UNIPOLAR BRUSH CELL: A POTENTIAL FEEDFORWARD EXCITATORY INTERNEURON OF THE CEREBELLUM

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Abstract—Unipolar brush cells are a class of interneurons in the granular layer of the mammalian cerebellum that receives excitatory mossy fiber synaptic input in the form of a giant glutamatergic synapse. Previously, it was shown that the unipolar brush cell within granule cell axon branches, giving rise to large terminals. Single mossy fiber stimuli evoke a prolonged burst of firing in unipolar brush cells, which would be distributed to postsynaptic targets within the granular layer. Knowledge of the ultrastructure of the unipolar brush cell terminals and of the cellular identity of its postsynaptic targets is required to understand how unipolar brush cells contribute to information processing in the cerebellar circuit. To investigate the unipolar brush cell axon and its targets, unipolar brush cells were patch-clamped in fresh parasagittal slices from rat cerebellar vermis with electrodes filled with Lucifer Yellow and Biocytin, and examined by confocal fluorescence and electron microscopy. Biocytin was localized with diaminobenzidine chromogen or gold-conjugated, silver-intensified avidin. Light microscopic examination revealed a single thin axon emanating from the unipolar brush cell soma that gave rise to 2–3 axon collaterals terminating in mossy fiber-like rosettes in the granular layer, typically within a few hundred μm of the soma. In some cases, axon collaterals crossed the white matter within the same folium before terminating in the adjacent granular layer. Electron microscopic examination of serial ultrathin sections revealed that proximal unipolar brush cell axons and axon collaterals were unmyelinated and devoid of synaptic contacts. However, the rosette-shaped enlargements of each collateral formed the central component of glomeruli where they were surrounded by dendrites of granule cells and/or other unipolar brush cells, with which they formed asymmetric synaptic contacts. A long-latency repetitive burst of polysynaptic activity was observed in granule cells in this cerebellar region following white matter stimulation. The unipolar brush cell axons, therefore, form a system of cortex-intrinsic mossy fibers.

The results indicate that synaptic excitation of unipolar brush cells by mossy fibers will drive a large population of granule cells, and thus will contribute a powerful form of distributed excitation within the basic circuit of the cerebellar cortex. © 2000 IBRO. Published by Elsevier Science Ltd. All rights reserved.

Key words: cerebellar glomerulus, cerebellar granule cell, cerebellar mossy fibers, patch-clamp recording, Biocytin.

Unipolar brush cells (UBCs) are a class of putative interneurons found in the granular layer of mammalian cerebellum and dorsal cochlear nucleus. At the light microscope level, they are characterized by: (a) a cell body diameter intermediate between granule and Golgi cells; (b) a typically single, short, stocky dendrite which terminates in a paint brush-like structure of dendrioles; (c) a differential distribution in the cerebellar cortex that favors the flocculo-nodular lobe in rodents, and, in some species, also the cerebellar vermis, intermediate cortex and ventral paraflocculus.

Ultrastructural studies using electron microscopy have demonstrated that the UBC dendritic brush forms the core of a glomerulus where its dendrioles interdigitate with the mossy fiber rosette and establish a giant excitatory synaptic contact estimated to cumulatively measure 12–40 μm² in adult rats. These glomeruli may be comprised exclusively of the mossy fiber terminal and UBC dendrioles. Typically, however, the same glomerulus will also contain several granule cell dendrites, and an occasional Golgi cell dendrite, that receive synaptic contact from the mossy fiber rosette. Golgi cells also make axo-dendritic contact with UBC and granule cell dendrites, and UBC dendrioles form dendro-dendritic synapses with granule cells. The mossy fiber–granule cell synapse and the mossy fiber–Golgi cell synapse are typical of most excitatory synapses in brain, with a small area of postsynaptic density at which ionotropic glutamate receptors (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate/kainate (AMPA/KA) and N-methyl-D-aspartate (NMDA)) are localized. However, the mossy fiber–UBC synapse, which also contains both AMPA/KA and NMDA receptors, is unusually extensive, with multiple presynaptic release sites opposed to continuous or fragmented regions of postsynaptic density.

This unique ultrastructure permits glutamate, released from the presynaptic terminal, to be entrapped within the synapse, allowing for greater interaction between glutamate and its postsynaptic receptors. Moreover, the UBC dendrioles are provided with numerous spinous appendages devoid of synaptic junctions, but rich in metabotropic, mGluR1 and mGluR2, glutamate receptors. The unusual structure of the mossy fiber–UBC synapse is thought to underlie the unique synaptic responses observed in UBCs. Patch-clamp recordings of UBCs in thin rat cerebellar slices have shown that single mossy fiber stimuli evoke biphasic excitatory postsynaptic currents (EPSCs) that last from several hundreds of milliseconds to several seconds, which are associated with a prolonged depolarization and train of action potentials. In order to understand how
such unique properties of the UBC may influence motor control pathways that are modulated by cerebellar circuits, it must first be determined what afferent information is received by UBCs, as well as identifying what cells serve as their efferent targets. Previous studies have demonstrated that the UBC axon branches locally within the granule layer and gives rise to several large terminals, suggesting that UBCs might serve as a feedforward interneuron that projects to granule cells. We sought to confirm this hypothesis through an ultrastructural examination of the axonal terminals and their synaptic targets derived from physiologically characterized UBCs. Here, we report that UBC terminals form cortex-intrinsic mossy fiber rosettes that comprise the central components of glomeruli within the granular layer, and are presynaptic to granule cell dendrites and UBC dendrites.

The results have been reported elsewhere in abstract form.

EXPERIMENTAL PROCEDURES

Young male and female Sprague–Dawley rats purchased from a commercial breeder were used for this investigation. All efforts were made to minimize both the suffering and the number of animals used. All experiments conformed to protocols approved by the Northwestern University Center for Experimental Animal Resources (CEAR), an AAALAC-accredited facility, and followed guidelines issued by the National Institutes of Health and the Society for Neuroscience.

Electrophysiological methods

Putative UBCs, with cell somata 9–14 μm in diameter, were visually identified in the nodulus of 200–μm-thick parasagittal slices cut from the cerebellar vermis of rats at postnatal days 15–28. Animals were anesthetized with isoflurane and killed with a small animal guillotine. The brain was rapidly removed and placed in ice-cold external solution. The cerebellar vermis was removed, and slices were cut in the sagittal plane using a vibrating tissue chopper (Vibratome). After preparation, the slices were transferred to an incubation chamber at room temperature (22–24°C) until required. For recording and cell filling, slices were placed in a submersion chamber on the stage of an upright microscope (Leitz Laborlux) and continuously perfused with an external medium containing (mM): NaCl 126, KCl 3, CaCl2 2.5, MgSO4 1.3, NaHPO4 1.25, NaHCO3 26 and n-glucose 10 (gassed with 95% O2 and 5% CO2; pH 7.4; osmolality adjusted to 310 mOsm). Putative UBCs were visually identified in the granular layer of the nodulus using a water immersion objective (Zeiss, £) with Hoffman Contrast Optics. Patch-clamp recordings were made to minimize both the suffering and the number of animals used.

RESULTS

Morphology of intracellularly labeled unipolar brush cells

More than 50 physiologically identified, Lucifer Yellow/Biotin-filled UBCs were screened by light and electron microscopy and were selected for this study if the tracer appeared strictly intracellular and ultrastructure was well preserved. Eleven UBCs that met these criteria were subjected to further analysis. Three of these were stained with the DAB chromogen and eight with gold-conjugated avidin followed by silver intensification. All the UBCs in the sample were obtained from 15–28-day-old rats. The cells had a single dendrite terminating with a brush formation, which appeared compact in the stacked confocal images. A single axon emanated from the cell body, usually opposite the dendritic stem. In one case the axon arose near the emergence of the dendritic stem. Two UBCs from the cerebellar nodulus, filled with a patch pipette containing Lucifer Yellow/Biotin, that were imaged with a laser-scanning confocal fluorescence microscope and volume rendered with Voxel View software, are shown in Fig. 1. In Fig. 1A only the somatodendritic compartment is shown, while Fig. 1B represents also the axon, which branches in the granular layer in the vicinity of the parent cell body. In some of the UBCs, the brush tip surrounded a hollow center, which we interpreted as the site of entrance of the presynaptic mossy fiber among the brush dendrites (Fig. 1A). Axons usually had 1–3 branches, which typically ended with elongate, knobby endings resembling mossy fiber rosettes and measuring 5–8 μm and 10–16 μm along the short and long axes. En passant rosettes were observed along the course of the main axon or one of the collaterals. The constricted portions of the UBC axon measured 0.2–1.5 μm in diameter. The distance of individual terminals from the parent cell body usually varied between 50 and 250 μm. In one case, the axon of a UBC, filled with Lucifer Yellow and not analysed further by electron fiber stimulation, as previously described.

Anatomical methods

After recording, slices were then fixed in buffered aldehyde (4% freshly depolymerized formaldehyde/1.25% glutaraldehyde in 0.12 M phosphate buffer, pH 7.4) for 2 h. Biotin was localized with avidin-biotin complex (ABC) (Vector Laboratories, Burlingame, CA, U.S.A.) methods and a diaminobenzidine (DAB) chromogen or 1 nm gold-conjugated avidin followed by silver intensification. Slices were then post-fixed in 1% osmium tetroxide, stained en bloc with 1% aqueous uranyl acetate, dehydrated and infiltrated with a mixture of Epon-TAAB resins (Ted Pella Inc., Redding, CA, U.S.A.) and flat embedded for 24–36 h at 60°C between two Mylar sheets. Polymerized slices were mounted on a resin block. Labeled UBCs were visualized by trans-illumination and sectioned on a Reichert ultramicrotome. Ultrathin sections (80–95 nm) were mounted on single-hole grids covered with Formvar, counterstained with uranyl acetate and lead citrate, and analysed by electron microscopy.

Some of the slices containing individually labeled neurons were whole mounted by fixation in buffered formaldehyde, alcohol drying, clearing in methyl salicylate, and mounting on a glass slide under a coverslip with interposed glass spacers. UBCs with well defined dendritic brushes and axonal projections were viewed on a laser-scanning confocal microscope (BioRad MRC600). A fluorescein filter set, an excitation line of 488 nm from an argon ion laser, and a ×40 Nikon CFU Fluor objective lens (numerical aperture 1.3) were used. Sequential optical sections were taken at 1.0 μm intervals in the Z plane to build an image volume in three dimensions on a Silicon Graphics work station using Voxel View software (Voxel Images).
microscopy, formed branches provided with a total of 12 endings, with one of the branches extending well over 300 μm from the parent soma. In another case, an axon branch crossed the white matter and terminated in the granular layer of the opposite side of the folium. Terminals of UBC axons were isolated from each other, except in one case where terminals of two branches abutted (Fig. 1B).

Characterization of unipolar brush cells by patch-clamp recording

Cells approximately 10–12 μm in soma diameter were visually identified near the surface of fresh slices mounted on a recording chamber. Their identification as UBCs was determined with white matter stimulation and patch-clamp recordings following criteria established by Rossi et al.25 (see Experimental Procedures), and successively confirmed by laser-scanning confocal fluorescence microscopy (Fig. 1). Mossy fiber activation with a single white matter stimulus evoked a long-lasting excitatory postsynaptic potential (EPSP) and associated burst of action potentials in the postsynaptic UBC. These EPSPs are mediated in UBCs via glutamate receptors, and the time-course of the EPSC is unusually prolonged (>2 s), with a long-lasting NMDA receptor-mediated component, and an unusual, biphasic AMPA receptor-mediated component.25,29,30 Smaller cells with a granular soma differed from UBCs as previously described (see Experimental Procedures).

In regions of the cerebellar vermis in which UBCs are located most densely (nodulus and ventral uvula), granule cells can be observed (n = >15) that have highly unusual synaptic responses as illustrated in Fig. 2. The arrow indicates the time at which a single shock was applied to mossy fibers in the white matter. The long latency to the first EPSC (>50 ms) indicates that this is a polysynaptic response. The single shock stimulus evoked a train of inward currents lasting over 1500 ms. Granule cells with this type of synaptic response are only rarely observed in other regions of the vermis. This type of response contrasts with the very short-latency, fast rise-time monophasic EPSCs evoked by monosynaptic activation of mossy fibers.22,25,28 This electrophysiologic observation provides suggestive evidence that UBCs may serve as feedforward excitatory interneurons within the granular layer.

To provide direct evidence for UBC–granule cell connections, we initially attempted dual recordings in the slice. UBCs were first filled with Lucifer Yellow as described and then the axon terminals located in the slice by fluorescence microscopy. A second recording was then obtained from granule cells in the vicinity of the axon terminal, and the UBC soma depolarized to elicit a train of action potentials. Attempts to perform dual-cell recordings in this way proved unsuccessful, presumably due to the sheer numbers of granule cells and the low probability of obtaining a postsynaptic follower cell. Consequently, the postsynaptic targets of the UBC axon were identified by electron microscopic examination of filled cells (see below).
Ultrastructure of identified unipolar brush cells

Verification of the synaptic arrangement between mossy fiber and unipolar brush cell brush. An electron micrograph of the brush from a patch-clamped UBC filled with Biocytin and visualized with gold-conjugated/silver-intensified avidin is shown in Fig. 3 (main panel). The accompanying schematic (inset A, top left) shows a line passing through the brush to indicate the orientation of the electron micrograph relative to the UBC. The UBC dendrioles emit several non-synaptic appendages, occupy a large portion of the glomerulus, and are innervated by a single mossy fiber rosette (highlighted yellow), which typically contains several mitochondria, numerous small, clear round synaptic vesicles, and scattered dense core vesicles. The small granular deposits within the UBC dendrioles permit clear identification of the dendriolar postsynaptic densities of the multiple mossy fiber–UBC synapses. The synapses are of the asymmetric category. Some of these consist of short individual junctions (main panel), while others are disposed in series (inset B, bottom right), as typically observed at sites of contact between extrinsic mossy fibers and UBCs. A small number of unlabeled, postsynaptic processes in contact with the mossy fiber rosette represent presumptive granule cell dendrites, which are characteristically bound to each other by puncta adherentia and share in common the glomerulus with the filled UBC. Occasional Golgi axonal boutons with pleomorphic synaptic vesicles are present at the glomerular periphery, which is lined by a discontinuous sheath of astrocytic lamellae.

Identification of the unipolar brush cell axon terminals and their targets. To provide direct confirmation that UBCs in the cerebellar nodulus do indeed project to granule cells, the UBC axon terminals and their targets were examined at the ultrastructural level. In 11 of the UBCs, the axon was sufficiently labeled to allow tracing in the electron microscope. For economy, cells were analysed in serial ultrathin sections as follows. Labeled cells were gradually approached by cutting successive 1-μm-thick sections. When a labeled profile was identified light microscopically in a semithin section, this was followed by a short series of ultrathin sections. Only occasional ultrathin sections were collected from portions of the axons devoid of large en passant or terminal swellings. Fourteen UBC axon terminals were analysed in the electron microscope. All of them were situated at the core of cerebellar glomeruli and had the rosette-like appearance typical of

Fig. 2. Polysynaptic responses of granule cells to mossy fiber stimulation. In regions of the cerebellar vermis in which UBCs are densely distributed (nodulus and ventral uvula), granule cells with unusual, polysynaptic responses to mossy fiber stimulation can be observed. The trace illustrates a long-latency, repetitive train of EPSCs elicited in a granule cell in the nodulus to a single mossy fiber stimulus (arrow; stimulus artifact has been removed for clarity). The interpretation of this is schematically illustrated above. Granule cells (GC) which are postsynaptic targets of UBCs will be driven by a burst of action potentials in the UBC following activation of the giant mossy fiber–UBC synapse. This input burst will produce a train of individual fast EPSCs reminiscent of the fast EPSCs evoked by direct mossy fiber stimulation. Under normal conditions, such a burst will in turn produce a summating EPSP in the granule cell that is mediated primarily by NMDA receptors.
Fig. 3. Ultrastructure of the dendritic brush of a Biocytin-labeled unipolar brush cell (UBC). The cartoon (top, inset A) represents an idealized, single, Biocytin-labeled UBC synapsing with a mossy fiber (mf). The line passing through the artist-rendered brush approximates the orientation of the electron micrograph illustrated in the main panel relative to the UBC. Biocytin was localized with gold-conjugated/silver-intensified avidin, which appears as small granular deposits within the UBC dendrioles (d). These contain numerous mitochondria, emit small, non-synaptic appendages (stars) and are innervated by a single mossy fiber (highlighted yellow), which occupies the core of the glomerulus. Granule cell dendrites (g), bound by puncta adherentia (arrowheads), and a Golgi bouton (triangle) are also included in this glomerulus, which is bordered by astrocytic lamellae (asterisks). Postsynaptic densities of the mossy fiber–UBC synaptic junctions (thick arrows); dense core vesicles (open arrowheads). Inset B (bottom) shows a complex synapse (thick arrows) between a dendriole and the mossy fiber rosette, from a serial section.
Fig. 4. Rosette-shaped, Biocytin-labeled terminals of the unipolar brush cell (UBC) axon form synapses with granule cell dendrites and putative Golgi cell dendrites. (A) UBC axon terminal in the cerebellar nodulus visualized with the ABC protocol and DAB chromogen (electron dense reaction product). The terminal occupies the center of a glomerulus. Digitiform tips of granule cell dendrites (g); short postsynaptic densities (arrowheads); Golgi bouton (triangle). (B) UBC axon terminal visualized with gold-conjugated/silver-intensified avidin (electron dense granular deposits). The terminal winds within a glomerulus, passing several times through the plane of section. Most of the remaining volume of the glomerulus is occupied by numerous granule cell dendrites and a putative Golgi cell dendrite (go?). The dendritic profiles receive short asymmetric synaptic contacts (arrowheads) from the UBC rosette. Punctum adherens (arrows) between two granule cell dendrites; small Golgi boutons (triangles). Scale bars = 0.4 μm (A), 0.5 μm (B).
mossy fiber endings. As shown in Figs. 4 and 5, these rosettes were surrounded by numerous dendritic profiles. The glomerular periphery consisted of astrocytic lamellae and scattered small boutons with pleomorphic vesicles presumably belonging to the Golgi axonal plexus.

Electron microscopy demonstrated that all the UBC axons were unmyelinated, contrary to extrinsic mossy fibers, which have a discrete myelin sheath. Proximal and constricted portions of the axons and axon collaterals were devoid of synaptic contacts. The en passant and terminal rosette-like enlargements of the UBC axons represented true presynaptic, axonal endings. Like the extrinsic mossy fiber rosettes previously described, the endings of the UBC axons resided inside glomerular arrays, contained several mitochondria, numerous small clear round synaptic vesicles, and scattered dense-core vesicles. The majority (71%, 10/14) of the UBC terminals were surrounded by large numbers of small dendritic profiles, whose features were characteristic of the digitiform tips of granule cell claws in young rats. The UBC terminals typically formed short asymmetric synaptic junctions with the adjoining postsynaptic dendrites. A Biocytin-filled UBC axon terminal in the cerebellar nodulus, reacted with the ABC method and DAB chromogen, is shown in Fig. 4A, and a Biocytin-filled UBC axon terminal stained with gold-conjugated/silver-intensified avidin, also from the cerebellar nodulus, is shown in Fig. 4B. The terminals contain numerous small round synaptic vesicles and mitochondria, partly obfuscated by the electron dense reaction product in Fig. 4A, but clearly evident in Fig. 4B. In the glomerulus of one of the labeled UBC terminals we observed an occasional large postsynaptic dendritic profile (labeled go? in Fig. 4B), that might represent a section through a Golgi dendrite coursing through the glomerulus. This dendrite, however, was noticed at the end of
the study and could not be traced in the serial ultrathin sections.

Some of the UBC axon terminals (29%, 4/14), however, participated in glomeruli that differed from those shown in Fig. 4 by containing large postsynaptic dendrioles (Figs 5 and 6) provided with numerous non-synaptic appendages, in addition to smaller and smoother granule cell dendrites. With serial ultrathin sections, it was possible to trace distinctly some of these dendrioles to a neuronal soma emitting a single dendritic stem (Fig. 6). The soma contained a nucleus rich in dispersed chromatin, differing from the heterochromatic nucleus of the granule cell (Fig. 6A). The dendrioles formed asymmetric synaptic junctions with the labeled UBC axon terminal. Many of these synapses were more extensive than those formed by mossy fiber rosettes with granule cell dendrites (cf. Fig. 5). Occasionally, the dendrioles were presynaptic to granule cell dendrites, forming small dendrodendritic junctions (Fig. 6B). As demonstrated elsewhere, this set of features is typical of the somatodendritic compartment of UBCs.

**DISCUSSION**

In this paper, we provide the first demonstration that UBC axon terminals target multiple granule cell dendrites and also brush dendrioles of other UBCs. We confirm that the UBC brush participates in a glomerular array where its dendrioles are innervated by a mossy fiber rosette, and demonstrate that branched UBC axons form rosette-shaped endings occupying glomeruli within the granule cell layer of the mammalian cerebellum. The UBC axons form asymmetric small synaptic junctions with granule cells and large or serial asymmetric synaptic junctions with other UBCs. These two categories of synapses are morphologically similar to those formed by extrinsic mossy fibers with granule cells and UBCs, which is in line with the concept that the postsynaptic element determines the main configuration of the synapse.

The tracer filled terminals of the UBC described in this study could not originate from accidentally labeled, extrinsic mossy fibers, because in the selected cell fills there was only a single parent fiber and this originated clearly from the labeled UBC.

The general organization of the glomeruli containing the UBC axon terminals is similar in principle to that of the glomeruli containing extrinsic mossy fiber rosettes; i.e. the labeled rosette-shaped ending forms the central element of the glomerulus, and it is surrounded by several postsynaptic dendritic profiles, while the glomerular periphery is formed by astrocytic processes and scattered Golgi cell boutons. Although UBC axon terminals may also form synapses with occasional large dendrites that could represent Golgi dendrites, a UBC axon–Golgi dendrite connection remains to be definitively proved.

Typically, we observed about 2–3 terminals (see also 11 in Ref. 25) with each labeled UBC axon in a short radius (50–250 μm) from the parent cell body. In a Golgi study of young rats, Berthié and Axelrad described UBC axons extending for more than 300 μm and provided with several mossy-like endings. In the present study, we have also observed a UBC whose axon entered the white matter to re-enter the granular layer on the opposite side of the folium. Although this particular axon was not measured, it must have extended for at least 500 μm. The small number of UBC axon terminals and their short distance from the parent perikaryon observed in the present study are most likely underestimated averages obtained in 200-μm-thick slices, since labeled UBCs were situated near the tissue surface, and the sectioning may have removed a portion of the axonal territory.

While direct demonstration of excitatory transmission using paired recordings proved unsuccessful, a number of lines of evidence point to the excitatory nature of the pathway. At the ultrastructural level, UBC terminal rosettes display asymmetric synapses with the target dendrites and contain numerous small clear round synaptic vesicles and scattered large dense-core vesicles. These same presynaptic features are shared by the dendro-dendritic synapses the UBC brush forms with granule cells near the afferent mossy fiber synapse. UBCs are immunonegative for GABA and glycine, and in organotypic cultures the terminals are immunopositive for glutamate. Furthermore, in this region of cerebellum granule cells are encountered which display long-latency polysynaptic discharges to white matter stimulation (Fig. 2). This type of response to white matter stimulation is consistent with the notion that the recorded granule cell was the recipient of feedforward excitation from a UBC, although direct proof is pending. Thus, activation of a mossy fiber in the white matter would produce a burst discharge in the UBC as previously observed. The granule cell targets of the UBC axons would be expected to display a burst of fast EPSCs in response to activation of the UBC, for unlike the giant mossy fiber–UBC synapse, synapses between UBCs and granule cells are of smaller diameter (Fig. 4). This volley of afferent input to the granule cell would, in turn, give rise to a summating EPSP, as observed in response to repetitive action of single mossy fiber inputs to these cells. The latency to onset of firing of granule cells postsynaptic to UBCs will be highly variable. Previous studies have shown that the fast AMPA receptor-mediated EPSC in UBCs acts primarily to charge membrane capacitance and does not itself trigger an action potential. The onset of firing in the UBC occurs during the rising phase of the slow EPSP, whose latency to peak may vary from 200 to 1000 ms. This long latency to firing is greatly diminished during repetitive activation of the extrinsic mossy fiber, as successive bursts fuse to form a plateau depolarization (see 5 of Ref. 29).

As a typical glomerulus may involve dendrites from about 50 granule cells, several hundred granule cells would be affected by the activation of a single UBC. UBCs, thus, could prime a large number of granule cells for activation by other coincident mossy fiber input. This input may either drive the massed firing of a large ensemble of granule cells, or provide an excitatory synaptic weight leading to an increased probability of those granule cells firing an action potential in response to other mossy fiber input.

The observation that UBCs make synaptic contact with one another (Fig. 6) demonstrates that the differentiated UBC dendritic brush makes two distinct forms of synapse (in any given UBC only one type was observed): a giant synapse with extrinsic mossy fibers as previously reported, and one with intrinsic mossy fibers originating from other UBCs. In the vestibulo-cerebellum of short-term organotypic slice cultures derived from mice at postnatal day 8, Nunzi and Mugnaini determined that 33% of non-degenerating axons, identified as UBC axons, form synapses with granule cells and that 66% form synapses with other UBCs. Although these counts were, respectively, 70% and 30% in our small
Fig. 6. Axon terminal (gold–avidin labeled) of a unipolar brush cell (UBC) forms synapses with another unipolar brush cell. (A) The labeled UBC axon terminal contacts the brush of a short dendrite emerging from an unlabeled UBC. The UBC nucleus contains predominantly dispersed chromatin. Portion of a granule cell nucleus (N) contains abundant heterochromatin. Boxed area approximates equivalent region in a subsequent serial section (right). (B) The labeled UBC axon terminal forms synapses (arrows) with a dendriole (d1), near its point of emergence from the main dendrite, and with other dendrioles (d) whose continuity with the same dendrites was observed in other serial sections. The UBC dendrioles are provided with characteristic non-synaptic appendages (stars). Dendriole d1 also forms a dendro-dendritic synapse (arrowhead) with a granule cell dendrite (g). Dense-core vesicles (open arrowheads) in the labeled UBC terminal and dendrioles.
data sample ($n = 14$) from 2–4-week-old rat cerebellar slices, the data indicate that UBC–UBC synapses occur frequently in the vestibulo-cerebellum. Like granule cells that are postsynaptic to a UBC (Fig. 2), a burst of action potentials in the presynaptic UBC would result in an output train in the follower UBC. However, unlike granule cells, this feedforward excitation would presumably be distributed to a large population of granule cells, acting to extend the numbers of granule cells driven by the original extrinsic mossy fiber. UBCs that are the recipients of intrinsic mossy fibers do not usually receive other synaptic inputs, as UBCs with two brushes are rare. Whether UBC–UBC contacts are
connected in a way that would support reverberating loops within the granular layer is also an intriguing notion.

These data, taken together, suggest that a single action potential in an extrinsic mossy fiber projecting to a UBC would be capable of generating a prolonged burst discharge in a population of many hundreds of granule cells. The projections of intrinsic mossy fibers originating from this UBC would in turn further amplify the recruitment of additional granule cell clusters. This provides an anatomic substrate for the powerful feedforward excitation of a large neuronal ensemble by a single synaptic input.

Cerebellar granule cells in situ are the recipients of GABAergic input from Golgi cells that takes the form of both tonic, spontaneous inhibition and recurrent inhibitory feedback via parallel fiber–Golgi cell loops. The tonic component arises both from a background activity of both tonic, spontaneous inhibition and recurrent inhibitory GABAergic input from Golgi cells that takes the form of granule cell clusters. This provides an anatomic substrate for generalized role in signal processing in humans than in rodents.

Thus, UBCs form a network of intrinsic mossy fibers (schematically illustrated in Fig. 7) that contributes a powerful form of distributed, feed forward excitation within the basic cerebellar circuit.

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