

# Ultrastructural Study of the Granule Cell Domain of the Cochlear Nucleus in Rats: Mossy Fiber Endings and Their Targets

DIANA L. WEEDMAN, TAN PONGSTAPORN, AND DAVID K. RYUGO

Center for Hearing Sciences, Departments of Otolaryngology-Head and Neck Surgery and Neuroscience, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

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## ABSTRACT

The principal projection neurons of the cochlear nucleus receive the bulk of their input from the auditory nerve. These projection neurons reside in the core of the nucleus and are surrounded by an external shell, which is called the granule cell domain. Interneurons of the cochlear granule cell domain are the target for nonprimary auditory inputs, including projections from the superior olivary complex, inferior colliculus, and auditory cortex. The granule cell domain also receives projections from the cuneate and trigeminal nuclei, which are first-order nuclei of the somatosensory system. The cellular targets of the nonprimary projections are mostly unknown due to a lack of information regarding postsynaptic profiles in the granule cell areas. In the present paper, we examined the synaptic relationships between a heterogeneous class of large synaptic terminals called mossy fibers and their targets within subdivisions of the granule cell domain known as the lamina and superficial layer. By using light and electron microscopic methods in these subdivisions, we provide evidence for three different neuron classes that receive input from the mossy fibers: granule cells, unipolar brush cells, and a previously undescribed class called chestnut cells. The distinct synaptic relations between mossy fibers and members of each neuron class further imply fundamentally separate roles for processing acoustic signals. © 1996 Wiley-Liss, Inc.

**Indexing terms:** chestnut cell, Golgi cell, granule cell, synapse, unipolar brush cell

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The cochlear nucleus is the site of the first central synapse in the auditory system. The auditory nerve enters the cochlear nucleus and bifurcates, sending branches to the dorsal cochlear nucleus (DCN) and the ventral cochlear nucleus (VCN). Surrounding these two core areas are regions of small cells, called granule cells, which do not receive terminals from the myelinated auditory nerve fibers but, instead, are the target for a variety of nonprimary inputs. The granule cell areas receive projections from neurons in higher auditory nuclei, including the inferior colliculus (Caicedo and Herbert, 1993; Saldaña, 1993) and the primary auditory cortex (Feliciano et al., 1993; Weedman et al., 1995), as well as from the olivocochlear neurons (Brown et al., 1988b). The type II auditory nerve fibers, which carry information from the outer hair cells of the cochlea, terminate among the granule cells (Brown et al., 1988a), although the myelinated type I nerve fibers do not. The granule cell domain also receives nonauditory inputs, including projections from the somatosensory cuneate nucleus (Weinberg and Rustioni, 1987), the trigeminal nuclei (Itoh et al., 1987), and the vestibular organ (Burian and Goesttner, 1988; Kevetter and Perachio, 1989). The granule cells project their axons through the superficial

layer of the DCN as parallel fibers and synapse on pyramidal and cartwheel cells of the DCN (Mugnaini et al., 1980b; Ryugo et al., 1995). Cartwheel cells project onto pyramidal cells (Berrebi and Mugnaini, 1991), whereas pyramidal cells project to the inferior colliculus (Spirou et al., 1993). The granule cells, therefore, conceivably integrate a wide spectrum of information carrying cues about attention, head position, sound localization, or sound recognition, all of which may influence the function and output of the DCN. If we are to understand the neuronal mechanisms underlying such diverse operations, it is important to understand the afferent organization of these inputs and the identity of their postsynaptic targets.

The DCN has been compared to the cerebellum based on anatomical, developmental, and immunocytochemical studies (i.e., Mugnaini et al., 1980a,b; Berrebi et al., 1990; Floris et al., 1994; Ryugo et al., 1995). The foundation of this similarity is the presence of distinct granule cell systems in each, and most studies of cell types in the cochlear granule

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Accepted January 5, 1996.

Address reprint requests to David K. Ryugo, 510 Traylor Building, 720 Rutland Avenue, Baltimore, MD 21205. E-mail: dryugo@bme.jhu.edu

cell areas have used cerebellar anatomy as their frame of reference. Both the cerebellar and cochlear granule cell domain are named for their principal cell type, the small, excitatory granule cells. However, there are several other cell types interspersed among the granule cells, including the Golgi cell and the recently described unipolar brush cell (UBC; Floris et al., 1994; Mugnaini and Floris, 1994; Mugnaini et al., 1994). The Golgi cell is hypothesized to be an inhibitory,  $\gamma$ -aminobutyric acid (GABA)ergic interneuron (Mugnaini et al., 1980a; Mugnaini, 1985), whereas the UBC is thought to be an excitatory, glutamatergic interneuron (Rossi et al., 1995). In the cochlear nucleus, their function and projection patterns are unknown, and even their anatomical characteristics are not yet fully described. Because projections to the granule cell domain tend to synapse on anonymous dendritic profiles, any of the above cells could be candidate targets.

In the present study, we sought to establish ultrastructural criteria that could differentiate between the dendrites of these separate cell types, thereby enabling the determination of the recipients of the various inputs. We focused on one distinct class of synaptic endings in the granule cell domain, the mossy fiber endings. Mossy fibers are large, vesicle-filled terminals that are surrounded by postsynaptic dendrites and are distributed in granule cell areas of both the cerebellum (Mugnaini, 1972; Palay and Chan-Palay, 1974) and the cochlear nucleus (McDonald and Rasmussen, 1971; Mugnaini et al., 1980a; Osen et al., 1984). We have previously shown that projections from auditory cortex to the granule cell domain synapse among the granule cells, often on dendrites, which, in turn, are synapsed upon by mossy fibers (Weedman et al., 1995). It was also demonstrated that projections from the cuneate nucleus terminate as large mossy fibers or small boutons in the granule cell areas. Although cuneate boutons were observed to synapse on the cell bodies of granule cells, it was not possible to identify unambiguously the targets of the cuneate mossy fibers (Wright and Ryugo, 1996). The origin of most mossy fibers in the cochlear nucleus is unknown, but, by analogy to the cerebellum, these terminals are hypothesized to arise from multiple sources and exert a significant influence over their targets (for review, see Ito, 1984). In this study, we describe mossy fiber targets in the cochlear nucleus granule cell regions that include three different cell types: the granule cell, the UBC, and the previously undescribed chestnut cell.

## MATERIALS AND METHODS

### Animals and surgical preparation

Ten male albino rats were used in this study. Rats were anesthetized with sodium pentobarbital (45 mg/kg body weight), and the skin and muscle overlying the head and neck were reflected. The skull over the cerebellum was drilled away, and the dura was reflected. Part of the cerebellum was aspirated to expose the cochlear nucleus. A 10% solution of biotinylated dextran amine (BDA; 10,000 m.w.; Molecular Probes; in five rats) or a 5% solution of biocytin (Sigma Chemicals; in four rats) was injected iontophoretically into the superficial dorsal cochlear nucleus by direct visual control by using an operating microscope. Injections were made through a glass micropipette (15  $\mu$ m, o.d.) which was lowered 0.25 mm below the pial surface. Then, 5  $\mu$ A of positive current in pulses of 7 seconds (50%

duty cycle) was applied. After a 5-minute injection, the animal was sutured and allowed to recover.

### Tissue processing

Twenty-four to forty-eight hours after the DCN injection, each rat ( $n = 9$ ) was administered a lethal dose of sodium pentobarbital and perfused transcardially with 0.1 M phosphate buffer containing 0.1% sodium nitrite and 0.9% sodium chloride followed by 4% paraformaldehyde in phosphate buffer. One normal rat was perfused for optimal ultrastructure with 0.1 M cacodylate buffer containing 0.1% sodium nitrite and 0.9% sodium chloride, followed by 0.5% paraformaldehyde, 1% glutaraldehyde, and 0.015% calcium chloride in cacodylate, followed by a final wash with 0.5% paraformaldehyde, 3% glutaraldehyde, and 0.015% calcium chloride in cacodylate. After perfusion, each brain was dissected from the skull and postfixed in the same fixative for several hours. Tissue was cut on a Vibratome in the coronal plane, and 50- $\mu$ m-thick sections were serially collected. The normal brain was processed immediately by using the electron microscope protocol described below. The BDA or biocytin tissue sections were rinsed in 0.1 M phosphate buffer, pH 7.3, and incubated overnight with ABC (Vector). The following day, they were reacted with diaminobenzidine (DAB) to visualize the label as follows: Biocytin tissue was incubated in a solution of 0.05% DAB, 0.025% cobalt chloride, 0.02% nickel-ammonium sulfate, and 0.01% hydrogen peroxide in phosphate buffer for 1 hour. BDA tissue was preincubated with a solution of 0.0125% DAB, 0.25% nickel-ammonium sulfate, and 0.35% imidazole in 0.1 M cacodylate buffer for 10 minutes; hydrogen peroxide was added to yield a final concentration of 0.003%, and the activated solution was incubated with the sections for 5–15 minutes more. Both BDA and biocytin yielded equivalent staining, producing a Golgi-like fill of the retrogradely labeled neurons.

### Electron microscopic tissue processing

The BDA and biocytin sections were rinsed of DAB and then processed identically to those of the normal rat. A brief protocol for electron microscopic processing is as follows: the sections were incubated with 1% osmium tetroxide for 15 minutes, rinsed, stained with 1% uranyl acetate overnight, dehydrated through graded alcohols and propylene oxide, infiltrated with Epon embedding medium, and sandwiched between two pieces of Aclar (Ted Pella Inc.) for light microscopy. Sections of interest were drawn with a camera lucida, and appropriate structures and landmarks were mapped onto the drawings. Selected granule cell areas were cut out of the Aclar and embedded in a BEEM capsule for ultramicrotome sectioning. Silver sections, approximately 75 nm thick, were collected on Formvar-coated grids, stained with uranyl acetate and lead citrate, and photographed with an electron microscope. All photographic negatives were digitized (Leafscan 45), the contrast and/or exposure were adjusted if necessary (consistent with standard darkroom techniques; Adobe Photoshop), and they were printed in high resolution format (Fuji Pictography 3000).

## RESULTS

The granule cell domain of the cochlear nucleus encompasses up to seven different subdivisions (Mugnaini et al., 1980b). These subdivisions are generally found around the

perimeter of the core VCN and DCN, including the superficial layer of the VCN, the lamina dividing DCN from VCN, the subpeduncular dorsal corner of the VCN, the dorsal strial corner of the DCN, and layer II of the DCN. To what extent the populations of granule cells are functionally distinct, aside from their location, remains to be determined, although it has been reported that some projections, such as olivocochlear and cortical efferents, innervate the granule cell regions of the VCN but not the DCN (Brown et al., 1988b; Weedman et al., 1995). We concentrated on studying the superficial layer of granule cells overlying the VCN and the lamina of granule cells separating the DCN from the VCN, because these regions are contiguous and appear to receive similar inputs. Another advantage of studying the VCN granule cell subdivisions is that cell types surrounding the VCN are not mixed with the cells of the core nucleus, such as the cartwheel, stellate, and pyramidal neurons of the DCN.

### Granule cells

The lamina and superficial layer are dominated by granule cells, with a few other cell types scattered throughout. The granule cell somata are roughly 6–10  $\mu\text{m}$  in diameter, with scant, pale-staining cytoplasm, few synapses, and a centrally placed nucleus. The nuclear envelope exhibits some infolding and a thin but distinct layer of chromatin lining its inner perimeter. Several prominent patches of chromatin adhere to this lining, and other clumps are dispersed within the nucleus. Although granule cells appear to resemble one another structurally, the variations in afferent input to the separate subdivisions leave open the possibility that there are also subpopulations of granule cells that have yet to be discerned. This region is also marked by the presence of large (5–15  $\mu\text{m}$ ), irregularly shaped terminals that are filled with round synaptic vesicles and mitochondria. Each terminal is characteristically surrounded by dendritic profiles upon which it forms asymmetric synaptic contacts. By virtue of its structure, synaptic relationships, and resemblance to the mossy fiber terminals of the cerebellar cortex, this terminal in the cochlear nucleus is also called a mossy fiber (McDonald and Rasmussen, 1971; Mugnaini et al., 1980a). Undoubtedly, multiple types of mossy fibers exist that are classifiable on the basis of origin, transmitter type, and/or postsynaptic target.

The most common type of mossy fiber profile in these granule cell areas consists of a large central mossy fiber terminal surrounded by small, round dendritic profiles (Fig. 1A). The dendritic profiles are occasionally seen in longitudinal section, revealing that they are actually elongated claw-like structures (Fig. 1B). The dendrites are smooth and without spines, usually have round-to-oblong shapes when viewed in transverse section, and abound with mitochondria and microtubules. Fine (<0.1  $\mu\text{m}$  diameter), nonsynaptic hairs arise from the dendrites and penetrate the mossy fiber (Fig. 1C). These mossy fiber glomeruli are similar to those described in the cerebellum (Szentágothai, 1970), and the dendritic profiles are hypothesized to belong to granule cells. To confirm the origin of these dendritic profiles, however, neurons throughout the granule cell domain were retrogradely labeled with biocytin or BDA following injections into the DCN. Individual cells with their dendrites, which were labeled in Golgi-like fashion, were then examined by light and electron microscopy.

Granule cells were the most frequent retrogradely labeled cells of the lamina and superficial layer and have a

characteristic appearance when viewed with light microscopy in 50- $\mu\text{m}$ -thick tissue sections (Fig. 2). Usually, two primary dendrites (range from one to four dendrites) radiate from the cell body. There are few branches to these dendrites, and their tips expand into small claw-like terminations that resemble those of cerebellar granule cells. In the center of each claw, there is a hollow (Fig. 2). In a number of cases, these claws were first identified by light microscopy (Fig. 3A,C, insets) and then examined with electron microscopy. It was readily apparent that each hollow was filled with a single mossy fiber, although the mossy fiber was not always completely contained within the claw (Fig. 3). There are multiple synaptic contacts between the mossy fiber and granule cell dendrite, but we did not observe synaptic specializations on the invaginating, hair-like dendritic processes (Fig. 3A–C). The labeled granule cell dendrites have smooth, regular surfaces; the labeled processes are virtually identical to the many unlabeled processes in contact with mossy fibers. Few synapses are observed on the proximal shafts of granule cell dendrites.

### UBCs

A second cell type that is less commonly labeled after an injection into the superficial layers of the DCN is the UBC. The labeled UBC, whose cell body is similar in size to that of a granule cell, is nevertheless unambiguously distinguishable in the light microscope by virtue of its single thick (3–6  $\mu\text{m}$  diameter) dendrite, which erupts after a short distance into a spray of fine (<1  $\mu\text{m}$  diameter) processes (Fig. 4A). This spray resembles a spiral whorl and seems to enfold a central empty space (Fig. 4A, arrow). When examined with the electron microscope, a single mossy fiber occupies the center of the whorl. The mossy fiber is almost completely enclosed by the labeled dendrite and its tangle of finger-like extensions (Fig. 4C). Some smaller protrusions are evident within the mossy fiber itself. An unusual feature of the UBC, which is best appreciated when studying a labeled cell, is the way in which the dendritic processes coalesce to form a mosaic that completely surrounds most of the mossy fiber (Fig. 4D). The components of this mosaic are stout and easily distinguishable from the thinner dendritic profiles of granule cells. It is also apparent that more than one UBC dendrite can be postsynaptic to a single mossy fiber, as illustrated by the unlabeled dendrite that participates in the UBC glomerular mosaic (Fig. 4E, labeled "d"). The long, undulating synapse as well as the fine dendritic hairs identify this unlabeled glomerular component as belonging to a neighboring UBC. The UBC soma and dendritic shaft are typically void of synaptic contacts.

### Golgi cells

A third cell type present in the granule cell domain is the Golgi cell (Fig. 5). This cell type has been previously described as a medium-sized, ribosome-rich cell with an irregular somatic surface, and it is named for its similarity to the Golgi cells of the cerebellum (Mugnaini et al., 1980a). In the rat cochlear nucleus, we confirmed and extended the original brief descriptions. Golgi cell somata are distinctly larger than those of granule cells, generally lacking synaptic contacts, and their cytoplasm is dense with Golgi apparatus. The cytoplasm of the Golgi cell also stains more darkly than the nucleus due to the high density of polysomes that

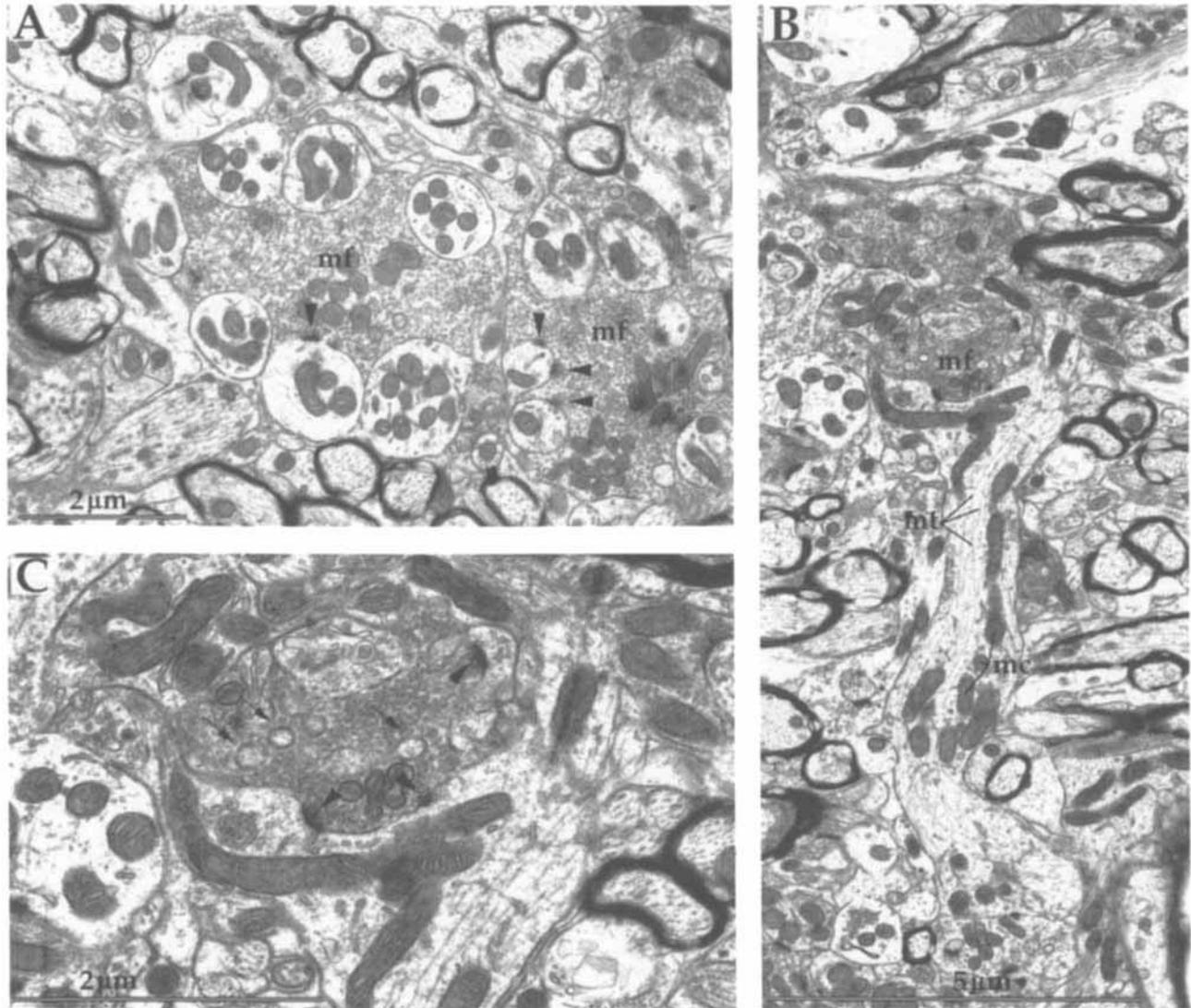


Fig. 1. Typical mossy fibers in the granule cell domain of the cochlear nucleus. **A:** Two small mossy fibers (mf) surrounded by round dendritic profiles. Synapses are indicated by arrowheads. **B:** Longitudinal section through a dendrite showing that the mossy fiber is actually

cupped in the dendrite. Note the abundant microtubules (mt) and mitochondria (mc) in the dendrite. **C:** Higher magnification of the synaptic glomerulus shown in B. Synapses are indicated by arrowheads. Note the fine projections embedded within the mossy fiber (arrows).

mostly appear as rosettes. The nucleus is irregular in shape, often highly invaginated, and is relatively free from the large clumps of condensed chromatin that are the hallmark of granule cells. No labeled Golgi cells were found following tracer injections into the DCN, and we did not observe mossy fiber interactions with this cell. We were unable to reconstruct Golgi cell dendrites and axons from their cell body; therefore, such descriptions remain to be made.

### Chestnut cells

We discovered a fourth cell in the granule cell domain whose characteristics do not fit into any previously described cell groups. This cell type was occasionally labeled by DCN injections, but it was studied most extensively in unlabeled tissue. The most striking light microscopic feature of this labeled cell is its unusually irregular somatic perimeter. One or two short, stubby dendrites emerge

directly from one side of the small ( $\sim 10 \mu\text{m}$ ) soma, with no intervening dendritic stalk like that seen in the UBC. Finger-like projections emanate both from the dendritic tuft and from the soma (Fig. 6).

Examination with the electron microscope revealed that the cell body and dendrite are rich in Golgi apparatus, rough endoplasmic reticulum, and free ribosomes. Unlike the Golgi cell, the large pale nucleus is centrally located, with no invaginations, and no condensed chromatin is visible. The irregular surface of the cell body becomes patently obvious when viewed with an electron microscope. The somatic perimeter displays scalloped edges, blunt protrusions, filiform appendages, and irregular blebs (Figs. 7–9). Synapses, which are marked by asymmetric postsynaptic densities, characteristically and reliably occur at each convexity on the soma (Figs. 7–9). Due to the extraordinary appearance of the irregular cell body, which resembles a

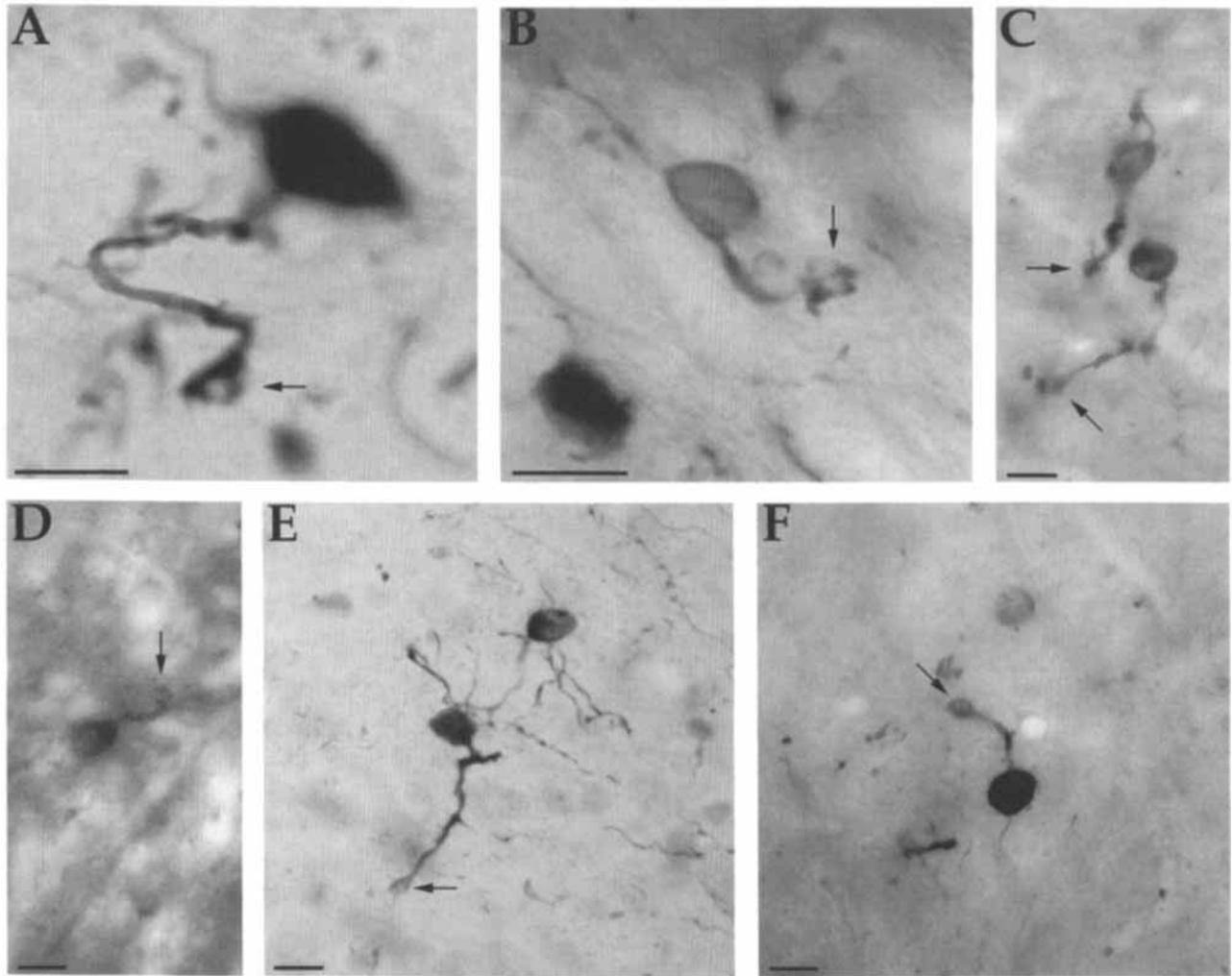


Fig. 2. Light micrographs of labeled granule cells (A-F). Note the distal dendritic claws (arrows) on each granule cell. Scale bars = 10  $\mu$ m.

chestnut still in its husk, we refer to this cell as the chestnut cell.

In contrast to the Golgi cell or the UBC, the chestnut cell body is invariably surrounded by terminals that range widely in size. These endings are uniformly dark in appearance due to their high density of round synaptic vesicles. By virtue of this similarity, the profiles are presumed to be part of neighboring mossy fibers (Figs. 7-9, asterisks). The thick irregular dendrite is surrounded by distinctly larger mossy fibers. The chestnut cell dendrite erupts from the soma without stalk or neck, resembling a turbulent wave (Figs. 8, 9). The dendrite, in fact, may be more an extension of the soma, given its high density of ribosomes, endoplasmic reticulum, and Golgi apparatus. The perimeter of the dendrite is even more irregular than that of the cell body, and features intricate hooks, claws, and protrusions that seem to entrap the mossy fibers (Fig. 9C,D). Although the apical dendritic brush somewhat resembles that of the UBC, the chestnut cell is unique in that every protuberance is prominently synaptic.

## DISCUSSION

### Cell types in the granule cell domain

There are at least four separate classes of neurons in the granule cell domain of the cochlear nucleus, three of which have been previously described—granule cells, Golgi cells, and UBCs—and a fourth type, which we describe in this paper, the chestnut cell. Although our study concentrated on the lamina and superficial layer of granule cells surrounding the ventral cochlear nucleus, it is our impression that these cells are distributed throughout the granule cell domain. The cell classes defined on the basis of cytologic criteria are reinforced by distinct differences in the types of synaptic relationships each class expressed with mossy fibers. The granule cells, UBCs, and chestnut cells all receive mossy fiber input and also project to the DCN, as evidenced by the retrograde labeling of these cells. Therefore, all of these cell types are situated to play a role in the integration of nonprimary input and in the processing of auditory information through the DCN. The Golgi cell was not observed to receive mossy fiber synapses or to project its

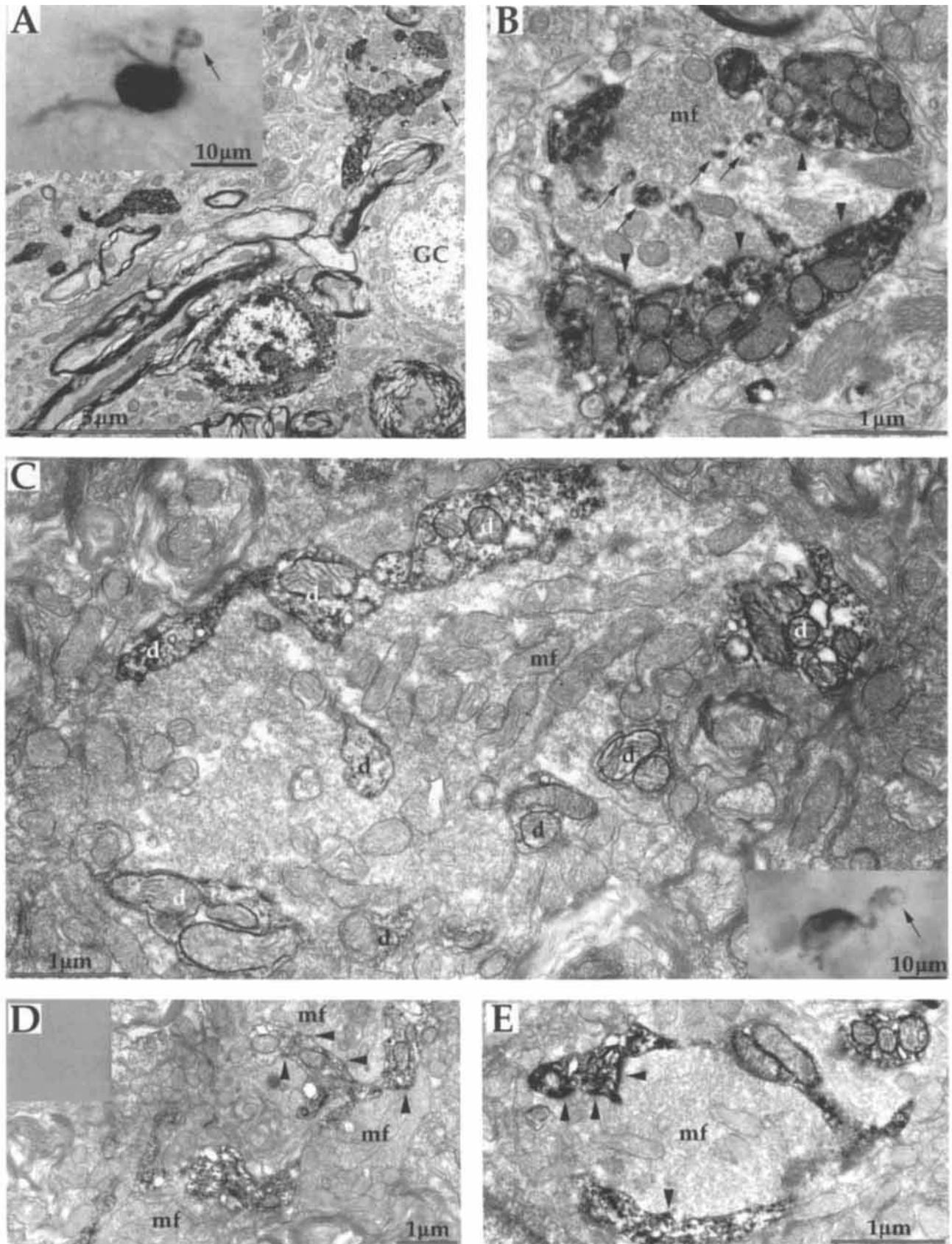


Figure 3

axon to the DCN, but it may be involved in local interactions with the first three cell types.

### Morphological distinctions between mossy fiber targets

Our data, in combination with other ultrastructural studies of granule cells and UBCs (Floris et al., 1994; Mugnaini and Floris, 1994; Mugnaini et al., 1994; Wright et al., 1996), indicate that there are major differences between the granule cell-mossy fiber glomerulus and the UBC-mossy fiber glomerulus (Fig. 10). The UBC dendrite is equal or larger in diameter than the mossy fiber with which it synapses, forms an irregular mosaic around the mossy fiber, and makes synapses that are long ( $> 1.0 \mu\text{m}$ ) and typically undulating or scalloped. The granule cell glomerulus is characterized by dendrites that are considerably smaller in diameter than the mossy fiber and synapses that are short ( $< 0.5 \mu\text{m}$ ) and widely spaced. Although both cell types send hair-like, nonsynaptic projections into the mossy fiber, granule cell dendrites have smooth, regular surfaces and rounded convex shapes, whereas the UBC dendrites are highly irregular and send projections out from every surface of the dendrite.

The mossy fiber-granule cell glomerulus of the lamina and superficial layer described in this paper is structurally different from the mossy fiber-granule cell glomerulus of the DCN. In the superficial regions of the DCN and along the DCN side of the lamina, mossy fibers arising from the cuneate nucleus were shown to form synaptic contacts not only on the dendrites of hypothesized granule cells but also on the dendritic hairs that penetrate the mossy fibers (Wright and Ryugo, 1996). The hair-like projections of these granule cell dendrites of the DCN are also thicker in caliber than the nonsynaptic dendritic hairs of granule cells distributed more towards the VCN. Because cuneate mossy fibers are distributed throughout the granule cell domain, these observations raise the possibility that there are indeed different subpopulations of granule cells that may be defined on the basis of differences in topologic location, synaptic organization of glomeruli, and source of the mossy fiber terminal. The granule cell domain is clearly a complicated region, and much more work is needed to unravel the mysteries of its neuronal composition and synaptic organization.

### Chestnut cells

The synapses between mossy fibers and chestnut cells are most easily recognized by the large area of the postsynaptic dendrite and the extensive Golgi apparatus and ribosomes present in the dendrite (Fig. 10). The chestnut cell synapses

are also unique, in that all occur on the convexities of somatic evaginations or dendritic protrusions. Although the chestnut cell has some similarities to the UBC, it is distinct from the UBC in several ways. The wave-like dendrite of the chestnut cell is not separated from the soma by a proximal stalk, as in the UBC. The chestnut soma is surrounded with large terminals that synapse on each convexity of the membrane, whereas the UBC rarely receives somatic synapses. No ringlet bodies, a type of nematosome unique to the UBC (Mugnaini et al., 1994), have been observed in the chestnut cell. Because ringlet bodies can be observed in UBCs even in the company of intracellular labeling, it is expected that they would be seen in chestnut cells if they are present in the section. There is still the possibility that ringlet bodies were missed by us because of incomplete sampling at the electron microscopic level, but we would argue strongly against it. The blebs and wave-like projections of the chestnut cell, unlike those of the UBC, are invariably synaptic (Fig. 10). In cross section, the dendritic protrusions of chestnut cells can appear similar to dendrites of granule cells, at least in terms of size and shape. They differ, however, in that the granule cell dendritic profiles are filled with mitochondria and exhibit short, flat, and infrequent postsynaptic densities (PSDs), whereas the profiles of chestnut cell processes are generally free of mitochondria but exhibit large, convex, and frequent PSDs.

All evidence leads us to conclude that the chestnut cell is a new and unique cell type. Why this cell type was not previously recognized may lie in its appearance when stained by BDA, biocytin, or Golgi methods. In essence, our initial interpretation of these stained cells in the light microscope was that they were artifacts. They are small and amorphous and are easily mistaken for truncated cells or background label. Once we became aware of their regular presence in our tissue, we could reliably locate them with an electron microscope. Immunocytochemical studies should further attempt to characterize the chestnut cell, because, at this time, it is not known whether the cell is excitatory or inhibitory. Due to the observation that the chestnut cell is labeled after BDA or biocytin injections into the DCN, it can be categorized as a local circuit neuron, similar to the granule cell.

There is a previous reference in the literature to a chestnut-like synapse in the cerebellum. The Golgi II neuron of the cerebellum receives crenated axosomatic synapses from mossy fibers, which Chan-Palay and Palay (1971) named synapses *en marron*, for their resemblance to the wrinkled surface of a Spanish chestnut ("marron" is French for chestnut). However, there are several significant differences between the cerebellar Golgi II synapses *en marron* and the mossy fiber synapses on the chestnut cell. First, the Golgi II cell has a deeply invaginated nucleus and does not receive a high proportion of axosomatic mossy fibers. The chestnut cell has a pale round nucleus, and the entire soma is surrounded by synaptic mossy fibers. Second, the mossy fiber of the cerebellum intrudes into the cytoplasm of the Golgi II cell at the *en marron* synapse, sometimes quite deeply, whereas outpockets of the chestnut cell commonly penetrate the body of the mossy fiber. The third and most striking difference is that, in the *en marron* synapse, the actual pre- and postsynaptic densities occur wherever the mossy fiber bulges or invaginates into the Golgi II soma, that is, in the valleys of the somatic undulations. In contrast, the synaptic specializations of the

Fig. 3. Electron micrographs of labeled granule cell claws. **A, inset:** Light micrograph of a labeled granule cell. An electron micrograph of the same cell shows the distinctive distal claw (arrow). The cell body has been cut in a grazing section, causing it to appear smaller than in the inset. An unlabeled granule cell is visible to the right (GC). **B:** Higher magnification of the claw shown in A. The entire claw appears to be occupied by a single mossy fiber (mf). Synapses are indicated by arrowheads. Nonsynaptic, labeled hairs are indicated by arrows. **C, inset:** Light micrograph of another labeled granule cell. An electron micrograph of the claw shows that the entire dendritic structure (labeled "d") encloses one mossy fiber. **D:** Later section of the claw shown in C. At this level, the mossy fiber surrounds the remains of the labeled dendrite. **E:** Micrograph of a third labeled granule cell claw, which encloses a mossy fiber.

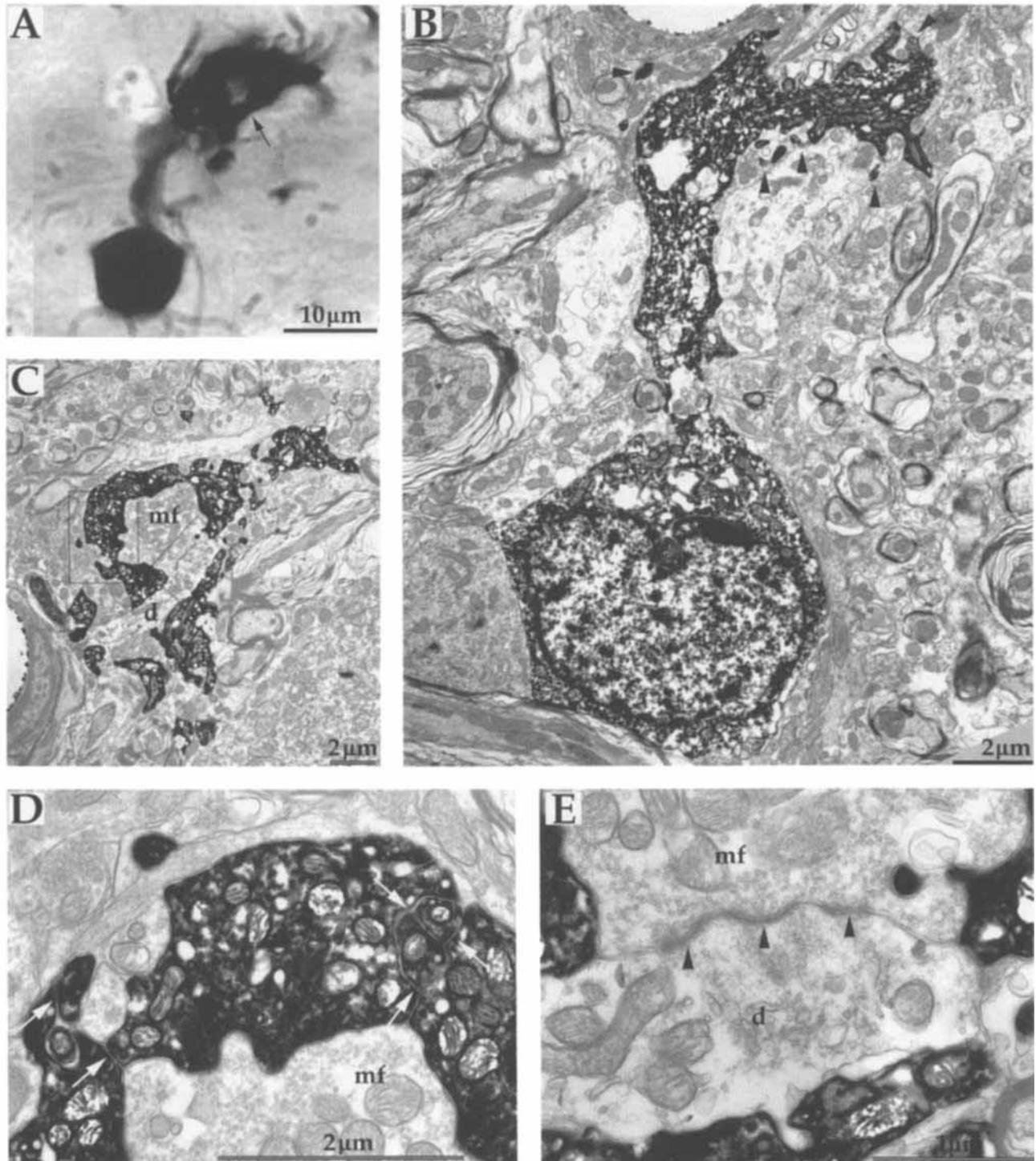


Fig. 4. Micrographs of a labeled unipolar brush cell (UBC). **A:** Light micrograph of a labeled UBC. The dendritic whorl is visible, and there appears to be a hollow (arrow) at its center. **B:** Micrograph of the same cell showing the spiny eruptions off the dendrite (arrowheads). This particular UBC has an unusually smooth somatic surface. **C:** Micrograph through the center of the whorl shown in A. A mossy fiber (mf) is in the center of the whorl. Extensions of the labeled dendrites project in all directions. An unlabeled dendrite (d) also participates in the

glomerulus. The rectangles on the left and right indicate the views shown in D and E, respectively. **D:** Higher magnification of the labeled dendrite shown in C. The mosaic effect of the interdigitating dendritic elements is evident (white arrows). **E:** Higher magnification of the unlabeled dendrite shown in C. The long, undulating synapse (arrowheads) and the hair at the bottom of the photograph identify this dendrite as belonging to another UBC.

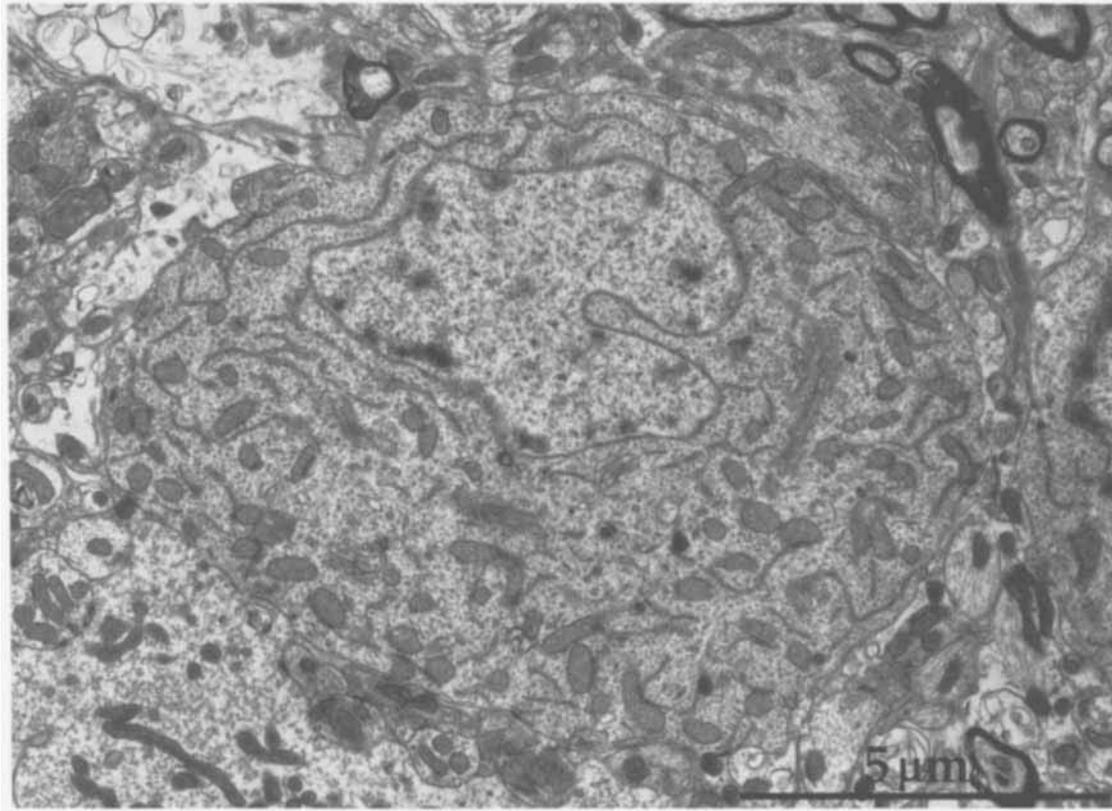


Fig. 5. Electron micrograph of a Golgi cell. This section shows the infolded nuclear envelope and dark cytoplasm speckled with ribosome rosettes. The perimeter of the cell is irregular, but no somatic synapses are visible.

chestnut cell are always found on the tips of the somatic or dendritic protuberances, sometimes deep in the mossy fiber. For these reasons, we are confident that, despite the superficially similar appearance of the chestnut-like synapses, the chestnut cell of the cochlear nucleus is unique from the Golgi II cell of the cerebellum.

#### Implications of varying synaptic structure

The mossy fiber contact on the distal dendritic claw appears to be the major source of input to the granule cells. Few synaptic contacts are observed on the cell body or proximal arms of the dendrites. Because there are so many active zones in a single granule cell claw, the synapse is likely to be very secure; an impulse in the mossy terminal would likely produce a response in the dendrite. The mossy fiber inputs from each dendrite would then be summated in the cell soma, an arrangement that may allow the granule cell to detect coincident inputs from limited but separate sources.

The UBC also makes a very secure synapse with a mossy fiber at its distal dendrite, sometimes even completely enveloping the mossy fiber. The UBC dendrite is short and relatively thick, suggesting that a single mossy fiber input could actually drive the UBC by itself. Patch-clamp recordings from cerebellar UBCs revealed that a single mossy fiber input resulted in long-lasting depolarizing potentials that produced a prolonged train of action potentials (Rossi et al., 1995). This long-lasting output implies that the UBC can serve as a kind of powerful signal amplifier whose

signal-processing role is strikingly different from that of the granule cell.

The chestnut cell receives virtually all of its input on or near its cell body. That is, there is major convergence of multiple mossy fibers onto the cell. This synaptic arrangement is obviously distinct from that of granule cells and UBCs, where a few mossy fibers contact individual granule cells, and a single mossy fiber contacts the UBC. The significance of such a large complement of mossy fiber input directly to the soma is not yet clear, but the chestnut cell could serve as a kind of "sumimator" of multiple mossy fiber inputs.

In summary, the granule cell domain contains three distinct cell types whose separate mossy fiber glomeruli endow the system with the structural substrate to subserve distinct physiological properties. A remarkable range of functions may be proposed for these interneurons: The UBC might serve as a "signal amplifier," the granule cell as a "coincidence detector," and the chestnut cell as a "sumimator." Understanding how these different features contribute to signal processing in the cochlear nucleus remains to be determined.

#### Golgi cells of the cochlear nucleus

The identity of the Golgi cell of the cochlear nucleus has been a confusing issue. The Golgi cell of the cerebellum is stellate-like in appearance, with a smooth soma and several radiating dendrites. Its apical dendrites receive input from

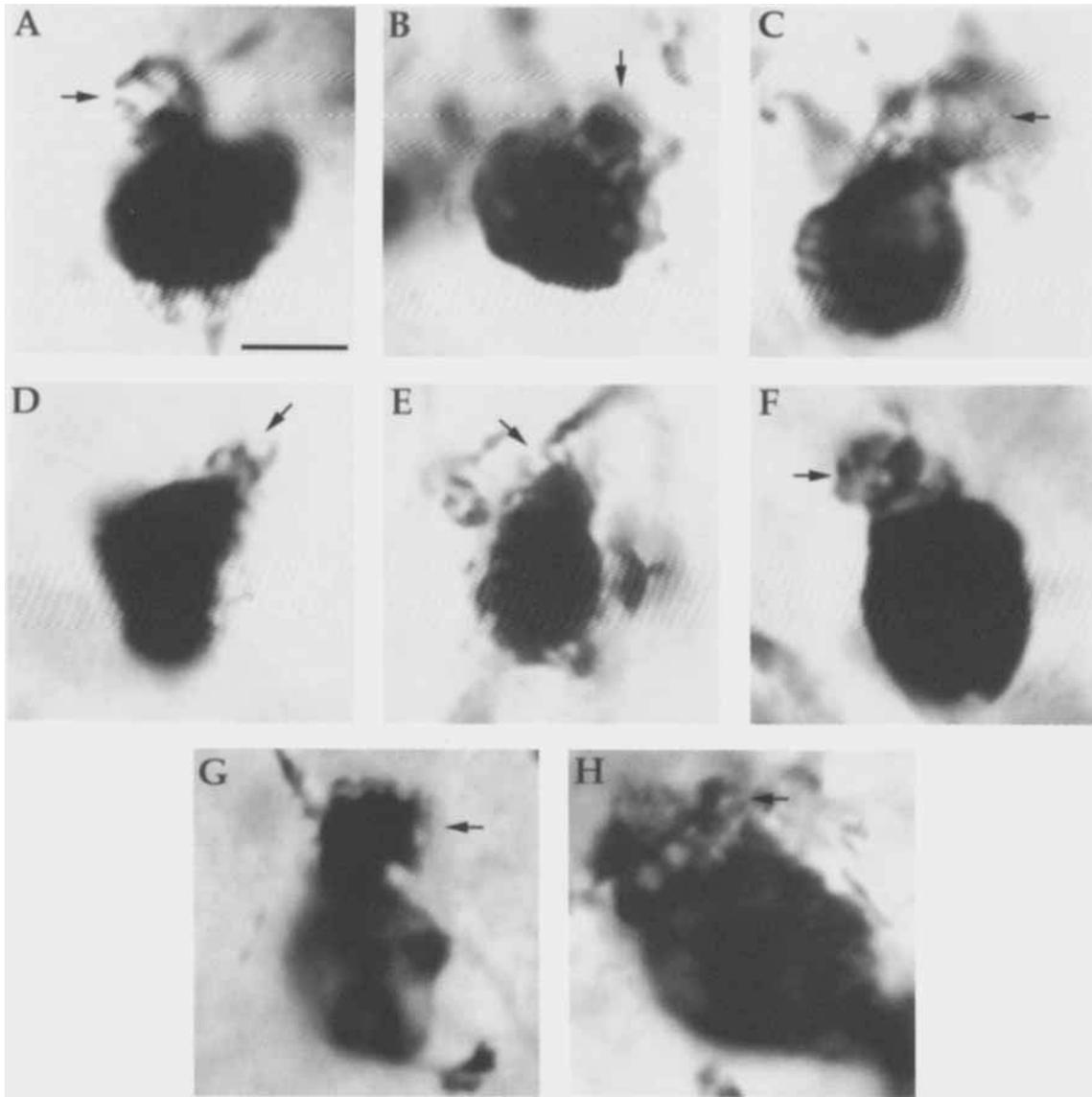


Fig. 6. **A–H:** Light micrographs of chestnut cells ( $\times 63$  oil-immersion lens, NA 1.4). These cells were labeled after injections of biotinylated dextran amine (BDA) or biocytin into the dorsal cochlear nucleus (DCN). Arrows indicate the dendritic tuft. Scale bar =  $5 \mu\text{m}$  in A–H.

parallel fibers, whereas its basal dendrites give rise to small protruding processes that participate in mossy fiber glomeruli with granule cell dendrites (Hámori and Szentágothai, 1966; Palay and Chan-Palay, 1974). It also sends a highly branched axon to the perimeters of mossy fiber glomeruli. The terminals of the Golgi cell axon are filled with pleomorphic synaptic vesicles, indicating an inhibitory function (Szentágothai, 1970). In the cochlear nucleus literature, only a single light microscopic description of a Golgi cell exists (see Fig. 6D of Mugnaini et al., 1980a). This Golgi-stained preparation was from a 1-week-old kitten, and its axon was not revealed. It is not known how maturation might affect the structure of this cell type. At the ultrastructural level, the cochlear nucleus Golgi cell is characterized by being larger than granule cells and by

having a highly invaginated nucleus and a high density of cytosolic ribosomes, among other characteristics (Mugnaini et al., 1980a). This cell type was tentatively identified as immunoreactive to antibodies directed against glutamic acid decarboxylase (Mugnaini, 1985). If this immunocytochemical interpretation is true, then the Golgi cell may use GABA to exert inhibitory influences and should exhibit synaptic endings with pleomorphic or flattened synaptic vesicles and symmetric postsynaptic densities. At least the cell body and nucleus illustrated in Figure 5 corresponds to descriptions for Golgi cells of the cochlear nucleus, although much more remains to be learned. Due to this scarcity of ultrastructural studies in the granule cell areas, other small interneurons may have been mistakenly named Golgi cells. What is critically needed is a combined light and

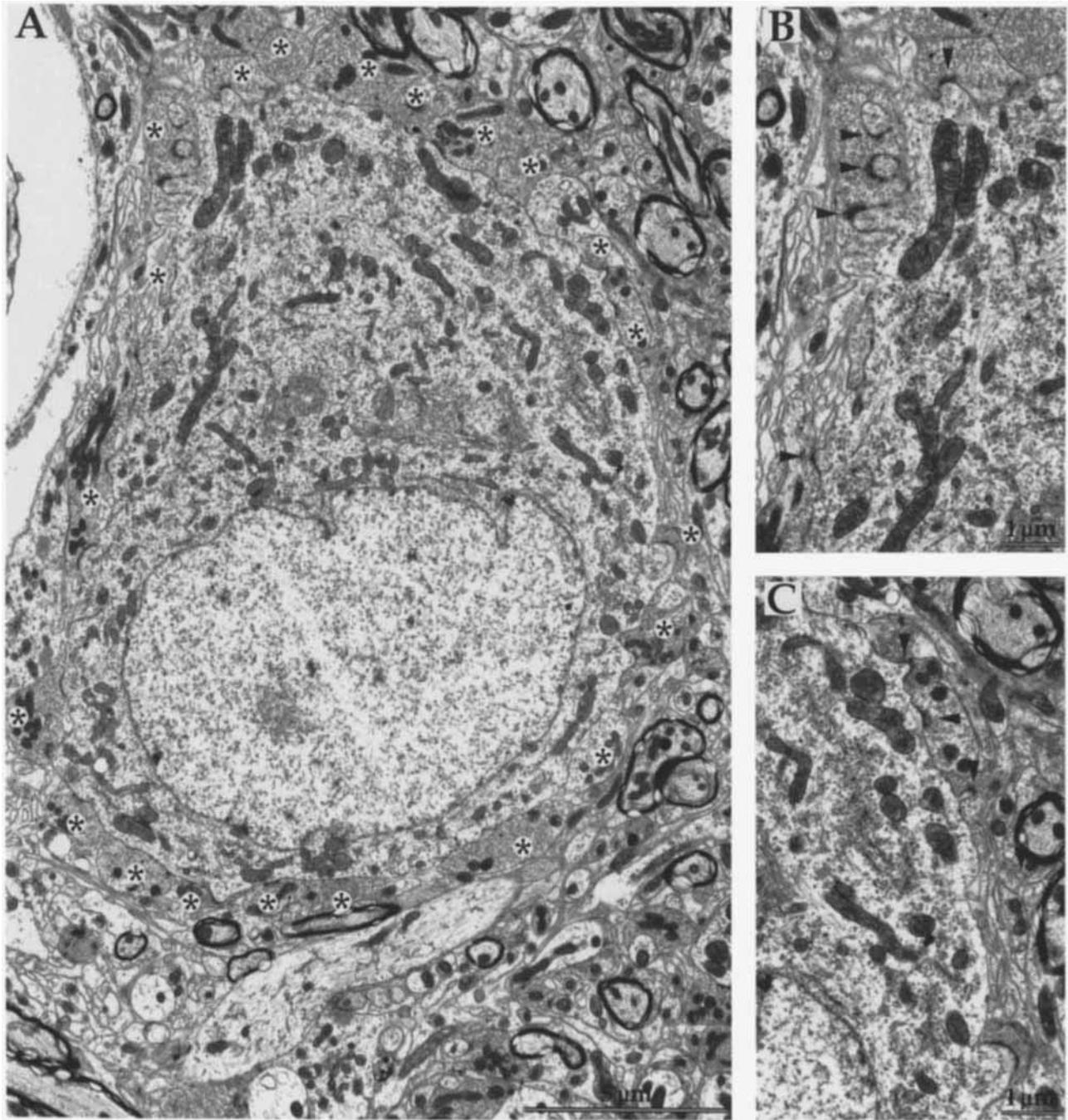


Fig. 7. Electron micrographs of a chestnut cell. **A:** Typical chestnut cell in the granule cell lamina. Note the large pale nucleus and the prominent ribosomes, mitochondria, neurofilaments, and Golgi apparatus. The cell perimeter is markedly irregular: synaptic nubs, blebs, and

protuberances emerge from every side. The surrounding mossy fiber terminals are marked by asterisks. **B,C:** Higher magnifications of the cell shown in A. Note the finger-like projections marked by arrowheads. Each projection forms a synapse at its apex.

electron microscopic examination of stained Golgi cells, complete with their axons and dendrites.

The UBC, which is found in both cerebellum and cochlear nucleus, was recently defined as unique from the Golgi cell (Mugnaini et al., 1994), clarifying the discrepancy between earlier light micrographs of Golgi cell somata that appeared smooth in Golgi-stained material and electron micrographs

that showed spiny irregular somata and dendrites (Mugnaini et al., 1980a). The UBC has an extremely irregular, hairy dendrite, and its mossy fiber glomeruli of the cochlear nucleus most likely correspond to what were once thought to be the Golgi cell glomeruli (Mugnaini et al., 1980a). It is unclear, as a result, whether the Golgi cell dendrites interact with mossy fibers at all. However, it is possible that

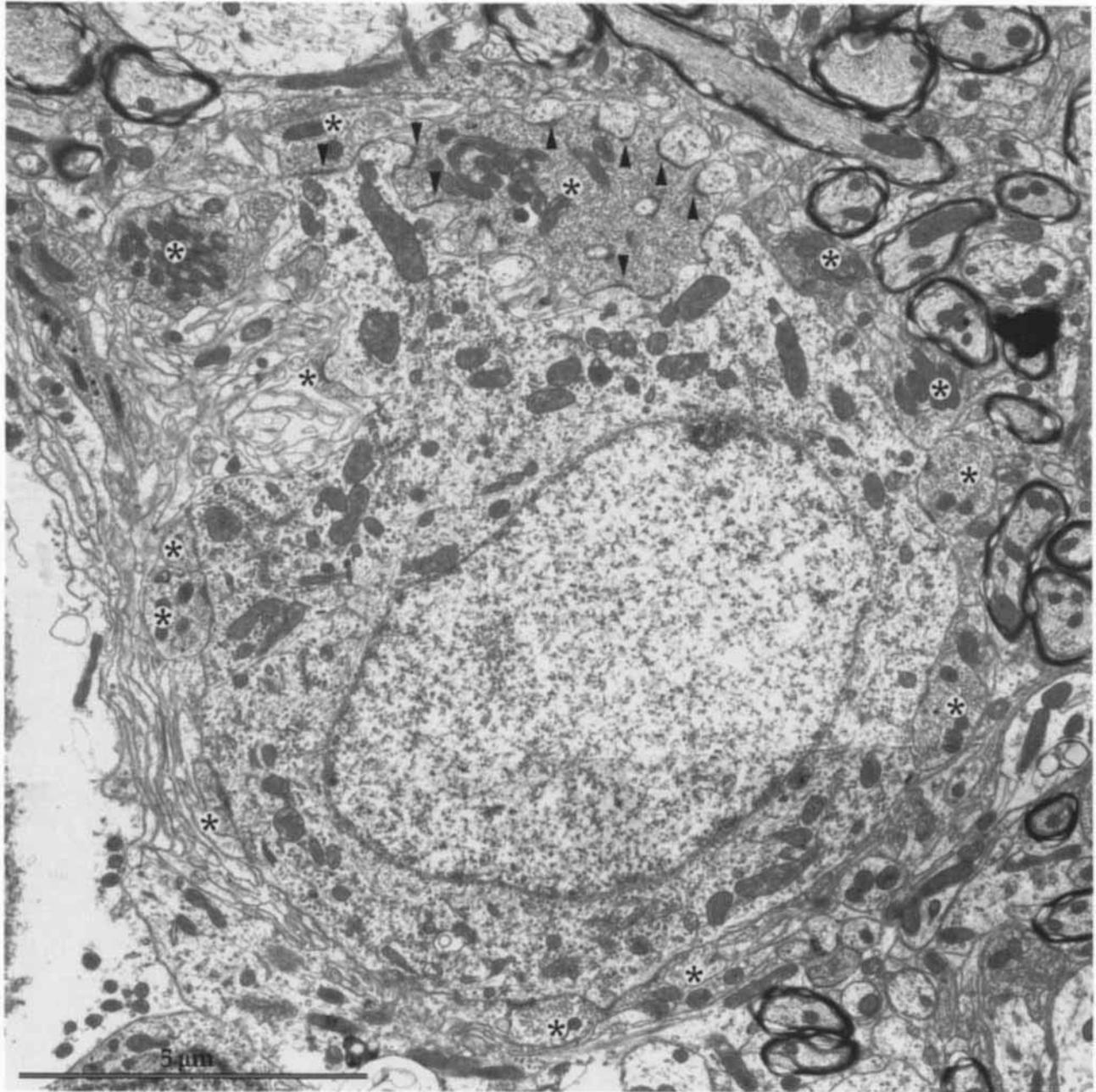


Fig. 8. Electron micrograph of a chestnut cell. The dendrite is especially irregular: It can be seen to wrap fingers around a mossy fiber like a fist. Mossy fibers are indicated by asterisks. Arrowheads indicate synapses.

their axons still participate in glomeruli of the cochlear nucleus, synapsing on the outer edges of granule cell dendrites, as in the cerebellum (Palay and Chan-Palay, 1974). The Golgi cell of the cochlear granule cell domain was not observed to be labeled following injections of retrograde markers in the DCN, indicating that its local circuit axon does not project to the DCN.

The functional role of the small digitiform projections from the dendrites of the UBCs, granule cells, and chestnut cells is not known. In the lamina and superficial layer of

granule cells, the thin, filiform appendages of UBCs and granule cells do not make synaptic contact with the mossy fibers. In contrast, the thicker protuberances of the chestnut cells are invariably synaptic, as are some of the dendritic hairs of DCN granule cells. These variations probably indicate a fundamentally different function for the digitiform structures. The synaptic appendages may serve to increase the synaptic surface area with the mossy fiber, to isolate individual active zones, or to provide a substrate with which to modify the strength of input by

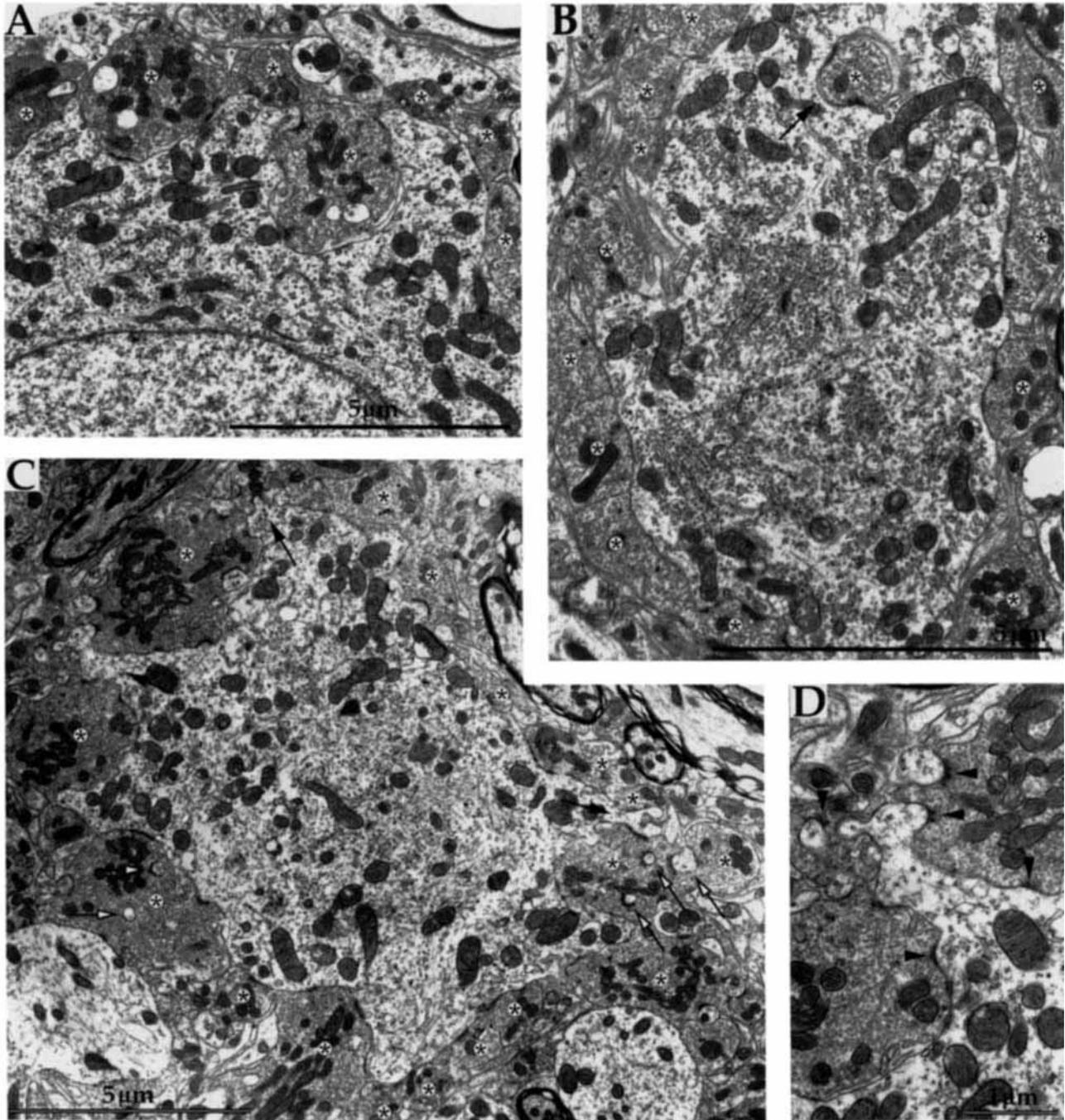


Fig. 9. Electron micrographs of chestnut cells. **A:** Micrograph of a chestnut cell showing the dendrite enfolding a mossy fiber. Mossy fiber terminals are indicated by asterisks. **B:** Grazing transverse section through a cell and dendrite (dendrite towards the top) showing the surrounding mossy fibers (asterisks) and the piece of mossy fiber, which has been completely engulfed (arrow). **C:** Section taken through a proximal dendrite, perpendicular to the plane shown in Figures 7 and 8.

The irregular expanse of the dendrite is clearly visible. Black arrows mark prominent projections into the surrounding mossy fibers (asterisks). Some fine finger-like synaptic projections are visible in cross section, embedded within the terminals (black and white arrows). **D:** Higher magnification of the dendrite shown in C. The irregular spine breaks into at least two other synaptic pieces, and every convexity is marked by a synapse (arrowheads).

altering the length or width of the postsynaptic crest or protuberance. The nonsynaptic hairs of the UBCs and granule cells, however, are more difficult to explain. It is possible that they serve to take up free neurotransmitter,

which would be trapped within the tightly contained glomeruli. Because uptake is the primary mechanism for transmitter removal at most central nervous system synapses, these fine processes would be ideally placed to

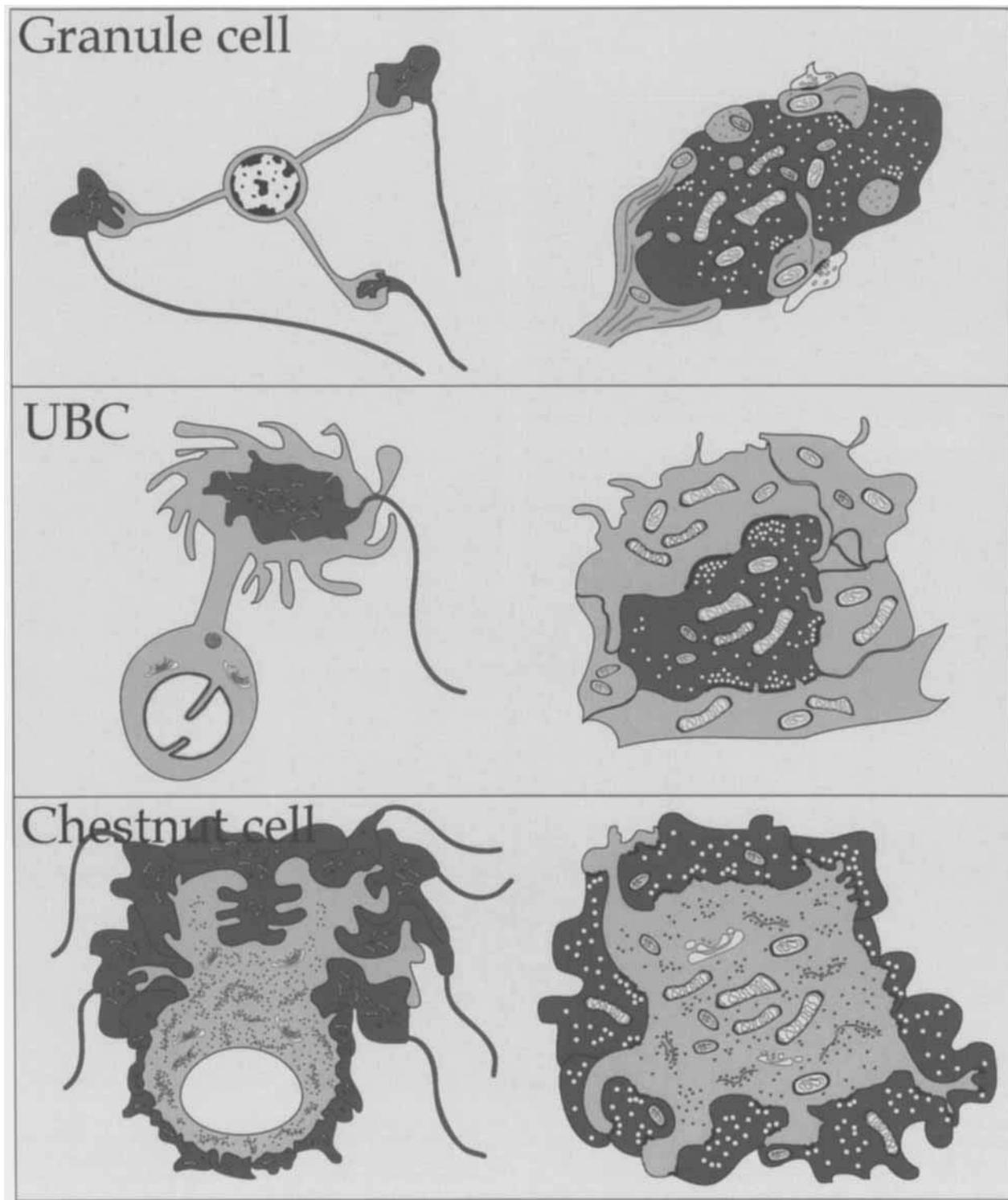


Fig. 10. Summary diagram illustrating the main characteristics of mossy fibers and their relationships to granule cells, UBCs, and chestnut cells at low (left) and high (right) magnification. Mossy fibers are indicated by dark gray, cells and dendrites are indicated by light gray. **Top:** The granule cell receives mossy fibers on each dendrite, and each dendritic claw is marked by microtubules, mitochondria, short synapses, and hair-like nonsynaptic projections. This arrangement may endow the granule cell with a sensitivity for coincident input from the separate mossy fiber endings. **Middle:** The UBC, with its distinctive organelle, the ringlet body, receives one mossy fiber at the dendritic

tuft. The UBC glomerulus is characterized by long synapses, wide interdigitated dendrites, and nonsynaptic hairs. A single input from the mossy fiber produces a prolonged UBC response, so that the UBC could serve as a potent signal amplifier. **Bottom:** The chestnut cell is dark with ribosomes and has a very short, irregular dendrite that, like the soma, is surrounded by mossy fibers. The chestnut dendrite is full of ribosome rosettes and Golgi apparatus, it is surrounded by smaller mossy fibers, and it sends irregular synaptic projections into the mossy fibers. The chestnut cell may serve as a kind of "summator" of mossy fiber activity.

accomplish that task. This possibility could be verified by ultrastructural localization of appropriate neurotransmitter uptake transporters.

### Significance for previous work

Progress is being made in identifying the postsynaptic targets of mossy fibers as a result of this study. Mossy fibers that project from the cuneate nucleus have been shown to involve glomeruli composed of small, round dendrites of the granule cell domain (Wright and Ryugo, 1996). These postsynaptic dendrites incompletely surround the mossy fiber and give rise to hair-like appendages, some of which are synaptic. In basic form, this organization conforms to what may be identified as granule cell glomeruli. Such an interpretation is also consistent with the demonstration of cuneate projections synapsing directly on granule cell somata. In addition, labeled projections from auditory cortex, although they do not terminate as mossy fibers, synapse on small, hairy dendritic profiles in the granule cell domain (Weedman et al., 1995). These same dendrites form an incomplete ring around and are synapsed upon by unlabeled mossy fibers. This characteristic structural arrangement involving mossy fibers strongly suggests that granule cells are the principal target of descending corticobulbar connections.

### Functional considerations

Based on the similarities between labeled granule cell claws and the most common unlabeled dendritic profiles, the granule cells are probably the major target of cochlear nucleus mossy fibers, as in the cerebellar cortex. This observation, along with the high density of granule cells and mossy fibers in the cochlear nucleus, implies a major synaptic system with the same degree of influence over the principal cells as is found in the cerebellar cortex. In the cerebellum, the mossy fibers constitute the main input to the Purkinje neurons. Mossy fibers synapse upon granule cell claws, and the granule cell axons then ascend to the superficial layer, where they synapse upon Purkinje cell dendrites (for review, see Ito, 1984). The result is that all mossy fiber input to the Purkinje cells is filtered through the granule cell domain. Another major input, the climbing fibers, seems to have a primarily modulatory function, adjusting the sensitivity of the Purkinje cells to mossy fiber stimulation. The climbing fibers are thought to make these adjustments in response to a mismatch between expected and executed signals when, for instance, an object being lifted turns out to be much heavier than expected (Gilbert and Thatch, 1977). The Purkinje cells send their inhibitory inputs to the deep cerebellar nuclei, which, in turn, send excitatory inputs to the rest of the brain (Eccles et al., 1967; Palay and Chan-Palay, 1974).

Because of the many similarities between the cerebellum and the DCN, there have been numerous attempts to describe the DCN as an "auditory cerebellum." One cell type, the cartwheel neurons of the DCN, are anatomically, developmentally, and cytochemically similar to the Purkinje neurons (Berrebi et al., 1990; Berrebi and Mugnaini, 1987). Although the cartwheel neurons are not projection neurons like the Purkinje cells, they do receive parallel fiber synapses, their output is inhibitory, and their axon targets are the major output cells of the DCN, the pyramidal cells. To make an accurate analogy between cerebellum and DCN, it can be proposed that DCN pyramidal neurons are

analogous of the deep cerebellar nucleus neurons. In both systems, therefore, the final output neurons (the pyramidal cells of the DCN and the deep cerebellar neurons) receive direct input from lower centers (the auditory nerve and the vestibular organs/somatosensory nuclei, respectively). This input also takes an indirect route, where it is combined with a wide range of information from other sources through a mossy fiber-granule cell system and translated to a major inhibitory neuron (the cartwheel cell and the Purkinje neuron), after which it is sent back to the final output neurons. This analogous circuitry suggests that the DCN is indeed performing a task similar to that of the cerebellum. One possibility is an auditory expected-vs.-executed comparison. There is evidence that the DCN is involved in the acoustic startle response (Lingenhöhl and Friauf, 1994), and it may be involved in an animal's orientation to an unexpected stimulus (Masterton and Sutherland, 1994). The DCN granule system could help in orienting to a sound by comparing the expected movement of the sound (equal and opposite to head movement) with the perceived movement of the sound. There is evidence that the DCN receives the information on head and pinna position necessary to make such a comparison. There is a large cuneate projection to the granule cell domain (Wright and Ryugo, 1996), and activity can be evoked in the DCN following tactile stimulation of the pinna (Young et al., 1995) or direct electrical stimulation of the dorsal column nuclei and spinal trigeminal nucleus (Saadé et al., 1989). Another possibility is that the DCN is involved in the processing of conspecific vocalizations and that the granule cell system is required to compare the expected vocalization with the executed, which is a continually modifiable process.

The DCN is organized into frequency planes (Spirou et al., 1993). One curiosity of the cochlear nucleus granule cells is that they project across this frequency organization. Because the parallel fibers run perpendicular to the isofrequency bands, it is difficult to explain how they could have a tonotopic function. However, there is evidence that the granule cells operate under an entirely different organizational principle. In the cerebellum, this principle is called fractured somatotopy. Single cell recordings of the receptive fields of cerebellar granule cells (and the mossy fibers that drive them) have shown that, although discrete clusters of granule cells are responsive to small areas on the skin, there are multiple representations of each single area of the skin (Shambes et al., 1978). This finding indicates that somatotopic specificity is preserved in the granule cell system but that there may be areas of separate and parallel processing. The same may be true in the cochlear nucleus granule cell domain, but it remains to be discovered.

The mossy fibers of the cochlear nucleus are a heterogeneous group. There are at least three cellular targets (granule cells, UBCs, and chestnut cells), and there may be subcategories of each cell class. There are probably many different sources of mossy fibers, including other auditory structures, nonauditory sensory nuclei, and reticular nuclei. There is also evidence that mossy fibers use at least two neurotransmitters, acetylcholine (McDonald and Rasmussen, 1971; Osen et al., 1984) and glutamate (Wright and Ryugo, 1996). All of these factors remain to be integrated with one another before we can understand fully how the granule cell regions contribute to the function of the DCN.

## ACKNOWLEDGMENTS

We thank Miqun L. Robinson and Zheng Yuan for technical assistance and Debora Wright Tingley and John R. Doucet for helpful discussions on the data and the paper. D.L.W. was supported by an NSF Predoctoral Fellowship. This work was supported in part by research grant 5 RO1 DC00232 from the National Institute on Deafness and Other Communication Disorders, National Institutes of Health.

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