

Projections From Auditory Cortex to the Cochlear Nucleus in Rats: Synapses on Granule Cell Dendrites

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ABSTRACT

Previous work has demonstrated that layer V pyramidal cells of primary auditory cortex project directly to the cochlear nucleus. The postsynaptic targets of these centrifugal projections, however, are not known. For the present study, biotinylated dextran amine, an anterograde tracer, was injected into the auditory cortex of rats, and labeled terminals were examined with light and electron microscopy. Labeled corticobulbar axons and terminals in the cochlear nucleus are found almost exclusively in the granule cell domain, and the terminals appear as boutons (1–2 μm in diameter) or as small mossy fiber endings (2–5 μm in diameter). These cortical endings contain round synaptic vesicles and form asymmetric synapses on hairy dendritic profiles, from which thin (0.1 μm in diameter), nonsynaptic “hairs” protrude deep into the labeled endings. These postsynaptic dendrites, which are typical of granule cells, surround and receive synapses from large, unlabeled mossy fiber endings containing round synaptic vesicles and are also postsynaptic to unlabeled axon terminals containing pleomorphic synaptic vesicles. No labeled fibers were observed synapsing on profiles that did not fit the characteristics of granule cell dendrites. We describe a circuit in the auditory system by which ascending information in the cochlear nucleus can be modified directly by descending cortical influences. © 1996 Wiley-Liss, Inc.

Indexing terms: centrifugal projections, electron microscopy, hearing, ultrastructure

The auditory cortex, which has often been regarded as the termination of ascending auditory pathways, has long been recognized as the origin of descending auditory pathways (Held, 1893). These descending pathways travel topographically from auditory cortex to the inferior colliculus (Andersen et al., 1980; Faye-Lund, 1985; Coleman and Clerici, 1987; Herbert et al., 1991) and from the inferior colliculus bilaterally to a wide array of brainstem auditory nuclei, including the nuclei of the lateral lemniscus, the periolivary nuclei, and the cochlear nucleus (Caicedo and Herbert, 1993; Saldaña, 1993). Historically, the inferior colliculus was considered to be an obligate synapse in the efferent pathway from cortex, just as it is in the afferent pathway (Ramón y Cajal, 1909; Geniec and Morest, 1971; Adams, 1976). Recently, however, sensitive anterograde tracers have shown that there are monosynaptic projections from the auditory cortex that bypass the inferior colliculus and directly innervate the cochlear nucleus and other auditory brainstem nuclei (Feliciano et al., 1995; Weedman et al., 1995). The existence of direct cortical projections to the source of central auditory pathways suggests that the auditory cortex influences ascending auditory information at this early level.

In a previous study, we demonstrated that the projections from auditory cortex to cochlear nucleus arise from layer V pyramidal neurons in primary auditory cortex (AI; Weedman and Ryugo, 1996) and terminate in the granule cell domain of the cochlear nucleus. The granule cell domain, although it is not a homogeneous structure, contains mostly granule cells, which are named for their morphological similarity to the cerebellar granule cells (Lorente de Nó, 1933). The granule cell domain consists of a continuous sheet of small cells that surrounds the magnocellular ventral cochlear nucleus (VCN) and penetrates into the dorsal cochlear nucleus (DCN) as layer II (Mugnaini et al., 1980b). The granule cell domain also houses a variety of other cell types, including unipolar brush cells (UBCs), Golgi cells, and chestnut cells (Mugnaini et al., 1980a; Mugnaini and Floris, 1994; Floris et al., 1994; Weedman et al., 1996; Wright et al., 1996).

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The inputs to the granule cell domain are diverse. Although the granule cell domain does not receive input from myelinated auditory nerve fibers, it does receive inputs from unmyelinated nerve fibers carrying information from outer hair cells (Brown et al., 1988a). The granule cell domain is also the target for auditory efferent projections. The olivocochlear neurons send collaterals into the granule cell domain en route to the cochlea (Brown et al., 1988b), where some form synapses upon the dendrites of small multipolar cells whose somata lie just outside the granule cell domain (Benson and Brown, 1990). There is a large descending projection to the granule cell domain from both the inferior colliculus (Caicedo and Herbert, 1993; Saldaña, 1993) and AI (Feliciano et al., 1995; Weedman et al., 1995). The granule cell domain receives nonauditory input as well, including projections from the cuneate and trigeminal nuclei, first-order somatosensory nuclei (Itoh et al., 1987; Weinberg and Rustioni, 1987; Wright and Ryugo, 1996), and fibers from the vestibular organs (Burian and Goettner, 1988; Kevetter and Perachio, 1989). Finally, the granule cell domain is the target of diffuse noradrenergic projections from the locus coeruleus (Kromer and Moore, 1980) and serotonergic projections from the raphe nuclei (Keppler and Herbert, 1991).

The cellular targets of these varied inputs are unknown. How we model signal processing in the cochlear nucleus will differ, for example, if the inputs are segregated with respect to cell type or if they are distributed across several cell types. In this paper, we examine the anterogradely labeled terminals of projections from auditory cortex to the granule cell domain of the cochlear nucleus by using light and electron microscopy to identify the postsynaptic targets. We provide evidence that the corticobulbar projection synapses on the dendrites of granule cells. Because granule cells, in turn, synapse with the principal cells of the DCN by way of parallel fibers (Mugnaini et al., 1980b; Manis, 1989), the auditory cortex may be able to modify the output of the DCN via the granule cell system.

MATERIALS AND METHODS

Corticobulbar projections were analyzed by unilaterally injecting a 10% solution of biotinylated dextran amine (BDA; 10,000 m.w.; Molecular Probes) into the auditory cortex of 12 adult albino rats. Animals were anesthetized by intraperitoneal injections of sodium pentobarbital (45 mg/kg body weight), and oral secretions were blocked by 0.05 mg intramuscular injections of atropine sulfate. When the animal was deeply anesthetized (areflexic to tail or paw pinch), the skin and soft tissue overlying the parietal bone were reflected, and the skull was opened by drilling. Auditory cortex was located on the basis of surface landmarks (Ryugo, 1976; Kelly, 1990), and a glass micropipette (30 μ m tip, inside diameter) filled with BDA was lowered 1 mm into the cortical surface by using a micromanipulator. A total volume of 0.25–1.0 μ l of BDA was injected by using a Nanoinject pressure injector (Drummond Scientific) at a rate of 50 nl/minute. The pipette was removed 10 minutes after the injection, and the animal was sutured and allowed to recover from anesthesia. After a 7–10 day survival period, rats were administered a lethal dose of sodium pentobarbital and were transcardially perfused with 0.1 M phosphate-buffered saline, pH 7.3, followed by 4% paraformaldehyde, 0.1 M lysine, and 0.01 M sodium periodate in 0.12 M phosphate buffer, pH 7.4. One animal was perfused

with a 2/2% solution of paraformaldehyde and glutaraldehyde to optimize ultrastructural integrity. The brainstems were dissected and allowed to postfix at 4°C for 1–12 hours. The cochlear nucleus was embedded in a gelatin-albumen mixture for stability, and the sections were cut at 50 μ m on a Vibratome.

Brainstem sections were incubated overnight at 4°C in avidin-biotin peroxidase complex (ABC; Vector) in 0.1 M phosphate buffer, pH 7.4. To improve membrane permeability, 0.1% Photo-Flo (Kodak) was added to the ABC solution. The following day, the sections were rinsed twice in phosphate buffer and twice in 0.05 M cacodylate buffer. The label was visualized with 0.0125% diaminobenzidine (DAB), 0.25% nickel-ammonium sulfate, and 0.35% imidazole in 0.05 M cacodylate, pH 7.2. Sections were preincubated in the DAB solution for 10 minutes, hydrogen peroxide was added to the sections at a concentration of 0.02%, and the tissue sections were incubated for approximately 15 minutes more. The nickel-intensified DAB reaction product is sharp and black and is excellent for the visualization of fine fibers. Tissue sections from seven rats were mounted and counterstained for light microscopy. Four of these cases were discarded, because the injection site was too small to produce labeled fibers or because the label was too faint for adequate study.

Tissue sections from five rats were processed for electron microscopy immediately following the DAB step of the procedure outlined above. Briefly, the sections were washed in phosphate buffer with 7% sucrose, incubated with 1% osmium tetroxide for 15 minutes, washed in maleate buffer, and stained with 1% uranyl acetate overnight. The next day, the sections were dehydrated through graded alcohols and propylene oxide, infiltrated with Epon embedding medium, and sandwiched between two pieces of Aclar (Ted Pella, Inc.). Hardened tissue was mounted onto microscope slides for light microscopic analysis. Regions of interest were photographed, then the appropriate structures and landmarks were drawn with the aid of a drawing tube and mapped. The granule cell areas were cut out of the Aclar and embedded in a BEEM capsule for sectioning. Ultrathin sections approximately 75 nm thick (silver) were collected on Formvar-coated, slotted 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 was adjusted (if necessary) to simulate standard darkroom techniques (Adobe Photoshop), and they were printed in high-resolution format (Fuji Pictography 3000).

The cerebral hemispheres were cut at a thickness of 75 μ m on a Vibratome and processed with DAB for light microscopy, as described above, to determine the extent of the injection site. Every other section through parietal cortex was drawn by using an overhead projector, and the injection site, as indicated by the presence of DAB reaction product, was traced. The boundaries of the auditory cortex were defined by using our own data (Weedman and Ryugo, 1996) and a stereotaxic atlas of the rat brain (Swanson, 1992). By aligning drawn sections with comparable atlas sections, a lateral view of the injected hemisphere was reconstructed.

RESULTS

A total of eight rats contributed to the cochlear nucleus data base in the present report: three strictly for light

microscopy and five for combined light and electron microscopy. All animals had injection sites confirmed to be in AI, as defined histologically (Swanson, 1992; Weedman and Ryugo, 1996; Herbert et al., 1991), with some portion usually extending beyond the AI boundary. Because a previous study demonstrated that cortical areas immediately surrounding AI do not contribute to projections to the cochlear nucleus (Weedman and Ryugo, 1996), this study was not compromised when an injection site extended slightly outside AI. In a few cases, the injection was completely confined within AI, and the pattern of labeling was identical to those cases with extra-AI injection sites. Smaller injection sites correlated to fewer labeled fibers.

Injections of BDA into AI labeled fine axons and terminals that were distributed throughout the superior olivary, periolivary, and cochlear nuclei. Terminals were found bilaterally, but they were more numerous ipsilateral to the injection. A typical injection site (Fig. 1A) occupied roughly 50% of AI, and labeled fibers in the cochlear nucleus were distributed primarily in the granule cell areas surrounding the VCN (Fig. 1B). The thin ($<0.5 \mu\text{m}$) labeled fibers entered the cochlear nucleus from its medial aspect by way of the dorsal and intermediate acoustic striae. These thin fibers branched repeatedly and distributed numerous en passant and terminal swellings throughout their specific target region (Fig. 1C). Because of their thin caliber and repeated branching, it was not possible to reconstruct individual fibers in their entirety. The few labeled fibers that were found in the DCN were localized in layer II, where granule cells congregate. In the present report, our focus is on the granule cell domain surrounding the VCN.

The granule cell domain consists of a continuous sheet of densely packed small cells, which aggregate in distinct regions of the nucleus (Fig. 1B). This sheet has been subdivided into several regions surrounding the VCN and DCN (Mugnaini et al., 1980b). The VCN is bounded laterally by the superficial granule cell layer, dorsally by the granule cell lamina that separates VCN from the DCN, dorsomedially by the subpeduncular corner of granule cells, and medially by the medial sheet of granule cells. A sheet of granule cells arises from the lamina to penetrate the DCN as layer II and continues through to the dorsomedial tip of the DCN, where it expands to form the strial corner.

The granule cell domain is not a homogeneous structure, and somata of at least four different cell types reside within: granule cells, UBCs, Golgi cells, and chestnut cells (Mugnaini et al., 1980a; Floris et al., 1994; Mugnaini and Floris, 1994; Weedman et al., 1996). The granule cells are the smallest of these ($\sim 6 \mu\text{m}$), and are characterized by two to five smooth, radiating dendrites that branch infrequently or not at all and that terminate in hairy, digitiform claws (Mugnaini et al., 1980a; Weedman et al., 1996). Ultrastructurally, the fine granule cell dendrites are marked by the presence of numerous microtubules (Fig. 2). Their cell bodies have small cytoplasmic-to-nuclear ratios, and the nucleus is distinctive by its pale staining and accumulation of peripheral chromatin. The cell bodies of UBCs are slightly larger than granule cells ($\sim 10 \mu\text{m}$) and characteristically exhibit one stout, relatively short dendrite, which erupts into a dense spray of fine dendritic processes (Floris et al., 1994; Mugnaini and Floris, 1994; Wright et al., 1996). The UBC somata frequently exhibit a ringlet body, and ribosomes and dense core vesicles are found throughout the cell body and dendrite (Fig 3B; Mugnaini et al., 1994). The Golgi cells have not been thoroughly characterized in the

cochlear nucleus. The Golgi cell body is identified by its dark appearance due to a high concentration of ribosomal rosettes (Fig. 4; Mugnaini et al., 1980a; Weedman et al., 1996); however, dendritic and axonal features are unknown, but they have been speculated upon by analogy to the cerebellar cortex (Mugnaini et al., 1980a). The recently described chestnut cell has a small cell body ($\sim 10 \mu\text{m}$) but is unique, in that it has one or two broad, stubby dendritic stalks and an irregular spray of filiform appendages that arises directly from the soma (Weedman et al., 1996). These appendages receive synapses and are characterized by the presence of a few polyribosomes and an absence of mitochondria.

In addition to the criteria described above, cytoskeletal elements can aid in the identification of ultrastructural profiles (Fig. 3A). Neurofilaments (Fig. 3A, arrowheads) are abundant in axons and are also characteristic of UBC dendrites (Mugnaini et al., 1994). Microtubules (Fig. 3A, arrows) are prominent in dendrites, especially those of granule cells. Finally, the very thin, tightly packed glial filaments (Fig. 3A, double arrows) identify glial processes.

The mossy fiber glomerulus is a characteristic structure of the granule cell domain (Fig. 3B). Mossy fiber endings are typically large ($5\text{--}20 \mu\text{m}$) synaptic endings that are densely packed with synaptic vesicles and surrounded by dendrites (McDonald and Rasmussen, 1971; Kane, 1974; Mugnaini et al., 1980a; Wright and Ryugo, 1996). These large endings were named in the cochlear nucleus due to their similarity to the mossy fiber rosettes of the cerebellum. Mossy fibers are often surrounded by granule cell claws, the distal tips of granule cell dendrites. In cross section (Fig. 3B), these claws appear as oblong profiles around the perimeter of the mossy fiber. Other elements in this interaction are glial processes, which envelop most of the glomerulus, and small axon terminals, which synapse on the outside surfaces of the granule cell dendrites. These small terminals with pleomorphic vesicles form symmetric synapses, indicating an inhibitory function; by analogy to the cerebellar cortex, such endings may be inferred to be Golgi cell axons. Our goal in this study was to use what is known about these cell types and profiles at the ultrastructural level to identify which cell groups receive the descending cortical synapses.

Labeled swellings, whether they are en passant or terminal, seem to fall into two groups on the basis of size and synaptic relationships. The small swellings ($0.5\text{--}1.0 \mu\text{m}$ in diameter) are most numerous and may be defined as boutons, where each bouton forms a single synapse with the postsynaptic structure (Fig. 4B,C). In contrast, larger swellings ($2\text{--}8 \mu\text{m}$ in diameter) are less frequent and may be defined as mossy fibers, because they form a central structure filled with round synaptic vesicles, they are surrounded by dendritic profiles, and they make several separate synapses with surrounding dendrites (Fig. 5). Labeled terminals display asymmetric membrane specializations, where the postsynaptic density is much more prominent than the presynaptic thickening. This asymmetry plus the presence of numerous round synaptic vesicles in the terminal suggest an excitatory synapse.

The typical postsynaptic targets are small, round profiles containing microtubules and mitochondria. All of the labeled swellings are penetrated by very thin ($\sim 0.1 \mu\text{m}$), nonsynaptic, hairlike extensions that originate from the surrounding postsynaptic dendrites (Figs. 4–7). These dendritic tendrils are characteristic of the distal claws of granule cells (Weedman et al., 1996). The presence of these

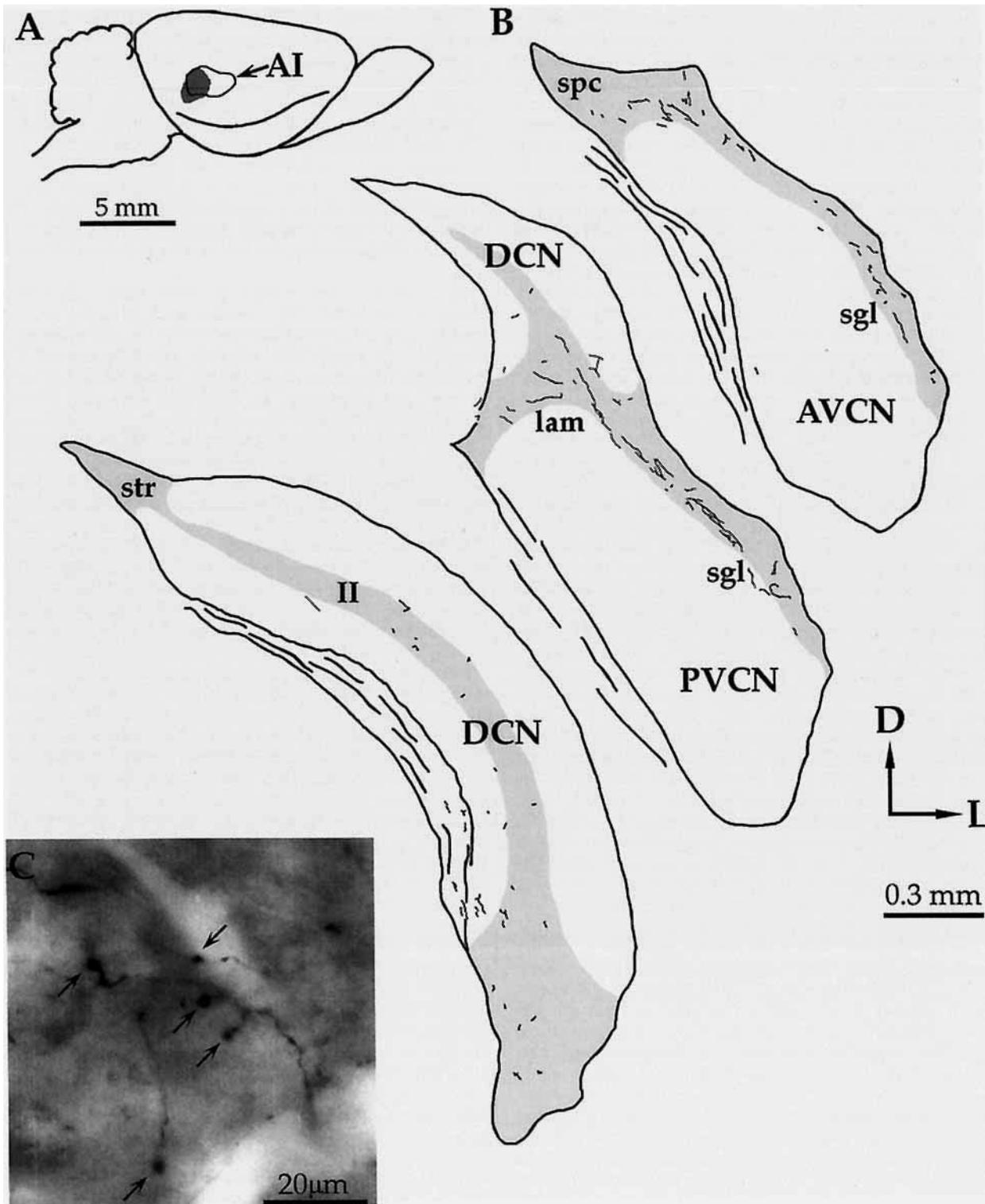


Fig. 1. **A:** Schematic drawing of reconstructed injection site, typical for the present study. Primary auditory cortex (AI) is outlined, and the injection has been shaded with gray. **B:** Camera lucida drawings of three coronal sections through the cochlear nucleus illustrating the distribution of labeled fibers and terminals after an injection of biotinylated dextran amine (BDA) into the auditory cortex. All labeled fibers in each section are shown and are almost completely contained

within the granule cell domains (shaded areas). **C:** Light micrograph of a typical grouping of labeled fibers in the granule cell areas. Several boutons are visible (arrows). AVCN, anteroventral cochlear nucleus; DCN, dorsal cochlear nucleus; PVCN, posteroventral cochlear nucleus; II, layer II of the DCN; lam, granule cell lamina; sgl, superficial granule cell area; spc, subpeduncular corner of granule cells; str, strial corner of granule cells.

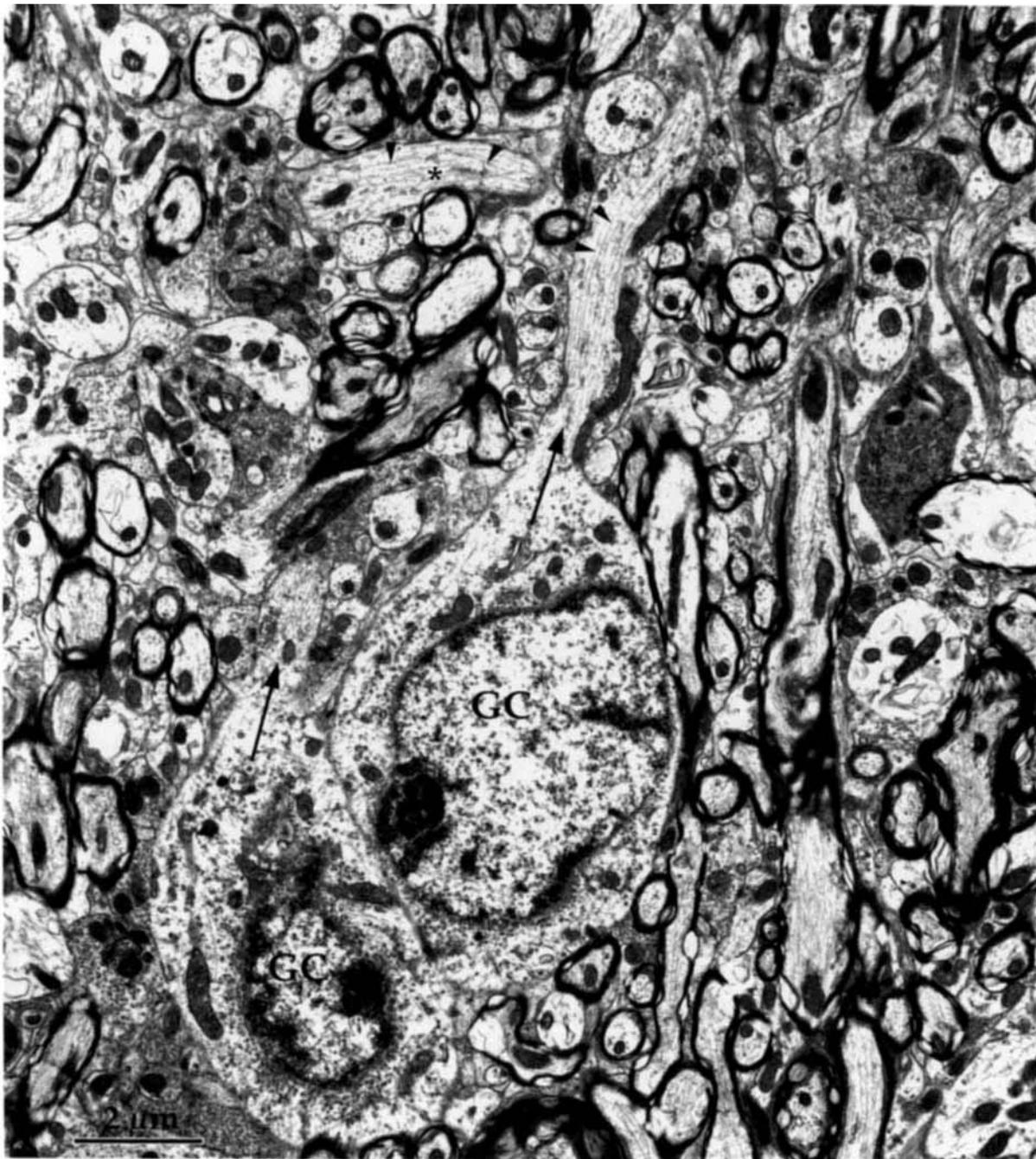


Fig. 2. Electron micrograph of granule cells (GC) in the granule cell lamina separating the ventral cochlear nucleus (VCN) from the DCN. Dendrites emerging from each cell body (arrows) abound with microtubules (arrowheads). Another granule cell dendrite is identifiable (asterisk) by its complement of microtubules.

tendrils plus the morphology of the dendrites strongly suggest that they are granule cell dendrites. The morphology of the postsynaptic dendrites is not consistent with UBCs, which contain ribosomes and dense core vesicles in their dendritic stalks (Fig. 3B) and emit large, tufted dendrites that completely enclose a single mossy fiber terminal. Chestnut cells are not likely candidates, because such cells are mostly adendritic, and their postsynaptic

processes are void of mitochondria and thread-like tendrils (Weedman et al., 1996).

The labeled endings and their postsynaptic targets form consistent relationships with mossy fiber glomeruli in the neuropil. The major components of this assemblage are the labeled ending, small hairy dendritic profiles, mossy fiber endings, glial processes, and axon terminals containing pleomorphic vesicles and forming symmetric synapses. In

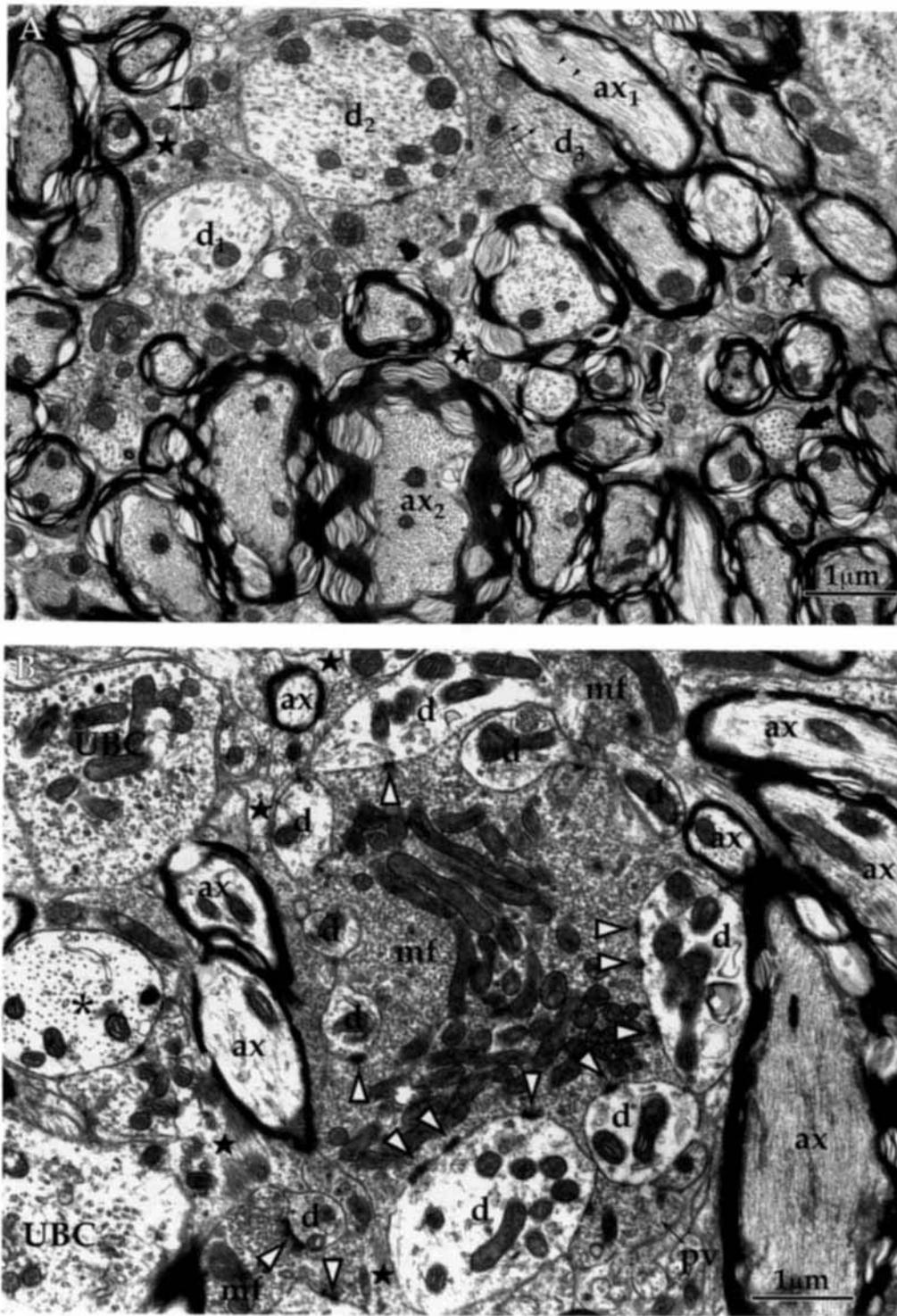


Fig. 3. **A:** Electron micrograph of dendrites (d), axons (ax), and astrocytic processes (stars). Neurofilaments (arrowheads), appearing as fine threads, are visible in myelinated axons both in longitudinal section (ax₁) and in cross section (ax₂). Microtubules (small arrows) appear as thin hollow tubes in all three dendrites. A fourth dendrite (large arrow) shows the microtubules in cross section. Astrocytic processes (stars) are identifiable by the dense tangles of very fine glial filaments (double arrows). **B:** Electron micrograph of a mossy fiber glomerulus. The

central mossy fiber (mf) is surrounded by granule cell dendrites (d), astrocytic processes (stars), and terminals with pleomorphic vesicles (pv). There are numerous synaptic contacts (arrowheads) between the mossy fiber and granule cell dendrites. Myelinated axons (ax) are prominent but do not interact with the glomerulus. Three dendrites are visible at left: The central dendrite (asterisk) is a granule cell dendrite (UBC). The others have the features of a unipolar brush cell dendrite (UBC).

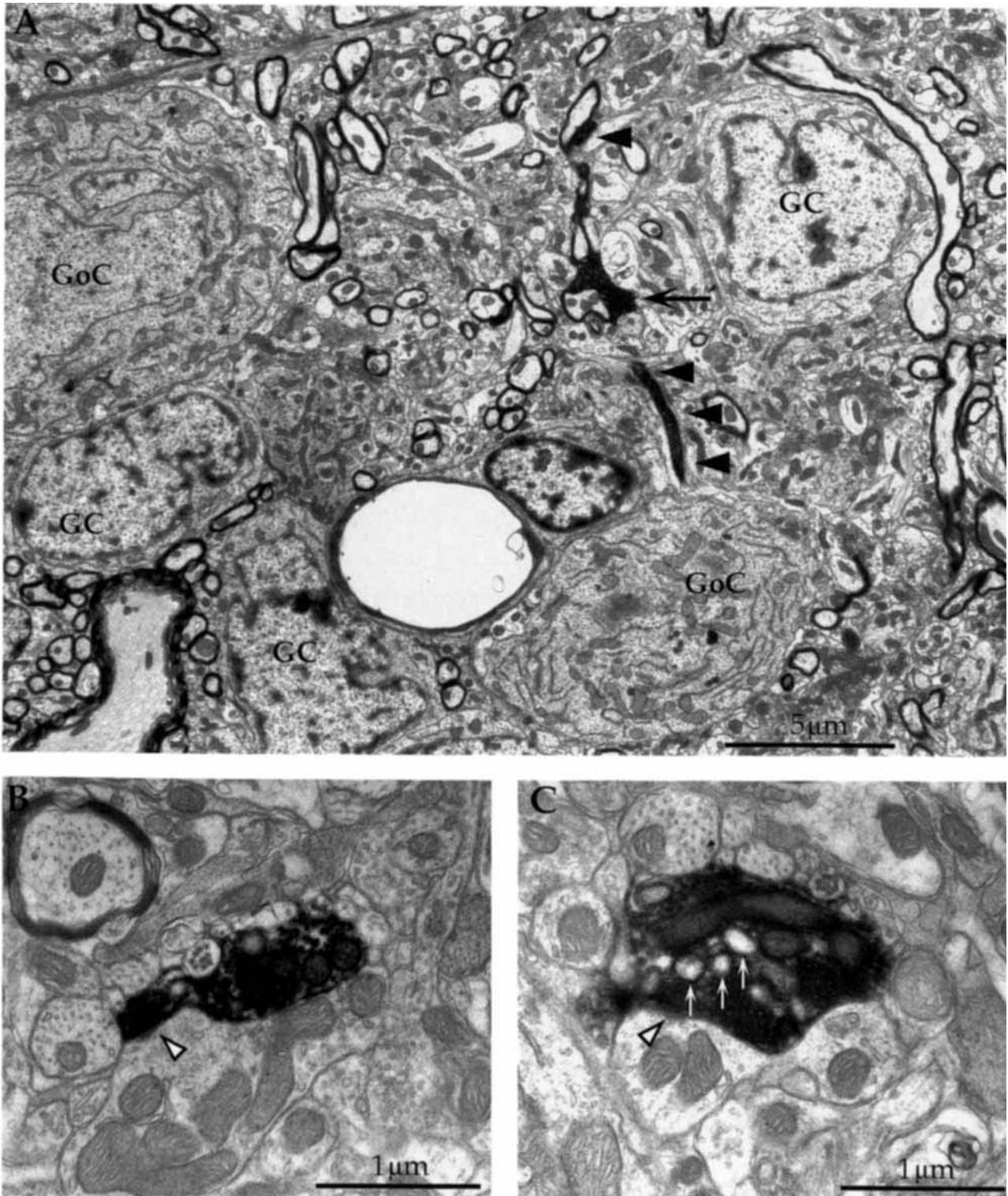


Fig. 4. **A:** Electron micrograph of a labeled fiber coursing through the superficial granule cell layer. Both granule cells (GC) and Golgi (GoC) cells are present. The length of the fiber is indicated by arrowheads, and the synaptic bouton is indicated by an arrow. **B,C:** Adjacent sections of the bouton shown in A. Synapses (arrowheads) are

identified by the presence of the postsynaptic density because the vesicles are mostly obscured by the label. Distinctive "hairs" are visible in C and are marked by the white arrows. These fine ($\sim 0.1 \mu\text{m}$), hairlike processes are characteristic of granule cell dendrites.

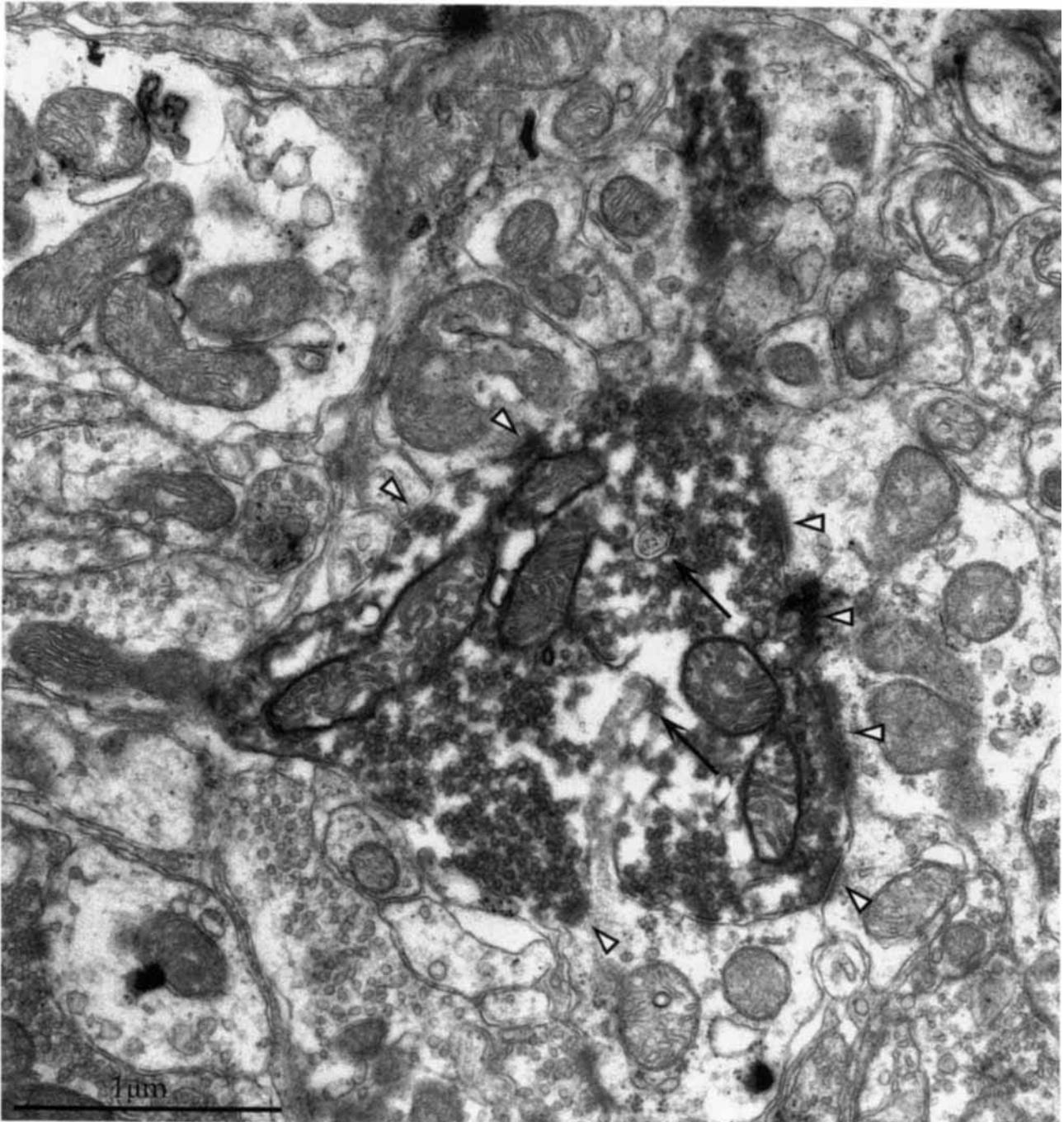


Fig. 5. Electron micrograph of a small labeled mossy fiber ending in the granule cell lamina. Multiple synapses are visible with the surrounding dendritic processes (arrowheads). Two hairlike projections are

embedded in the ending (arrows), one of which clearly arises from a dendrite at the bottom of the figure. Round vesicles are visible throughout the ending.

fortuitous sections, all of these elements are visible. In one such section, a granule cell dendrite was cut in longitudinal section, revealing its claw-like shape and its relationship with the mossy fiber (Fig. 6). The corticobulbar terminals have a spatial relationship to the glomerulus similar to the terminals with pleomorphic vesicles, in that they both synapse on the outer surface of dendrites in the granule cell glomerulus. Due to the many possible planes of section, not

all elements of the glomerulus are always visible in a single micrograph. Because we followed corticobulbar axons through serial sections, however, it was possible to verify that these glomerular relationships were preserved.

Occasionally, the corticobulbar fiber itself appears to form a mossy fiber ending. The fiber expands into a relatively large terminal surrounded by small, round, hairy dendritic profiles (Figs. 5, 7). Even in these cases, however,

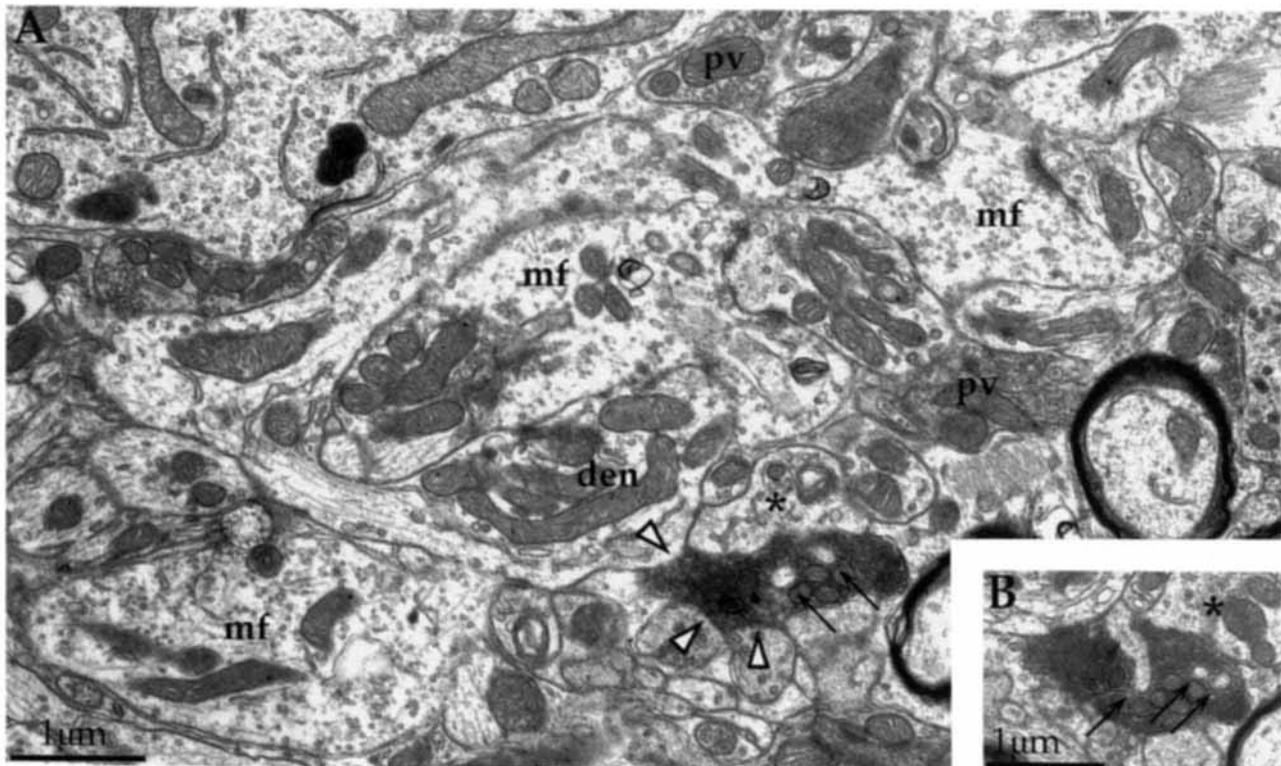


Fig. 6. **A:** Electron micrograph of labeled bouton synapsing on a granule cell claw in the superficial granule cell layer. The curling claw-like dendrite (den) is wrapped around a large mossy fiber ending (mf). A labeled bouton synapses on the claw as well as on other neighboring small dendrites (arrowheads). The faint synapse onto the claw was confirmed in later sections. Round vesicles are clearly visible in this lightly labeled bouton. A second dendrite (asterisk) sends

hairlike projections (arrows) into the labeled bouton. Two terminals with pleomorphic vesicles (pv) synapse on the outer faces of granule cell dendrites. **B:** Adjacent section of the same labeled bouton showing that the dendrite marked by an asterisk is continuous with the hairs (arrows). All of the dendritic profiles show features characteristic of granule cell dendrites and may all be part of the same claw.

there are nearby mossy fiber endings whose size dwarfs these corticobulbar mossy fiber endings, and these large mossy fiber endings also form synapses upon the same hairy dendrites. The result is that the fundamental relationship of corticobulbar endings and the granule cell glomerulus remains intact, irrespective of their size.

DISCUSSION

In the present report, we describe the projections to the cochlear nucleus that originate from AI by using anterograde tracing methods combined with light and electron microscopic analysis. From a previous study, we know that the cells of origin are layer V pyramidal cells (Weedman and Ryugo, 1996). The terminal field of this projection is concentrated in the granule cell domain of the ipsilateral cochlear nucleus, and the synaptic endings appear primarily as small boutons and occasionally as slightly larger mossy fiber endings.

In every example examined with electron microscopy, the postsynaptic targets of descending corticobulbar fibers were either small, round, dendritic profiles viewed in cross section or distinctive claw-like dendrites, all with fine, hairlike appendages. The most logical and parsimonious interpretation is that all postsynaptic targets are granule cell dendrites, as viewed from different perspectives. On the basis of structural features and mossy fiber relationships of

these small dendrites (Mugnaini et al., 1980a; Weedman et al., 1996), and in those cases where the postsynaptic target is clearly a stereotypic granule cell dendrite (i.e., Fig. 6), we conclude that the major target of the descending corticobulbar system is the granule cell.

To date, there have been four cell types described in the granule cell domains of the cochlear nucleus: the granule cell, the Golgi cell, the UBC, and the recently described chestnut cell (Mugnaini et al., 1980a; Floris et al., 1994; Wright et al., 1996; Weedman et al., 1996). Of these, the UBCs and the chestnut cells have large, irregular dendrites, so they are unlikely candidates as recipients of the corticobulbar fibers. Because so little is known about the dendrites of Golgi cells, these cells cannot be ruled out as the origin of some of the small round dendrites.

Corticobulbar fibers are found to synapse at the perimeter of the granule cell-mossy fiber glomerulus (Fig. 8). These terminals contain round synaptic vesicles and form asymmetric synapses, indicating that they exert an excitatory postsynaptic effect. Because the corticobulbar ending gives rise to a single synapse in the case of the bouton and to only a few synapses in the case of the small mossy fiber endings, its effect on the postsynaptic target is hypothesized to be relatively small, especially when compared with that of the large mossy fiber endings. Thus, auditory cortex by itself probably does not activate the granule cells; instead, we propose that it modulates the sensitivity of the

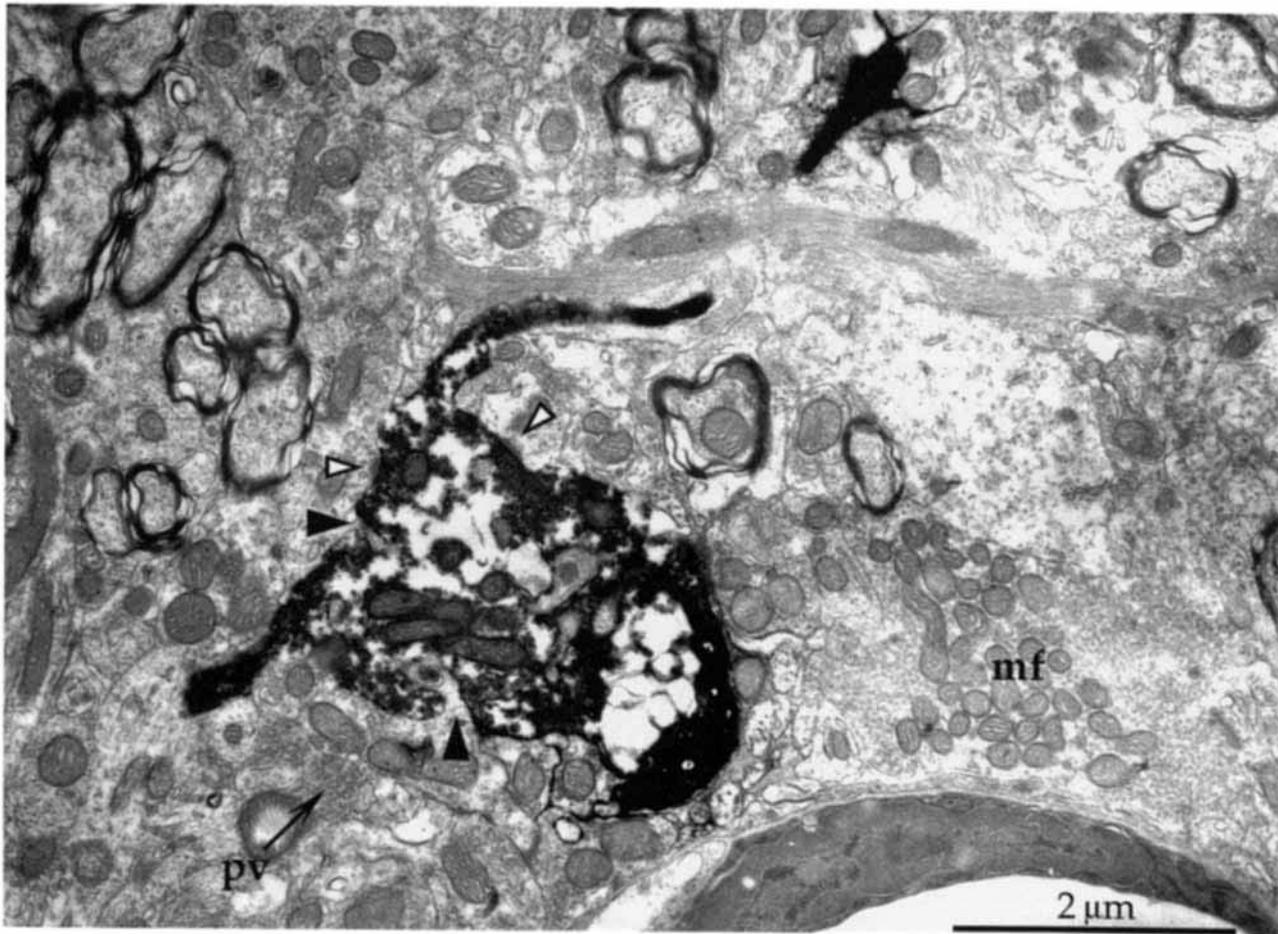


Fig. 7. Electron micrograph of a small labeled mossy fiber. This cortical mossy fiber synapses on at least two dendrites (white arrowheads) and is penetrated by dendritic hairs (black arrowheads). An unlabeled mossy fiber (mf) is closely apposed to the terminal, as is a terminal with pleomorphic vesicles (pv).

granule cell dendrites with excitatory depolarizations. The descending cortical system might therefore fine tune the responses of the granule cells to mossy fiber inputs. Terminals containing pleomorphic synaptic vesicles and forming symmetric synapses also contact granule cell dendrites along the glomerular perimeter. Analogous to the corticobulbar terminals, they synapse on the granule cell dendrites facing opposite the mossy fiber synapses. The source of such presumably inhibitory terminals is unknown, but it has been suggested that they arise from Golgi cells (Mugnaini et al., 1980a). Given the array of cell types involved in the glomerulus, there is a striking interplay of excitatory and inhibitory influences that modulate the mossy fiber inputs and, thus, granule cell outputs.

A schematic view of the circuitry as described in this paper is illustrated (Fig. 9). The pyramidal cells receive auditory nerve terminals from the myelinated fibers on their basal dendrites (Gonzalez et al., 1993; Ryugo and May, 1993) and send their axons out of the cochlear nucleus through the dorsal acoustic stria (Osen, 1972; Adams and Warr, 1976). The pyramidal cell axons project onto second-order neurons in the inferior colliculus (Ryugo et al., 1981; Willard and Martin, 1983; Oliver, 1985; Ryugo and Willard, 1985), which, in turn, project to the medial geniculate

nucleus (Andersen et al., 1980; Oliver, 1984; Rouiller and de Ribaupierre, 1985) and, from there, to the auditory cortex (Ryugo and Killackey, 1974; Roger and Arnault, 1989; Clerici and Coleman, 1990). Cortical neurons in layer V project back to the cochlear nucleus (Weedman and Ryugo, 1996), where they synapse on the granule cell dendrites. Thus, highly processed information from auditory cortex and, for example, somatosensory cues from the pinna and neck muscles by way of cuneate mossy fiber endings (Wright and Ryugo, 1996) are sent along granule cell axons through the superficial layer of the DCN. Parallel fibers from granule cells traverse the DCN, synapsing upon the apical dendrites of cartwheel and pyramidal cells. In this manner, auditory cortex can regulate pyramidal cell output by adjusting granule cell activity as well as by activating inhibitory circuits through cartwheel cells.

A recurrent theme in the auditory system is the division of pathways into a "core" and a "belt" (Ryugo, 1976; Patterson, 1977). The core includes the auditory nerve, the VCN and DCN, the central nucleus of the inferior colliculus, the ventral nucleus of the medial geniculate, and AI and, together, constitutes the main ascending flow of auditory information. This auditory core is surrounded by a belt region, which seems to deal with multimodal sources of

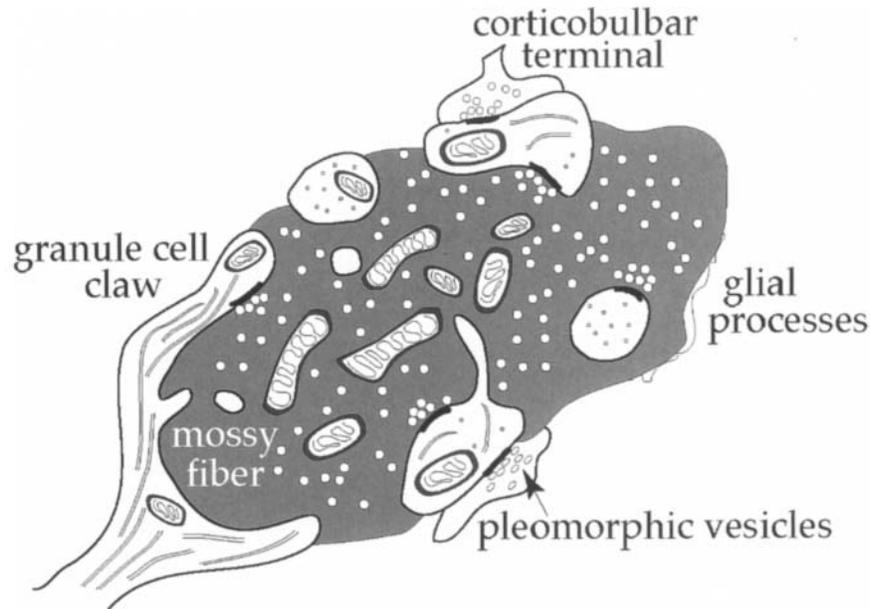


Fig. 8. Schematic drawing of a typical granule cell-mossy fiber glomerulus and its relationship to the corticobulbar terminal.

information (Wepsic, 1966; Schroeder and Jane, 1971; RoBards, 1979; Ryugo and Killackey, 1974; Ryugo and Weinberger, 1978; Willard and Ryugo, 1983; Winer and Morest, 1983; Roger and Arnault, 1989). This belt includes the granule cell domains of the cochlear nucleus, the dorsal (ICd) and external (ICe) nuclei of the inferior colliculus, the medial and dorsal nuclei of the medial geniculate nucleus, and the secondary or association areas of auditory cortex. Although the corticobulbar pathway originates in AI, a core area, much of the descending system seems to travel through the belt areas. The corticocollicular projection terminates in the ICd and the ICe, avoiding the central nucleus (ICc; Faye-Lund, 1985; Herbert et al., 1991). Similarly, the corticobulbar projection terminates among the granule cells, not in the central magnocellular regions of the cochlear nucleus. This "core vs. belt" dichotomy is not without exceptions, but, in general, belt areas may be seen to have a modulatory effect on pathways ascending through the core.

A large descending projection from the auditory cortex to the cochlear nucleus travels via the inferior colliculus, in contrast to the smaller but direct corticobulbar pathway. The two pathways are anatomically distinct, implying functional segregation. The corticocollicular pathways terminate in the ICd and ICe, as described above (Faye-Lund, 1985; Herbert et al., 1991). In contrast, the colliculocochlear nucleus pathways arise predominantly from the ICc (Caicedo and Herbert, 1993). The descending information, therefore, must travel from auditory cortex to the ICd or ICe, and then to the ICc, before finally reaching the cochlear nucleus. Descending information must synapse at least twice in the inferior colliculus, suggesting that the inferior colliculus does not merely relay this information but significantly alters it. Such an arrangement would result in a substantially different stream of information traveling through the inferior colliculus compared with information that travels directly to the cochlear nucleus.

The many structural similarities between the granule cell systems of the DCN and the cerebellum have led to some speculation on possible similarities in function. Both the cerebellum and the DCN have a primary sensory input from the inner ear that is supplemented by a large population of mossy fibers from diverse sources. In both systems, the mossy fibers synapse on digitiform granule cell dendrites; the granule cells then send their axons toward the pial surface, where they branch and run as parallel fibers, perpendicular to the planar orientation of the dendrites residing in the molecular layer. In the cerebellum, the parallel fibers synapse on the inhibitory Purkinje cells, whose axons synapse, in turn, on excitatory neurons in the deep cerebellar nuclei (Palay and Chan-Palay, 1974). In the cochlear nucleus, the parallel fibers synapse on both the inhibitory cartwheel neurons and the excitatory pyramidal neurons (Mugnaini et al., 1980a; Berrebi and Mugnaini, 1991). The cartwheel neurons, which have many anatomical, developmental, and neurochemical similarities to the Purkinje neurons (Mugnaini and Morgan, 1987; Berrebi et al., 1990), project onto the pyramidal neurons (Berrebi and Mugnaini, 1991). The pyramidal neurons, therefore, may be analogous to the deep cerebellar nucleus neurons, because both cell types are the primary output neurons for the DCN and the cerebellum, respectively.

In the cerebellum, this elaborate system serves to compare an expected movement with an executed movement, and a mismatch causes the system to make a motor compensation (Allen and Tsukahara, 1974). A similar process may be occurring in the cochlear nucleus. Sounds from different locations in space have different spectral shapes at the tympanic membrane due to reflections off the asymmetrical pinna. A moving sound source, therefore, generates a constantly changing spectral pattern. However, this same changing pattern may be replicated by turning the head while listening to a stationary sound source. Even the most primitive auditory system must be able to differenti-

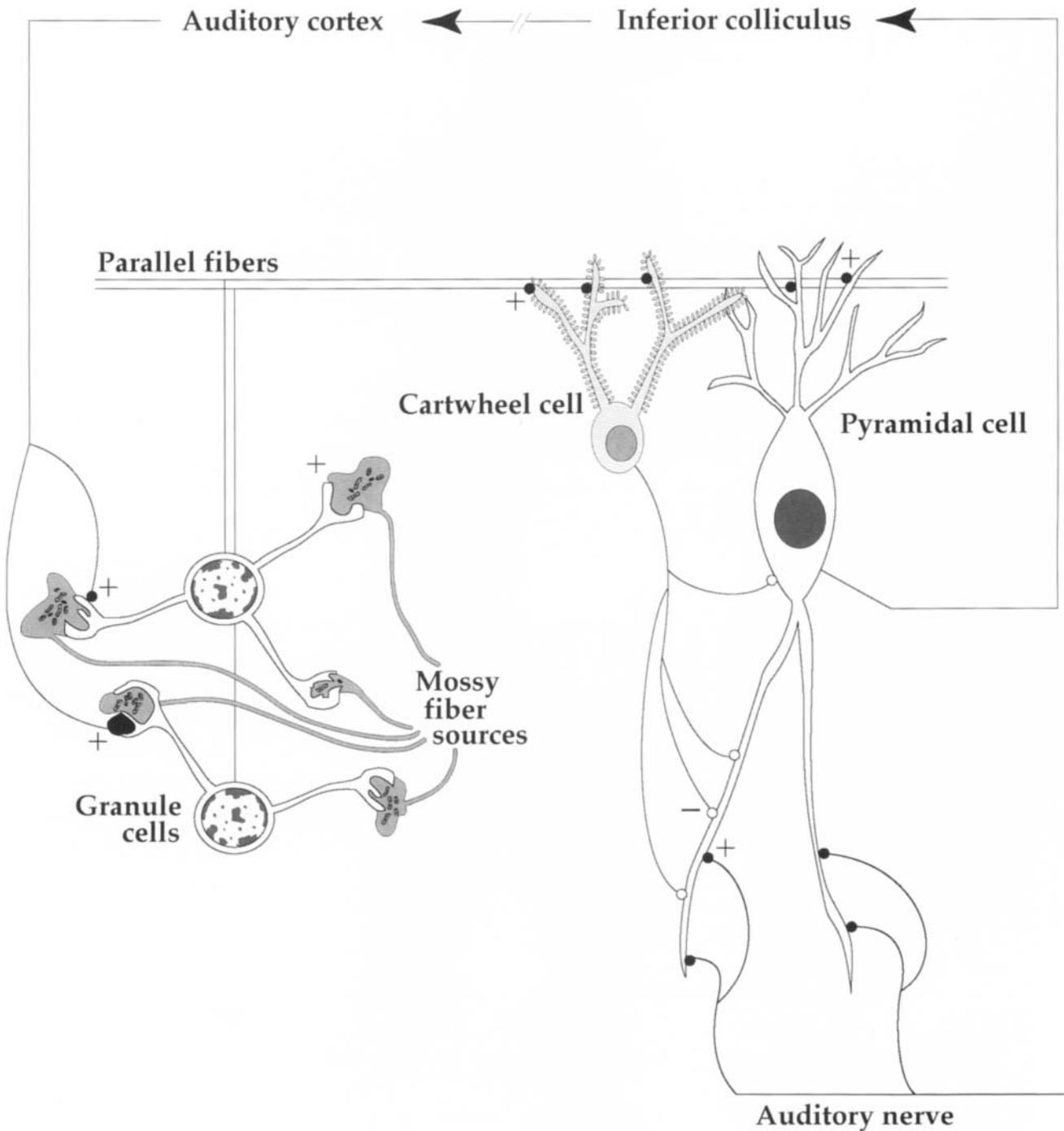


Fig. 9. Schematic drawing of the circuitry described in this paper. See Discussion for explanation.

ate between sound-source movement and head or body movement. One strategy might be to compare an expected change in spectral patterns, as determined by head movement, with the perceived change in spectral patterns. A mismatch would indicate that the sound source is actually in motion. The DCN granule cell system, which receives information from the somatosensory and vestibular systems, would appear to have much of the necessary information about head (or pinna) movement and position to make

this comparison. Descending projections, therefore, may serve to fine tune this tracking system based on attention, affective state, or learned behaviors.

There are several lines of evidence that are consistent with the hypothesis that the DCN performs a cerebellar-like function. The anatomical evidence consists of several studies that have shown projections to the cochlear nucleus from the cuneate (Weinberg and Rustioni, 1987; Wright and Ryugo, 1996) and trigeminal (Itoh et al., 1987) somato-

sensory nuclei. These nuclei carry tactile and proprioceptive information from the pinna, head, and upper body. At least one projection, that from the cuneate, has been shown to terminate as mossy fibers (Wright and Ryugo, 1996). The output of the DCN might also modulate a motor function (Lingenhöhl and Friauf, 1994). Giant neurons in the caudal pontine reticular nucleus are the sensorimotor interface of the acoustic startle circuit, and pyramidal and giant neurons of the DCN contribute over one-third of their input. The DCN neurons are unlikely candidates as the primary relay limb of the acoustic startle, because their response latencies (4–9 msec; Yajima and Hayashi, 1989) are longer than that of the startle-induced neck muscle response (5–6 msec; Hammond et al., 1972; Pellet, 1990) or the giant neurons of the reticular nucleus (2.6 msec; Lingenhöhl and Friauf, 1994). The acoustic startle reflex, however, is a nondirectional response to a loud sound, but it is typically followed by directional orientation to the sound source (Sokolov, 1975). The pyramidal cells may modulate this secondary orientation reflex, in which case, granule cell input, carrying information about head position, would be necessary for appropriate orientation.

Electrophysiological studies indicate that these anatomical connections are functional. Manipulations of the pinna or direct electrical stimulation of the somatosensory nuclei evoke responses in the DCN that are as strong as those evoked by moderate acoustic stimuli (Young et al., 1995). Data from single-unit and evoked potential recordings indicate that the granule cell system is activated by the somatosensory inputs (Davis et al., 1995). Type IV units, the physiological equivalent of pyramidal and giant cells of the DCN, are inhibited, although there is a small excitatory component buried in the inhibitory response. The proposed mechanism for the overall response is that the parallel fibers, activated by mossy fiber input, synapse on both cartwheel cells and pyramidal cells. The excitatory parallel fibers cause a small excitatory response in the principal cells, but, because the parallel fibers also synapse upon the inhibitory cartwheel cells, inhibition occurs following a short delay. These data suggest that considerable somatosensory input is delivered to the pyramidal cells in the form of inhibitory signals, presumably from the cartwheel cells. This situation is analogous to that of the cerebellum, in which mossy fiber input reaches the deep cerebellar nuclei by way of inhibitory Purkinje cells. One important caveat, however, is that, because this electrophysiological study was done in decerebrate cats, type IV responses may have been altered by the loss of descending input from auditory cortex or inferior colliculus.

Although there have been several behavioral studies that have lesioned the DCN or its output (Masterton and Granger, 1988; Sutherland, 1991; Masterton et al., 1994), few auditory deficits have been uncovered as a result of such a lesion. Structural redundancy in the auditory system, especially at the higher centers, may facilitate compensation for information lost in the periphery, providing that at least one ascending pathway remains intact. With the proper behavioral paradigms, it may be possible to extract those tasks that are best accomplished by the DCN.

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