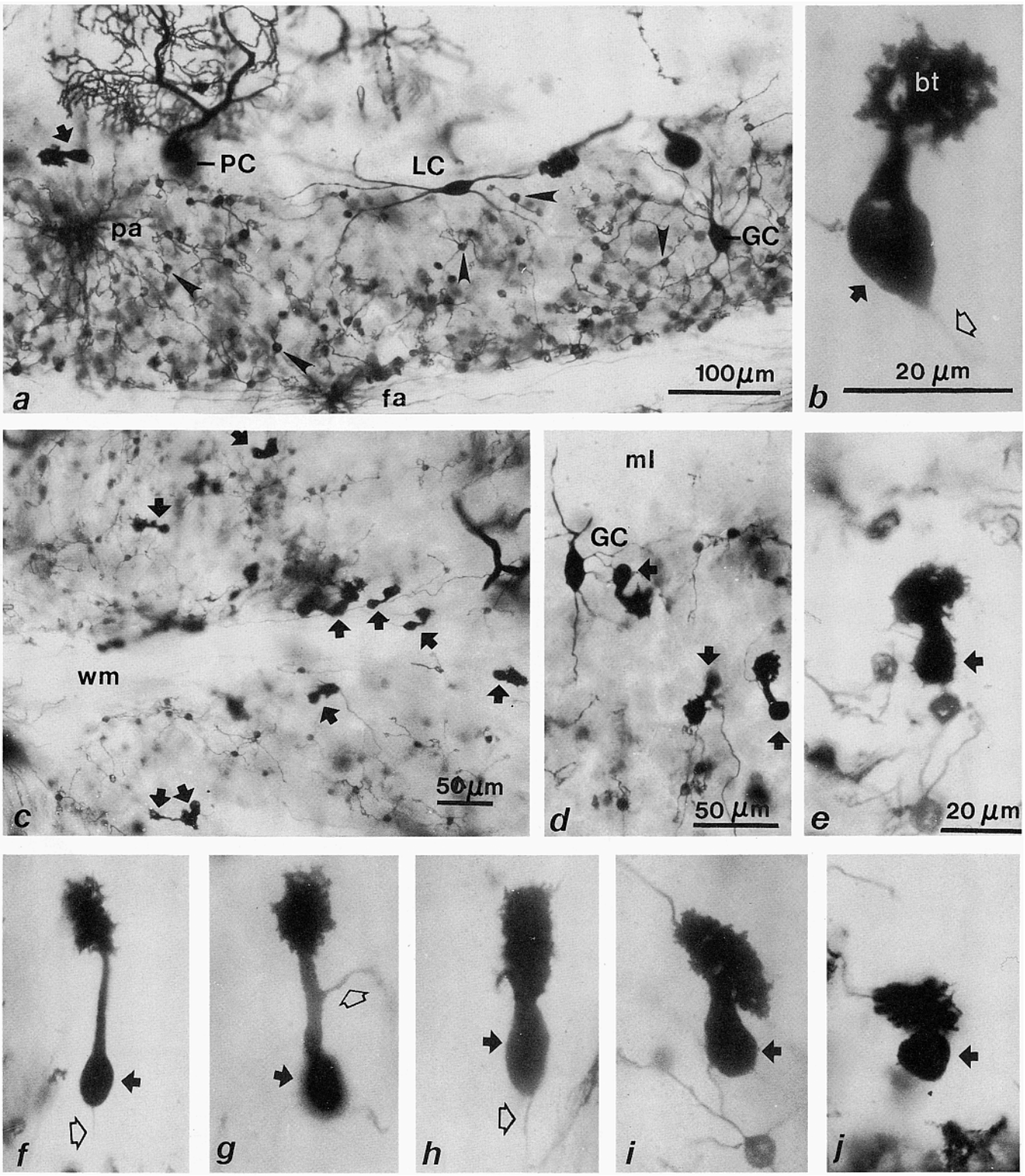

Fig. 1. Golgi impregnated unipolar brush cells (UBCs) in the cerebellum of macaque (a–c, e–i), cat (d), and rat (j). UBCs are indicated by arrows and their axons by open arrowheads. **a:** PC, Purkinje cell; GC, Golgi cell; LC, Lugaro cell; arrowheads, granule cells; pa, protoplasmic astrocyte; fa, fibrous astrocyte. **b:** UBC with a brush-like dendritic tip (bt) and the axon emanating from the cell body. **c:** High concentration of UBCs in the nodulus: Nine of these are in focus and eight additional ones, situated in other planes, appear as dark shadows. The brush tips of two UBCs interdigitate at bottom left; wm, white matter. **d:** Three UBCs with differently oriented dendrites. ml, Molecular layer; GC, Golgi cell. **e:** UBC with spinous appendages on the cell body. **f:** UBC in the white matter. **g:** UBC with the axon emanating from the stem dendrite. **h:** UBC with elongated brush tip. **i:** Mushroom-shaped UBC. **j:** UBC with brush tip originating very near the cell body. Scale bar in e applies to e–j.

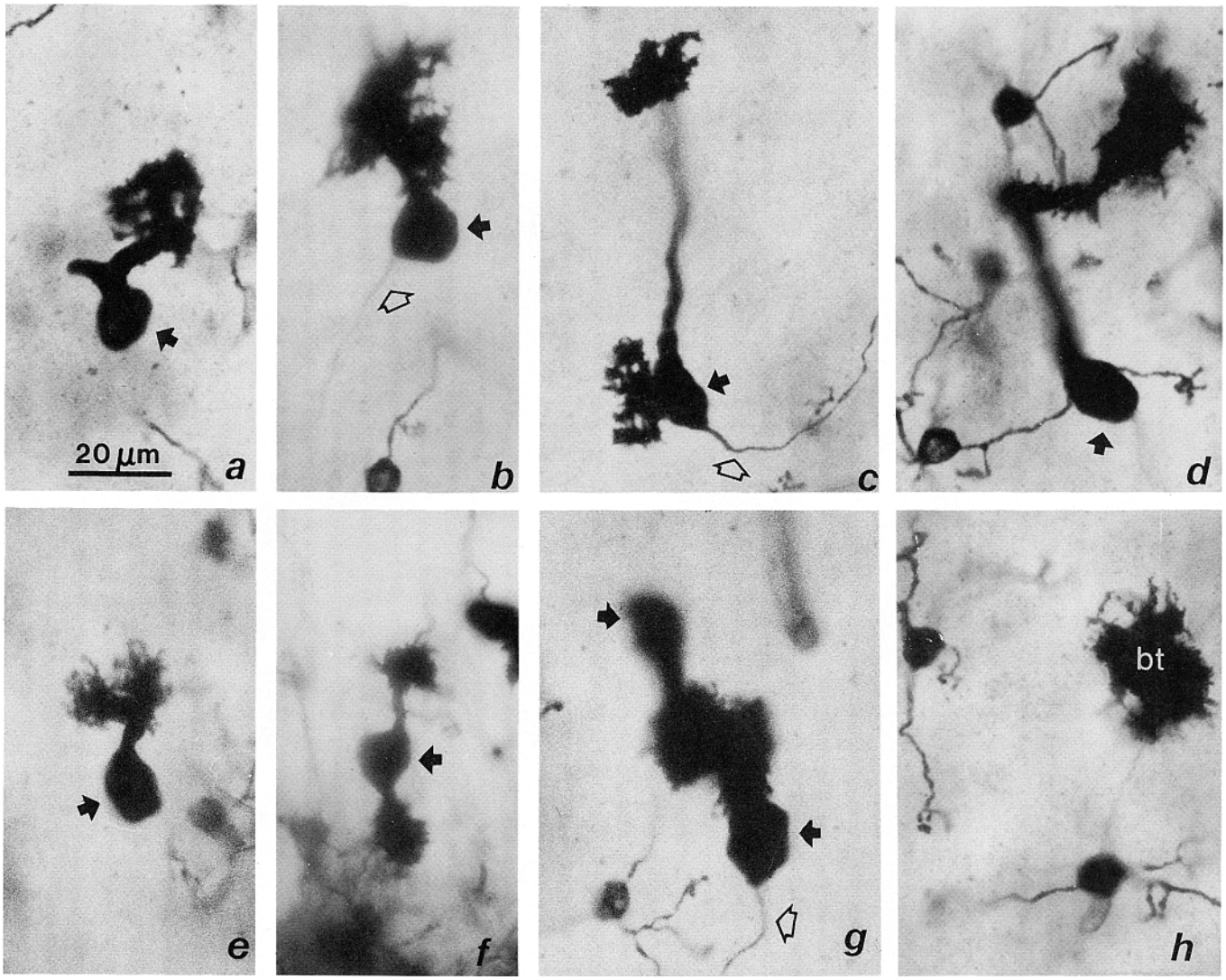

Fig. 2. Golgi impregnated UBCs from the macaque cerebellum. Arrows, UBC cell bodies; open arrows, emerging axons; granule cells are unlabeled; cells in a–f represent unusual forms of UBCs). **a:** UBC with a branching dendritic stem. One of the brush tips is not included in the section. **b:** UBC with a large brush at the end of the dendrite and a smaller brush originating from the dendritic stem. **c:** UBC with a brush at the tip of the dendrite and a brush originating from the cell body. **d:** UBC with a long, twisted stem dendrite covered with spinous appendages. **e:** UBC with a split brush tip. **f:** UBC with two dendrites; these arise from opposite poles of the cell body and both end with a brush tip. **g:** Two UBC with interlacing brush tips. **h:** Brush tip (bt) of a UBC as it appears when the cell body is not in the plane of section. Scale bar in a applies to a–h.

specific neuron classes, we estimated that these cells may number several thousand in each of these folia. UBCs similar to those of the cerebellar cortex were also observed in the rodent dorsal cochlear nucleus (data not shown), which is known to contain a microcircuit analogous to that of the cerebellum (Mugnaini and Morgan, '87; Berrebi and Mugnaini, '91).

DISCUSSION

Why have the UBCs not been recognized previously by practitioners of the Golgi method? We believe the main reason is the sampling problem. The rapid Golgi method, the most commonly utilized procedure for the analysis of neuronal microcircuits, usually impregnates no more than 2% of the neurons present in any given area, and the UBCs occur at high frequency only in the superficial folia that belong to the vestibulocerebellum and may not commonly be included in the well-impregnated portions of the cerebellar cortex. Moreover, the UBC is clearly identified only when its cell body and dendrite lie in the plane of section; when seen in isolation from the parent cell body (Fig. 2h), the brush tips are easily confused with impregnation artifacts. A compounding reason may be that the exhaustive description of the cerebellar cortex by Cajal and his contemporaries stifled the power of inquiry of successive investigators.

Evidence that the UBCs correspond to neurons incompletely characterized by previous authors with other techniques is accumulating in our laboratory. With the autoradiographic method, Altman and Bayer ('77) described neuronal cell bodies in the granular layer of the rat

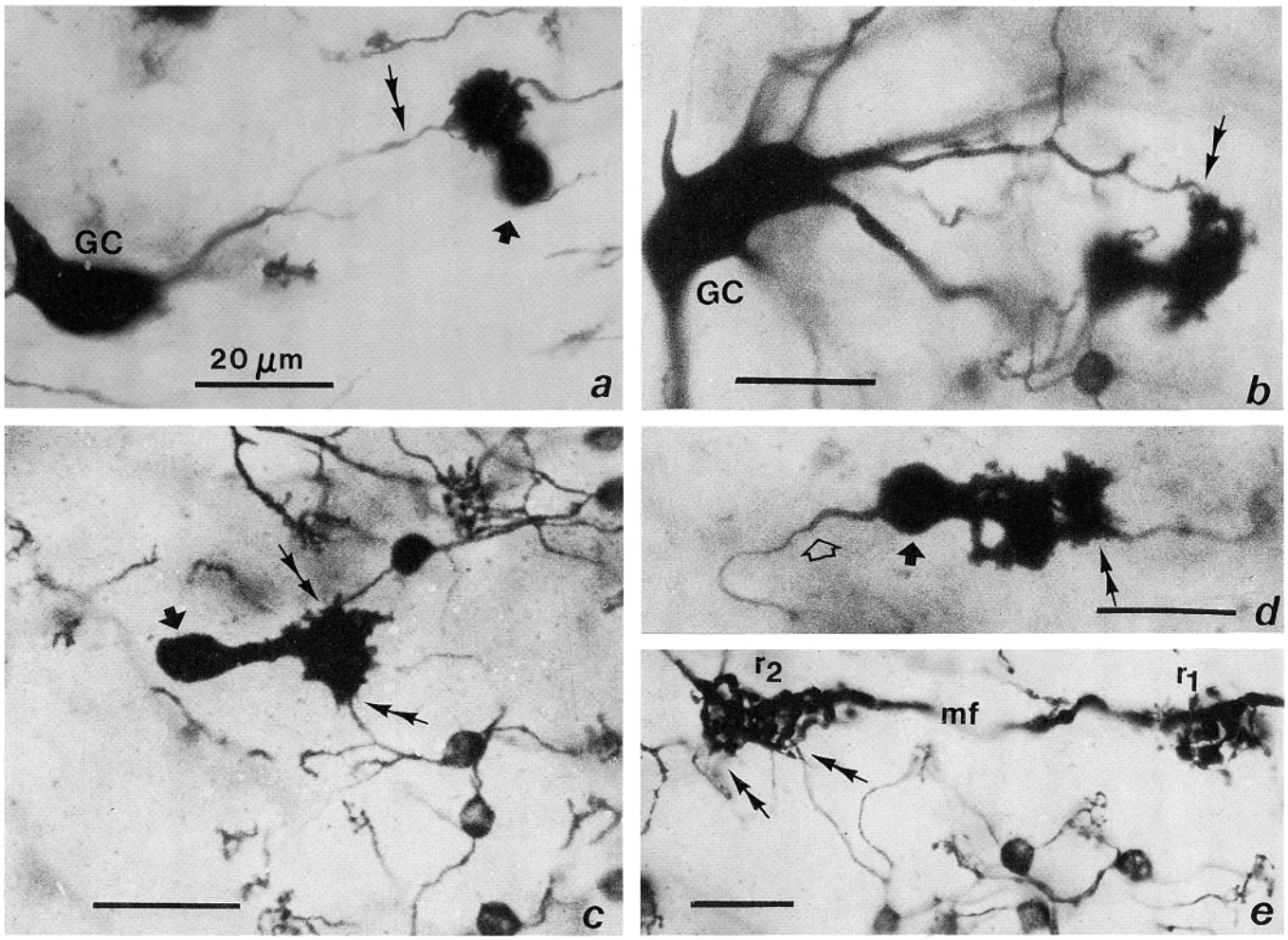

Fig. 3. Relations of UBCs with other neurons in the rhesus monkey (arrows, UBC cell bodies; granule cells are unlabeled). **a:** A dendritic trunk (double arrow) of a small Golgi cell (GC) traverses the brush tip of a UBC. **b:** A peripheral branch (double arrow) of a large Golgi cell (GC) intermingles with the branchlets of a UBC. **c:** The tips of two granule cells intermingle (double arrow) with the branchlets of a UBC. **d:** the rosette of a mossy fiber (mf) is tightly interlaced (double arrow) with the brush tip of a UBC. **e:** A rosette is impregnated in isolation (r1) and a second one (r2), from the same mossy fiber (mf), is interlaced with the tigitiform tips of granule cells (double arrowheads). The rosettes roughly match the sizes of the brush tips. Scale bars = 20 μm.

cerebellar cortex that were intermediate in size between granule and Golgi cells. These neurons were generated in the ventricular neuroepithelium between embryonic day 19 and postnatal day 2, i.e., after generation of the neurons of the cerebellar nuclei, the Purkinje cells, and the Golgi cells, and before generation of most granule and stellate-basket cells. The authors termed these neurons pale cells, because similar cells in adjacent sections stained with hematoxylin-eosin had pale nuclei, distinguishable from those of other neurons in the granular layer. Like the UBCs, the pale cells were ten times more frequent in the nodulus and the ventral uvula than in other vermal folia. Successively, small cells provided with dendrites ending "in a spray of neurites" were reported in the vestibulocerebellum by immunostaining with monoclonal antibody (mAb) Rat-302 (Hockfield, '87), and by antisera to secretogranin II (or chromogranin C, Cozzi et al., '89), chromogranin A (Munoz, '90), and calretinin (Floris et al., '92; Braak and Braak, '93). Ultrastructural evidence that the Rat-302-positive cells and the calretinin-positive cells actually correspond to the UBCs is presented separately (Floris et al., '94; Harris et al., '93). Double label immunofluorescence studies intended to ascertain definitely whether the secretogranin II positive cells (Cozzi et al., '89) and the chromogranin A positive cells (monodendritic neurons of Munox, '90) are calretinin positive are in progress. Observations on the murine mutant *reeler* indicate that calretinin-positive UBCs originate from the ventricular neuroepithelium (Floris et al., '92), like pale cells.

Hockfield ('87) produced other mAbs, of which Rat-303 stains the Golgi cells but not the Rat-302 cells, and Cat-301 and Cat-303 (Sahin and Hockfield, '90) stain the intermediate cells of Lugaro, a type of relatively rare and little understood cerebellar cortical neuron, but not the Golgi or the Rat-302 cells. By contrast with the Golgi cells, which are stained with antisera to gamma-aminobutyric acid (GABA) and glycine (Ottersen et al., '88), the UBCs were immunonegative for both these neurotransmitters (Floris et al., '92, '94; Harris et al., '93). Furthermore, UBCs are distinctly immunostained by antibodies to high-molecular-weight neurofilament subunits, while the multipolar granule, Golgi, and Lugaro cells are not (Harris et al., '93). These data cumulatively add to the evidence that the UBCs differ from all other neurons of the granular layer. In none

of the species examined did we see transitional forms between UBCs and other cerebellar neurons. Like other neurons of the cerebellar cortex, the UBCs have features that are similar in all mammalian species, ranging from rodents to monkeys. Identical neurons are also present in the human cerebellum, where they have been recognized by their dense immunostaining with antibody to chromogranin A (Munoz, '90) and calretinin (Floris et al., '92, '94; Braak and Braak, '93). The UBCs, therefore, not only appear morphologically unique but also are provided with special chemical phenotypes. On the basis of the observation that the UBCs are immunonegative for the inhibitory neurotransmitters GABA and glycine, we suggest that they constitute a new category of cerebellar cortical neuron endowed with excitatory properties. One explanation could be that the UBCs are local circuit neurons provided with peculiar intracortical axons, but this is not supported by demonstrative observations. A second possibility is that the UBCs are a peculiar form of granule cell specialized in the transmission to the Purkinje cells of special inputs requiring a high safety factor of transmitter release (see also Hockfield, '87; Rogers, '89; Résibois and Rogers, '92). Three considerations militate against this hypothesis. 1) We could find no evidence that UBC axons synapse with Purkinje cell dendrites in the molecular layer. 2) Ordinary granule cells derived from the external granular layer are present in the flocculonodular lobe, while the UBCs originate from the ventricular neuroepithelium. 3) Our preliminary studies of UBCs in murine mutants indicate that these cells survive in *pcd* and *lurcher* (unpublished observations), where practically all Purkinje cells undergo cell death followed by a massive secondary degeneration of granule cells. On the other hand, all these considerations are compatible with the view, provisionally based on the finding of several UBC axons entering the folial white matter, that the UBCs represent special cortical efferent neurons analogous to neurons of the cerebellar nuclei. The UBCs may be wired somewhat similarly to the large multipolar neurons of the nuclei of the mammalian cerebellum (Ito, '84) and to the eurydendroid cells of the fish cerebellar cortex (Finger, '83), which are innervated by collaterals of cerebellar afferent fibers. In contrast to all these neurons, the UBC occupies a very limited tissue volume. Because of its size, the dendritic brush tip can only extend into one or two glomeruli and is able to perform little, if any, spatial integration of inputs. The UBCs provided with two brush tips, which may provide special modes of convergence, occurred only rarely (see also Braak and Braak, '93).

Functional considerations

What may be the function of UBCs? Their preferential distribution in the vestibulocerebellum and their extensive synapses with individual mossy fibers are striking and may provide important clues. The vestibulocerebellum is the phylogenetically oldest part of the cerebellum and does not have a typical cerebellar nucleus to serve as target of Purkinje cell inhibition (Eccles et al., '67). Instead, its Purkinje cells project out of the cerebellum to vestibular neurons in the vestibuloocular and vestibulocollic pathways (Ito, '84; Langer et al., '85). In more recent parts of the cerebellum, the cerebellar nuclei serve as a focal point for convergence of multiple recurrent pathways that interconnect the cerebellar nuclei, the primary motor cortex (via the thalamus), the magnocellular red nucleus, and the precerebellar nuclei in the brainstem (Allen and Tsukahara, '74; Houk, '89). Positive feedback transmitted through these loops probably serves as an important driving force for the

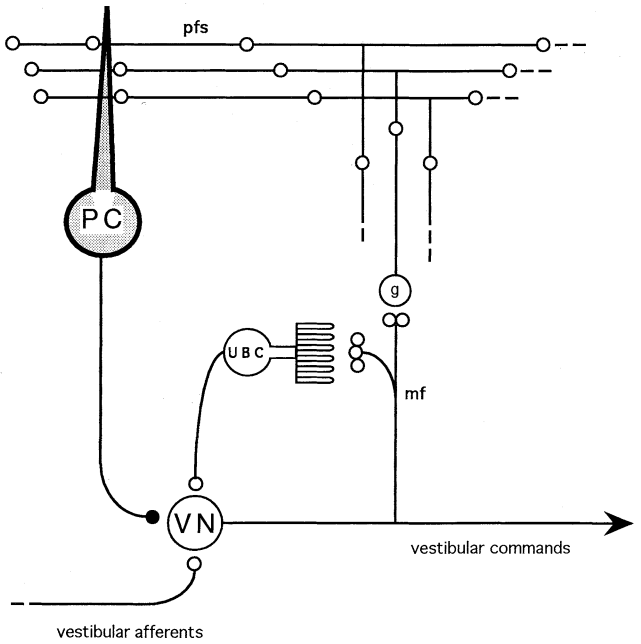

Fig. 4. Schematic diagram of the presumed wiring of the UBCs. PC, Purkinje cell; VN, projection neuron in the vestibular nucleus; mf, mossy afferents from the vestibular nerve and the vestibular nucleus; g, granule cell; pfs, parallel fibers; solid circle, inhibitory axon terminal; open circles, excitatory axon terminals.

generation of motor commands (Houk et al., '93). Pursuing this analogy, we suggest that UBCs may function as relays in the more specialized recurrent loop illustrated in Figure 4. The mossy fiber input to the flocculonodular lobe includes first-order (Korte and Mugnaini, '79; Barmack et al., '93) and second-order (Barmack et al, '92a,b) vestibular neurons, but only the secondary input is illustrated for simplicity. Although the targets of UBC projections are not yet known, the projection to the vestibular nuclei is a strong possibility. Positive feedback transmitted through this loop would be expected to enhance the intensity and extend the duration of vestibuloocular and vestibulocollic motor commands and perhaps also cardiorespiratory set points. Specifically, such a mechanism might help to explain 1) the maintenance of smooth pursuit eye movements in the absence of visual motion input, which was postulated to be mediated by an undisclosed positive feedback loop through the flocculus (Zee et al., '81; Langer et al., '85; Lisberger et al., '87; Morris and Lisberger, '87); 2) long-acting modulations of the velocity storage mechanism of eye movement control that depend on the integrity of the nodulus (Waespe et al., 85; Cohen et al., '92); and 3) the long-term regulation of cardiorespiratory drives that is thought to be mediated by the uvula (Paton and Spyer, '92; Hayashi et al., '93). The mossy fiber-UBC synaptic pathway is remarkable for its direct passage through the granular layer that bypasses information processing in the molecular layer. These features present a striking contrast to the convergent characteristics of the adjacent mossy fiber-parallel fiber-Purkinje cell pathway. Consistent with the principles of reciprocity enunciated by Lorente de Nó ('38), the vestibular neurons do not send excitatory axon collaterals back to themselves; rather, they use a long loop through the cerebellar cortex for self-reinforcement and/or counteraction of inhibitory inputs. One interpretation of the mossy fiber-UBC synaptic pathway, thus, is that it represents an exaggerated form of

reciprocity. Knowledge about the time course of transmission at this extraordinary synapse may determine whether this circuit is well suited for a special form of temporal integration, analogous to the mathematical operation of integration.

These considerations are by necessity speculative, because UBCs were previously unknown and their axonal projections remain to be demonstrated; nevertheless, they may serve to stimulate fresh ideas and produce testable hypotheses about the operating principles of the vestibulocerebellum. What is quite clear is that the UBC is a new and prominent cytoarchitectural element that needs to be considered in interpreting structure-function relationships of the cerebellum.

ACKNOWLEDGMENTS

This study was supported by U.S. PHS grants NINDS 09904-22 and NIDOCD 1805-01 (E.M.) and by a stipend of the Italian M.U.R.S.T. (A.F.). We thank Drs. Sanford L. Palay and James C. Houk for criticism and comments on a previous version of the manuscript.

LITERATURE CITED

Adams, J.C. (1979) A fast, reliable silver-chromate Golgi method for perfusion-fixed tissue. Stain Technol. *54:*225–226.

Allen, G.I., and N. Tsukahara (1974) Cerebrocerebellar communication systems. Physiol. Rev. *54:*957–1006.

Altman, J., and S.A. Bayer (1977) Time of origin and distribution of a new cell type in the rat cerebellar cortex. Exp. Brain Res. *29:*265–274.

Barmack, N.H., R.W. Baughman, and F.P. Eckenstein (1992a) Cholinergic innervation of the cerebellum of rat, rabbit, cat and monkey as revealed by choline acetyltransferase activity and immunohistochemistry. J. Comp. Neurol. *317:*233–249.

Barmack, N.H., R.W. Baughman, F.P. Eckenstein, and H. Shojaku (1992b) Secondary vestibular cholinergic projection to the cerebellum of rabbit and rat as revealed by choline acetyltransferase immunohistochemistry, retrograde and orthograde tracers. J. Comp. Neurol. *317:*250–270.

Barmack, N.H., R.W. Baughman, P. Errico, and H. Shojaku (1993) Vestibular primary afferent projections to the cerebellum of the rabbit. J. Comp. Neurol. *327:*521–534.

Berrebi, A.S., and E. Mugnaini (1991) Distribution and targets of the cartwheel cell axon in the dorsal cochlear nucleus of the guinea pig. Anat. Embryol. *183:*427–454.

Blackstad, T.W., K.K. Osen, and E. Mugnaini (1984) Pyramidal neurones of the dorsal cochlear nucleus: A Golgi and computer reconstruction study in the cat. Neuroscience *13:*827–854.

Braak, E., and H. Braak (1993) The new monodendritic neuronal type within the adult human cerebellar granule cell layer shows calretinin immunoreactivity. Neurosci. Letts. *154:*199– 202.

Cajal, S. Ramón y (1911) Histologie du Système Nerveux de l'Homme et des Vertébrés, Vol. I and II (trans. L. Azoulay). Paris: Maloine [reprinted 1955, Madrid: Consejo Superior de Investigaciones Cientificas].

Cohen, H., B. Cohen, T. Raphan, and W. Waespe (1992) Habituation and adaptation of the vestibuloocular reflex: A model of differential control by the vestibulocerebellum. Exp. Brain Res. *90:*526–538.

Cozzi, M.G., P. Rosa, A. Greco, A Hille, W.B. Huttner, A. Zanini, and P. De Camilli (1989) Immunohistochemical localization of secretogranin II in the rat cerebellum. Neuroscience *28:*423–441.

Eccles, J.C., M. Ito, M., and J. Szentágothai (1967) The Cerebellum as a Neuronal Machine. New York: Springer Verlag.

Finger, T.E. (1983). Organization of the teleost cerebellum. In R.G. Northcutt and R.E. Davis (eds): Fish Neurobiology, Vol. 1. Ann Arbor: The University of Michigan Press, pp. 261–284.

Floris, A., M.E. Dunn, A.S. Berrebi, D.M. Jacobowitz, and E. Mugnaini (1992) Pale cells of the flocculonodular lobe are calretinin positive. Soc. Neurosci. Abstr. *18:*853.

Floris, A., M. Diño, D.M. Jacobowitz, and E. Mugnaini (1994) The unipolar brush cells of the cerebellar cortex studied by calretinin immunocytochemistry: Light and electron microscopy. J. Neurosci. (in press).

Fox, C.A. (1959) The intermediate cell of Lugaro in the cerebellar cortex of monkey. J. Comp. Neurol. *112:*39–51.

Golgi, C. (1873) Sulla struttura della sostanza grigia del cervello. Gazz. Med. Ital. *31:*244–246.

Harris, J., S. Moreno, G. Shaw, and E. Mugnaini (1993) Unusual neurofilament composition in cerebellar brush neurons. J. Neurocytol. (in press).

Hayashi, F., S.K. Coles, K.B. Bach, G.S. Mitchell, and D.R. McCrimmon (1993) Time dependent phrenic nerve responses to carotid afferent activation: Intact versus decerebellate rats. Am. J. Physiol. *265:*R811–R819.

Hockfield, S. (1987) A Mab to a unique cerebellar neuron generated by immunosuppression and rapid immunization. Science *237:*67–70.

Houk, J.C. (1989) Cooperative control of limb movements by the motor cortex, brainstem and cerebellum. In R.M.J. Cotteril (ed): Models of Brain Function. Cambridge: Cambridge University Press, pp. 309–325.

Houk, J.C., J. Keifer, and A.G. Barto (1993) Distributed motor commands in the limb premotor network. Trends Neurosci. *16:*27–33.

Ito, M. (1984) The Cerebellum and Neural Control. Raven Press: New York.

Korte, G.E., and E. Mugnaini (1979) The cerebellar projection of the vestibular nerve in the cat. J. Comp. Neurol. *184:*265–278.

Langer, T., A.F. Fuchs, M.C. Chubb, C.A. Scudder, and S.G. Lisberger (1985) Floccular efferents in the rhesus macaque as revealed by autoradiography and horseradish peroxidase. J. Comp. Neurol. *235:*26–37.

Lisberger, S.G., E.J. Morris, and L. Tychsen (1987) Visual motion processing and sensorimotor integration for smooth pursuit eye movements. Annu. Rev. Neurosci. *10:*97–129.

Lorente de Nó, R. (1938) Analysis of the activity of the chains of internuncial neurons. J. Neurophysiol. *1:*207–244.

Lugaro, E. (1894) Sulle connessioni tra gli elementi nervosi della corteccia cerebellare con considerazioni generali sul significato fisiologico dei rapporti tra gli elementi nervosi. Riv. Sper. Freniat. *20:*297–331.

Morris, E.J., and S.G. Lisberger (1987) Different responses to small visual errors during initiation and maintenance of smooth-pursuit in monkeys. J. Neurophysiol. *58:*1351–1369.

Mugnaini, E. (1972) The histology and cytology of the cerebellar cortex. In O. Larsell and J. Jansen (eds): The Comparative Anatomy and Histology of the Cerebellum. The Human Cerebellum, Cerebellar Connections, and Cerebellar Cortex. Minneapolis: The University of Minnesota Press, pp. 201–264.

Mugnaini, E., and A. Floris (1994) The extraordinary synapses of the unipolar brush cells of the rat cerebellum. Synapse (in press).

Mugnaini, E., and J.I. Morgan (1987) The neuropeptide cerebellin is a marker for two similar neuronal circuits in rat brain. Proc. Natl. Acad. Sci. USA *84:*8692–8696.

Munoz, D.D. (1990) Monodendritic neurons: A cell type in the human cerebellar cortex identified by chromogranin A-like immunoreactivity. Brain Res. *528:*335–338.

Ojima, H., S. Kawajiri, and T Yamasaki (1989) Cholinergic innervation of the rat cerebellum: Qualitative and quantitative analyses of elements immunoreactive to a monoclonal antibody against choline acetyltransferase. J. Comp. Neurol. *290:*41–52.

Ottersen, O.P., J. Storm-Mathisen, and P. Somogyi (1988) Colocalization of glycine-like and GABA-like immunoreactivities in Golgi cell terminals in the rat cerebellum: A postembedding light and electron microscopic study. Brain Res. *450:*342–353.

Palay, S.L., and V. Chan-Palay (1974) Cerebellar Cortex. Cytology and Organization. New York: Springer Verlag.

Paton, J.F.R., and K.M. Spyer (1992) Cerebellar cortical regulation of circulation. News Physiol. Sci. *7:*124–129.

Résibois, A., and J.H. Rogers (1992) Calretinin in rat brain: An immunohistochemical study. Neurosci. *46:*101–134.

Rogers, J.H. (1989) Immunoreactivity for calretinin and other calcium-binding proteins in cerebellum. Neurosci. *31:*711–721.

Sahin, M., and S. Hockfield (1990) Molecular identification of the Lugaro cell in the cat cerebellar cortex. J. Comp. Neurol. *301:*575–584.

Sotelo, C., and S.L. Palay (1968) The fine structure of the lateral vestibular nucleus in the rat. I. Neurons and neuroglial cells. J. Cell Biol. *36:*151–179.

Sturrock, R.R. (1990) A quantitative histological study of Golgi II neurons and pale cells in different cerebellar regions of the adult and ageing mouse brain. Z. Mikrosk. Anat Forsch. *104:*705–714.

Waespe, W., B. Cohen, and T. Raphan (1985) Dynamic modification of the vestibulo-ocular reflex by the nodulus and uvula. Science *228:*199–202.

Zee, D.S., A. Yamazaki, P.H. Butler, and G. Gucer (1981) Effects of ablation of the flocculus and parafloccullus on eye movements in the primate. J. Neurophysiol. *46:*878–899.