

Brainstem Branches From Olivocochlear Axons in Cats and Rodents

M.C. BROWN, M.C. LIBERMAN, T.E. BENSON, AND D.K. RYUGO

Departments of Physiology and Otolaryngology (M.C.B., M.C.L.), and Department of Anatomy and Cellular Biology (T.E.B., D.K.R.), Harvard Medical School, Boston, Massachusetts 02115; Center for Hearing Sciences, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205 (D.K.R.); Eaton-Peabody Laboratory, Massachusetts Eye and Ear Infirmary, Boston, Massachusetts 02114 (M.C.B., M.C.L., T.E.B., D.K.R.)

ABSTRACT

Horseshoe peroxidase was used to label axons of olivocochlear (OC) neurons by intracellular injections in cats and extracellular injections in rodents. These axons arise from cell bodies in the superior olivary complex and project to the cochlea. En route to the cochlea, the thick axons ($> 0.7 \mu\text{m}$ diam.) of medial olivocochlear (MOC) neurons formed collaterals that terminated in the ventral cochlear nucleus, the interstitial nucleus of the vestibular nerve (in cats), and the inferior vestibular nucleus (in rodents). The thin axons ($< 0.7 \mu\text{m}$ diam.), presumed to arise from lateral olivocochlear (LOC) neurons, did not branch near the CN. Within the CN, the MOC collaterals tended to ramify in and near regions with high densities of granule cells, regions also associated with the terminals of type II afferent axons (Brown et al.: *J. Comp. Neurol.* 278:581-590, '88). These results suggest that those fibers associated peripherally with outer hair cells (MOC efferents and type II afferents) are associated centrally with regions containing granule cells, whereas those fibers associated with inner hair cells peripherally (LOC efferents and type I afferents) are not.

Key words: cochlear nucleus granule cell, vestibular nucleus, hair cell, axon collateral, efferent nerve

Rasmussen ('46, '53) published the initial descriptions of the olivocochlear bundle (OCB) from its sources in the superior olivary complex to its presumed endings in the cochlea. More recent work (Warr and Guinan, '79; Guinan et al., '83; White and Warr, '83) has divided the olivocochlear (OC) neurons into two systems according to soma location with respect to the medial superior olive: the medial olivocochlear (MOC) system and the lateral olivocochlear (LOC) system. Peripherally, the thick myelinated axons of the MOC neurons terminate mainly on outer hair cells, whereas the thin and apparently unmyelinated axons of LOC neurons terminate predominantly on afferent dendrites just beneath inner hair cells (Liberman, '80; Ginzberg and Morest, '83; Guinan et al., '83; Liberman and Brown, '86; Brown, '87). The anatomical differences between the MOC and LOC systems suggest at least two general types of feedback control of the periphery. At present, however, there is no consensus as to the function of either OC system in the awake, behaving animal. Further studies of the anatomical and physiological characteristics of the efferent systems may offer clues to their functional significance.

The present study concentrates on the morphology of the OC collaterals within the brainstem. These collaterals have not been described systematically, although they are known to innervate the ventral cochlear nucleus (VCN; Rasmussen, '60, '67; Liberman and Brown, '86). We used horseradish peroxidase (HRP) to label collaterals of OC neurons: intracellular injections for physiologically characterized single neurons, and focal extracellular injections for small populations of neurons. Both types of injections can also label primary afferent neurons in the same tissue (Liberman and Brown, '86; Brown et al., '88a). Thus, the methods permit us to directly compare the trajectories and terminations of afferent and efferent fibers in the CN. These descriptions are relevant to an understanding of the relationship of the feedback system to the afferent pathways.

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MATERIALS AND METHODS

Intracellular injections

Single OC units were recorded in the internal auditory meatus from the region near the vestibulocochlear anastomosis in seven Dial-anesthetized (0.73 ml/kg, i.p.) cats as described previously (Lieberman and Brown, '86). At the time of recording, fibers were classified as "efferent" on the basis of 1) position within the nerve bundles, 2) regularity of the interspike intervals, and 3) latency of responses (5–50 msec) to tone bursts (Lieberman and Brown, '86). Neurons were labeled by iontophoresis of HRP, and injections were limited to one or two fibers per animal. After a survival time of about 30 hours, the anesthetized cats were perfused intracardially with glutaraldehyde and paraformaldehyde. The cochlear nucleus was either separated from the brainstem and processed with diaminobenzidine (Fekete et al., '84) or was left attached to the remainder of the brainstem, sectioned on a freezing microtome, and processed with tetramethylbenzidine (Mesulam, '82). Axons in material processed with tetramethylbenzidine could be traced for long distances and their branching patterns could be reconstructed, but the crystalline nature of the label prevented detailed analysis of fiber or terminal morphology. For fine resolution of the terminal regions, material processed with diaminobenzidine was used.

Extracellular injections

Extracellular injections of HRP were made into the spiral ganglion region as described previously (Brown et al., '88a). Data described are from five gerbils (*Meriones unguiculatus*) and eight CD-1 mice (*Mus musculus*). All injections labeled both efferent and afferent fibers. Rodent material was processed with diaminobenzidine.

Electron microscopy

Electron microscopic observations were made from brainstems in two mice. After a brief intracardiac saline rinse, fixation was achieved by perfusion of a mixture of 0.5% paraformaldehyde and 1% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3), followed by 0.5% paraformaldehyde and 3% glutaraldehyde in cacodylate buffer. After 1-hour immersion in the stronger fixative, the brainstem was dissected and stored overnight in the 0.1 M cacodylate buffer. The tissue was sectioned at 50 μm on a Vibratome. Histological processing for HRP was as described by Fekete et al. ('84) with the addition of 1% dimethylsulfoxide to all solutions containing cobalt or diaminobenzidine. The sections were then stained en bloc with osmium tetroxide and uranyl acetate, dehydrated, and embedded in Epon. Labeled fibers and reference marks such as blood vessels were traced from the Epon-embedded tissue sections at the light microscopic level with the aid of a drawing tube. Selected areas containing drawn fibers were cut from the sections and mounted on Epon blanks for ultrathin sectioning and electron microscopy.

Acetylcholinesterase stains

A modification of the Koelle and Friedenwald method was used as described by Osen et al. ('84). Cochlear nuclei were embedded in gelatin-albumin, serially sectioned at 80 μm in either the coronal or modified sagittal plane, and processed as free-floating sections.

RESULTS

Classification and general brainstem course of OC fibers

In seven cats, nine OC fibers were labeled following intracellular injection near the vestibulocochlear anastomosis. Labeled cell bodies were found in brainstem loci that have been described as the origins of the medial olivocochlear (MOC) cell group (Warr and Guinan, '79; Guinan et al., '83). Single OC units respond to monaural sounds presented to either the ipsilateral or the contralateral or to both ears and have tuning curves with a well-defined characteristic frequency (Lieberman and Brown, '86). The units labeled in this study ranged in characteristic frequency from 1.9 to 21 kHz; six responded only to ipsilateral sound, and three responded only to contralateral sound.

The MOC axons course from their cell bodies (Fig. 1) in a dorsal direction, approaching the surface of the brainstem just beneath the fourth ventricle. As the axons travel laterally after approaching the midline, they follow the vestibular nerve root and run ventromedial to the anteroventral cochlear nucleus (AVCN). Within the vestibular nerve root, MOC axons produce collaterals. The MOC axons cross the inferior vestibular ganglion, enter the auditory nerve (at the vestibulocochlear anastomosis), and terminate peripherally on outer hair cells (Lieberman and Brown, '86). The MOC axons are thick (about 2–4 μm diameter in the vestibular nerve root) and have periodic constrictions that we interpret as nodes of Ranvier (Fekete et al., '84; Lieberman and Oliver, '84). Although thin unmyelinated efferent axons are also present at the recording site in the vestibulocochlear anastomosis (Arnesen and Osen, '84), they have not been labeled intracellularly, probably because they are too thin to be impaled with micropipette electrodes. Presumably, these thin axons are from LOC neurons and terminate peripherally near inner hair cells (Brown, '87).

In rodents, extracellular injections of HRP made into the spiral ganglion labeled both thin and thick efferent axons, as well as afferent axons. The reaction product within the efferent axons typically faded before reaching the cell body. These partially reconstructed fibers were identified as efferent because their course was similar to the course of fully reconstructed efferents labeled by intracellular injections. Medially, the efferent fibers could be retrogradely traced as far centrally as the ventral boundary of the vestibular nuclei (Fig. 2, panels 5–7). Laterally, they follow the vestibular nerve root, which forms the ventromedial boundary of the VCN, and exit the brain in the vestibular nerve. Near the inferior vestibular ganglion, the efferent fibers cross to the auditory nerve at the vestibulocochlear anastomosis. In the cochlea, they cross the spiral ganglion and enter the intraganglionic spiral bundle before disappearing into the dense accumulation of reaction product at the injection site. The collaterals given off to the inferior vestibular nucleus (Fig. 2, panels 3, 4) and to the CN (Fig. 2, panels 1, 2) are described below. Afferent fibers, in contrast, are restricted to the auditory nerve, bifurcate after entering the CN, and always end within the CN (Lorente de N3, '81; Fekete et al., '84; Fig. 5 in Brown et al., '88a). The afferent fibers labeled in the present study can often be traced from their cell bodies in the spiral ganglion.

The efferent fibers labeled in extracellular injections fall into two distinct populations based on their diameters (Fig. 3): thick fibers (diameter > 0.7 μm), and thin fibers (diam-

Abbreviations

| | | | |
|------|--------------------------------|---------|---------------------------------------|
| AN | auditory nerve | MSO | medial superior olive |
| ANR | auditory nerve root | MVN | medial vestibular nucleus |
| AVCN | anteroventral cochlear nucleus | N. V | nucleus of tr. V |
| Cb | cerebellum | OC | olivocochlear |
| DCN | dorsal cochlear nucleus | OCB | olivocochlear bundle |
| ICP | inferior cerebellar peduncle | OHC | outer hair cell |
| IHC | inner hair cell | PVCN | posteroventral cochlear nucleus |
| IVG | inferior vestibular ganglion | SCP | superior cerebellar peduncle |
| IVN | inferior vestibular nucleus | Tr. V | spinal tract of Vth cranial nerve |
| LC | locus coeruleus | VCN | ventral cochlear nucleus |
| LSO | lateral superior olive | VNR | vestibular nerve root |
| LOC | lateral olivocochlear | VNTB | ventral nucleus of the trapezoid body |
| LVN | lateral vestibular nucleus | VN | vestibular nerve or nuclei |
| MCP | middle cerebellar peduncle | V | Vth nerve root |
| MOC | medial olivocochlear | VIN | Vlth nucleus |
| MS | medial sheet | VII | VIIth nerve root |
| | | VIII n. | VIIIth nerve |

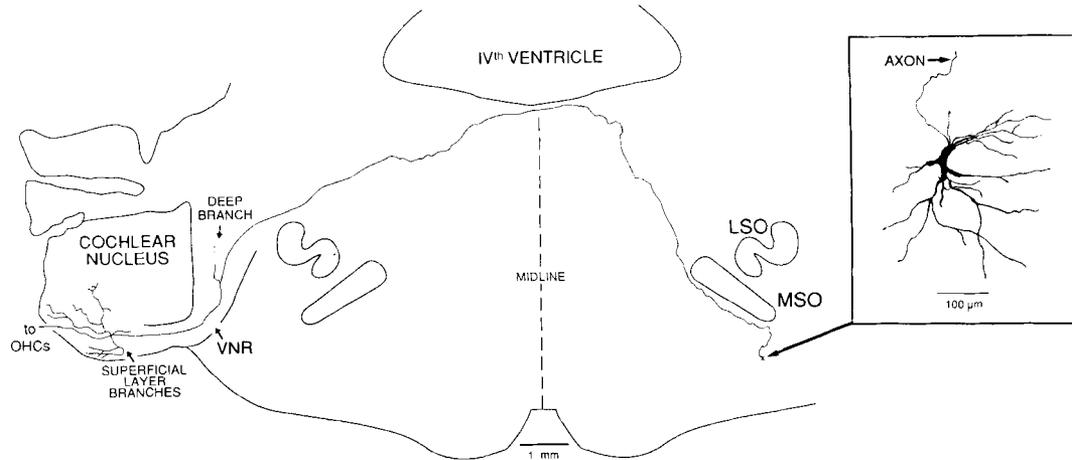


Fig. 1. Tracing the brainstem course and cochlear-nucleus branching pattern of an MOC efferent axon labeled by an intracellular injection of HRP in a cat. The injection site was in the "ipsilateral" vestibulocochlear anastomosis (not shown) and the neuron innervated OHCs in the ipsilateral ear. For monaural sound presentations, the unit responded only to ipsilat-

eral ear stimulation, and the characteristic frequency of its tuning curve was 4.5 kHz. Inset: Camera-lucida drawing of the labeled soma at high magnification. The figure has been reversed to make this illustration of an injection into the right ear similar to the succeeding figures, which are of injections into left ears.

eter $< 0.7 \mu\text{m}$). Throughout their course, from the injection site in the spiral ganglion to the region of the vestibular nuclei (where they usually fade), thick and thin efferent fibers are intermingled but retain their differences in diameter. On the basis of light microscopic criteria (e.g., presence of periodic constrictions that label darkly), the thick fibers appear to be myelinated (Fekete et al., '84) and thus probably correspond to MOC axons, which arise from cells in the medial periolivary region. Only two thick fibers in one mouse could be traced as far as somata in the ventral nucleus of the trapezoid body, a nucleus shown to be the major origin of MOC neurons in rodents (White and Warr, '83). In contrast, the thin fibers have numerous en passant varicosities, lack obvious constrictions (nodes of Ranvier), and label uniformly throughout their length. Most thin efferent fibers probably originate from LOC neurons (Guinan et al., '83; Brown, '87); however, we could not verify this, since the reaction product in these fibers faded before reaching the cell bodies.

In two mice, examples of thick and thin fibers that had been analyzed first in thick sections at the light microscopic level were resectioned for examination in the electron microscope to verify the presence or absence of myelin. For almost all fibers, it was possible to match single fibers drawn with the light microscope with single labeled profiles

in the electron micrographs (Fig. 4). The exceptions consisted of one thick efferent for which a profile could not be found in the electron micrographs and two faintly labeled thin axons found in the electron micrographs that were not seen in the light microscope. A total of 46 thick fibers and nine thin fibers were verified to be myelinated and unmyelinated, respectively. An analogous correspondence has been shown for auditory primary afferent fibers (Ryugo et al., '86).

One of the cases examined in the electron microscope contained two thick axons (about $1\text{-}\mu\text{m}$ diam.) that at the light microscopic level did not show the periodic constrictions characteristic of nodes and were labeled uniformly throughout their course. These axons proved to be unmyelinated at the electron microscopic level. They were the only such examples in our light microscopic examination of hundreds of OC fibers, and their origin is unclear.

Branching patterns of OC fibers

In mice and gerbils, individual thin OC axons were selected from the cluster of fibers labeled in the extracellular injections and followed centrally (Fig. 5). A total of 14 thin axons in four mice and one thin axon in a gerbil could be reconstructed centrally from the saccular ganglion beyond the CN before they faded. Two of these thin axons in mice

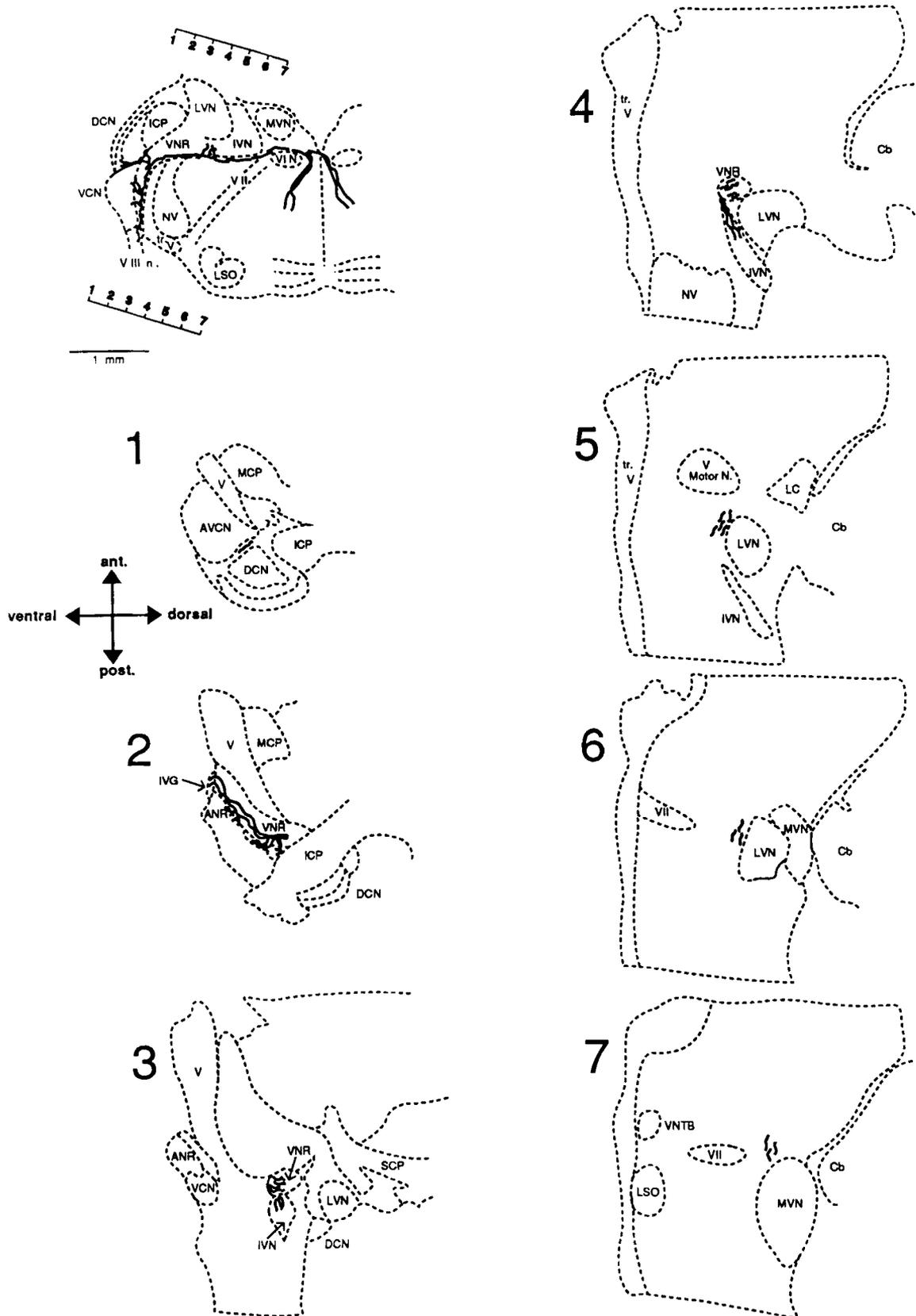


Fig. 2. The course of OC fibers (solid lines) labeled in an extracellular injection in a mouse, shown schematically in a coronal view (upper left), and as actual camera-lucida sketches (1-7) from the tissue sections. The tissue sections were cut in a "modified sagittal" plane as indicated in the

upper left panel. Numbers indicated on the coronal view refer to the positions of the modified sagittal sketches. Sections illustrated are 0.24 mm apart.

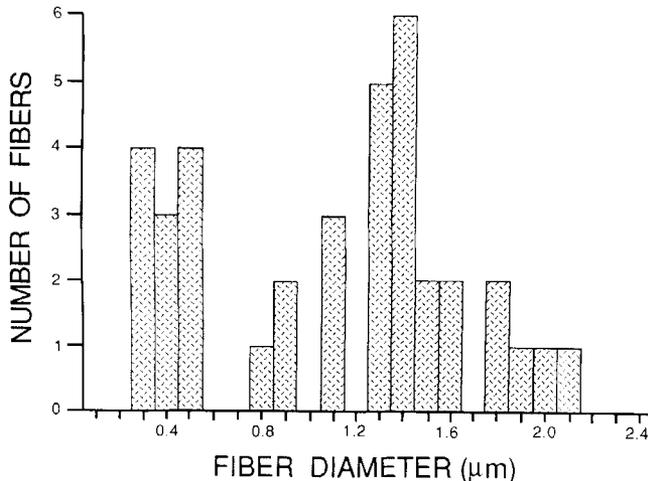


Fig. 3. Axonal diameters for 37 OC fibers that were labeled in a single mouse, and measured in the vestibular nerve root near the vestibular nuclei. Diameters were averaged over segments 10 μm in length and were measured from camera-lucida drawings using a light microscope.

could be reconstructed centrally past the vestibular nuclei. None of the 15 thin fibers formed collaterals in the brainstem.

In contrast, every thick efferent axon, in cats ($n = 4$) or in rodents ($n = 15$), that could be reconstructed from the saccular ganglion to the vestibular nuclei formed one or more brainstem branches (Table 1). Additionally, when efferent branches in the CN were traced back to their source in the OCB (a total of 101 branches), their parent fibers were always thick. The OC branches end within the cochlear or vestibular nuclei, with the few OC axons traceable to somata showing no further collaterals. The branches that end within the CN usually terminate in or near areas having high densities of granule cells, which lie along the lateral and medial edges of the nucleus (Fig. 6). Of the six granule-cell regions¹ described by Mugnaini et al. ('80a), OC branches have been found associated with those encompassing the VCN: 1) the superficial layer of granule cells over the surface of the VCN, 2) the lamina of granule cells dividing the dorsal cochlear nucleus (DCN) from the posteroventral cochlear nucleus (PVCN), 3) the medial sheet of granule cells dividing the VCN from the underlying vestibular nerve root and brainstem, and 4) the subpeduncular corner of granule cells at the dorsomedial edge of the VCN. No OC collaterals from any species were directed toward the granule-cell regions of the DCN—neither to the strial corner of granule cells near the dorsal acoustic stria nor to layer II (the pyramidal or granule cell layer). Most OC terminals were formed in the VCN between granule-cell regions and the subjacent large-cell regions. A few terminals were found within the granule-cell regions and others were within the large-cell regions. The large-cell regions innervated were AVCN, PVCN, and the deep DCN.

In cats, four thick efferent axons produced 13 total branches, with two to five branches produced per axon. They were primarily directed into the superficial granule-cell region of the AVCN (four out of four axons, ten of 13 branches) and to the interstitial nucleus of the vestibular

nerve root (three out of four axons, three of 13 branches), as shown for the neuron in Figure 1. Although the "deep" branch in Figure 1 could not be traced to any of its terminals, collaterals from other fibers clearly terminated in the interstitial nucleus of the vestibular nerve, as defined by Brodal and Pompeiano ('57). Axonal branching to both the CN and the interstitial nucleus of vestibular nerve was found for units with low or high characteristic frequencies and for units responsive to ipsilateral or contralateral sound.

In mice and gerbils, the number of collateral branches produced per axon is fewer than in cats and their regional distribution is also somewhat different (Fig. 7). Whereas collaterals in cat innervated the superficial granule-cell layer, branches to this region in rodents were very rare. In addition, the "deep" branches in cat ended in the interstitial nucleus of the vestibular nerve, whereas those in rodents ended in the inferior vestibular nucleus. In one gerbil, seven axons were completely reconstructed² from the saccular ganglion to the vestibular nuclei; these axons produced a total of 12 branches (one or two produced per axon). The branches were directed toward the medial sheet of granule cells in rostral AVCN (three of 12 branches), the medial sheet in the region of the auditory-nerve root (two of 12 branches), and the inferior vestibular nucleus (seven of 12 branches). Eight axons were completely reconstructed from one injection in a mouse, producing a total of 11 branches, one or two produced per axon. The branches were directed toward the lamina of granule cells dividing PVCN from DCN (three of 11 branches), the medial sheet of granule cells near the region of the auditory-nerve root (two of 11 branches), or the inferior vestibular nucleus (six of 11 branches). Some axons formed collaterals only to the cochlear nucleus—others only to the inferior vestibular nucleus; when there were two branches, both nuclei were innervated (Fig. 5).

OC collaterals formed in the vicinity of the vestibular nuclei in rodents were confined to the vestibular-nerve root region near the inferior vestibular nucleus and to the inferior nucleus itself. This nucleus extends posteriorly from the vestibular nerve root and contains small and medium-sized cells amongst the descending branches of the vestibular afferent fibers. OC collaterals terminated only in the rostral portion of the inferior nucleus (Fig. 2, panels 3, 4) and ran parallel to the vestibular fibers before terminating in neuropil, sometimes near small cells.

The finding of OC collaterals directed to vestibular nuclei raises the issue of whether OC collaterals are also directed into the vestibular periphery. We found no such collaterals in our material. In three mice and three gerbils, a total of ten thin and 40 thick fibers were traced from the injection site in the spiral ganglion centrally across the inferior vestibular ganglion and into the vestibular nerve root. None of these fibers formed branches directed into the vestibular nerves or vestibular ganglia. One of the thick fibers formed a branch near the inferior vestibular ganglion, but this branch travelled to the CN. Two other thick fibers and one thin fiber branched in the modiolus, but all branches were directed into the auditory nerve toward the cochlea. It is more difficult to address the issue of MOC fiber branching to the vestibular periphery in cats, since our cat tissue was divided to process the temporal bone separately from the

¹Although separate granule-cell regions can be defined, the regions merge at boundaries, forming a continuous granule-cell domain.

²A reconstruction was considered complete if there was no fading of any part of the axon and each branch could be traced to a terminal swelling.

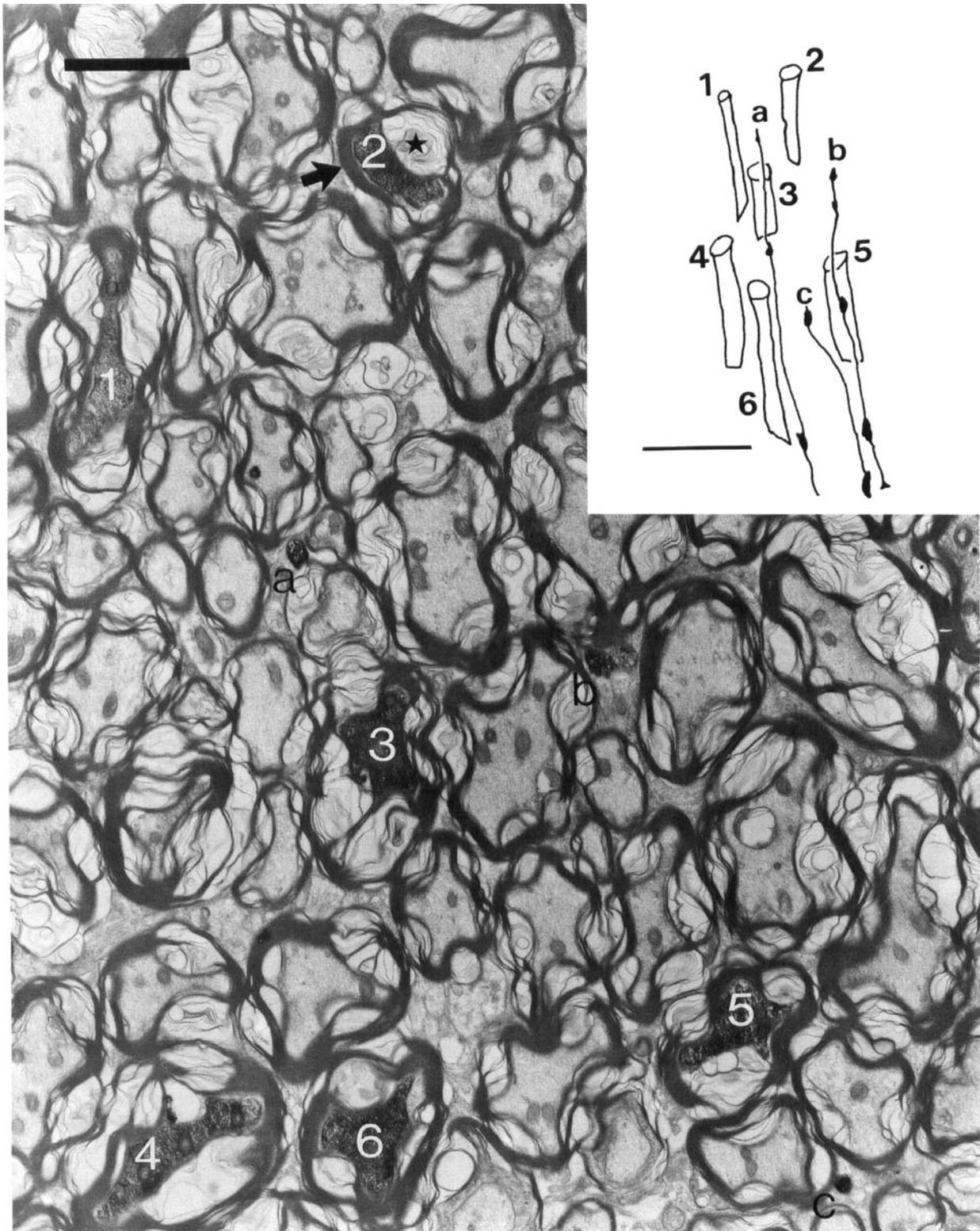


Fig. 4. Camera-lucida drawing of several labeled fibers as seen in the light microscope (inset), and an electron micrograph of a section through the same tissue. The section is from the OCB within the vestibular nerve root near the vestibular nuclei. Fibers classified in the light microscope as thick are numbered and those classified as thin are lettered. Fibers are only drawn near the top of the tissue section, and the electron micrograph was

taken from this same region. This micrograph is part of a montage $200 \times 280 \mu\text{m}$ in which 41 thick and 8 thin labeled profiles were found. Histological processing produces widespread myelin delamination (e.g., star at 2) although myelin lamellae relatively free of artifact are apparent (arrow at 2). The scale bar in the camera-lucida drawing represents $10 \mu\text{m}$ and the scale bar in the electron micrograph represents $1 \mu\text{m}$.

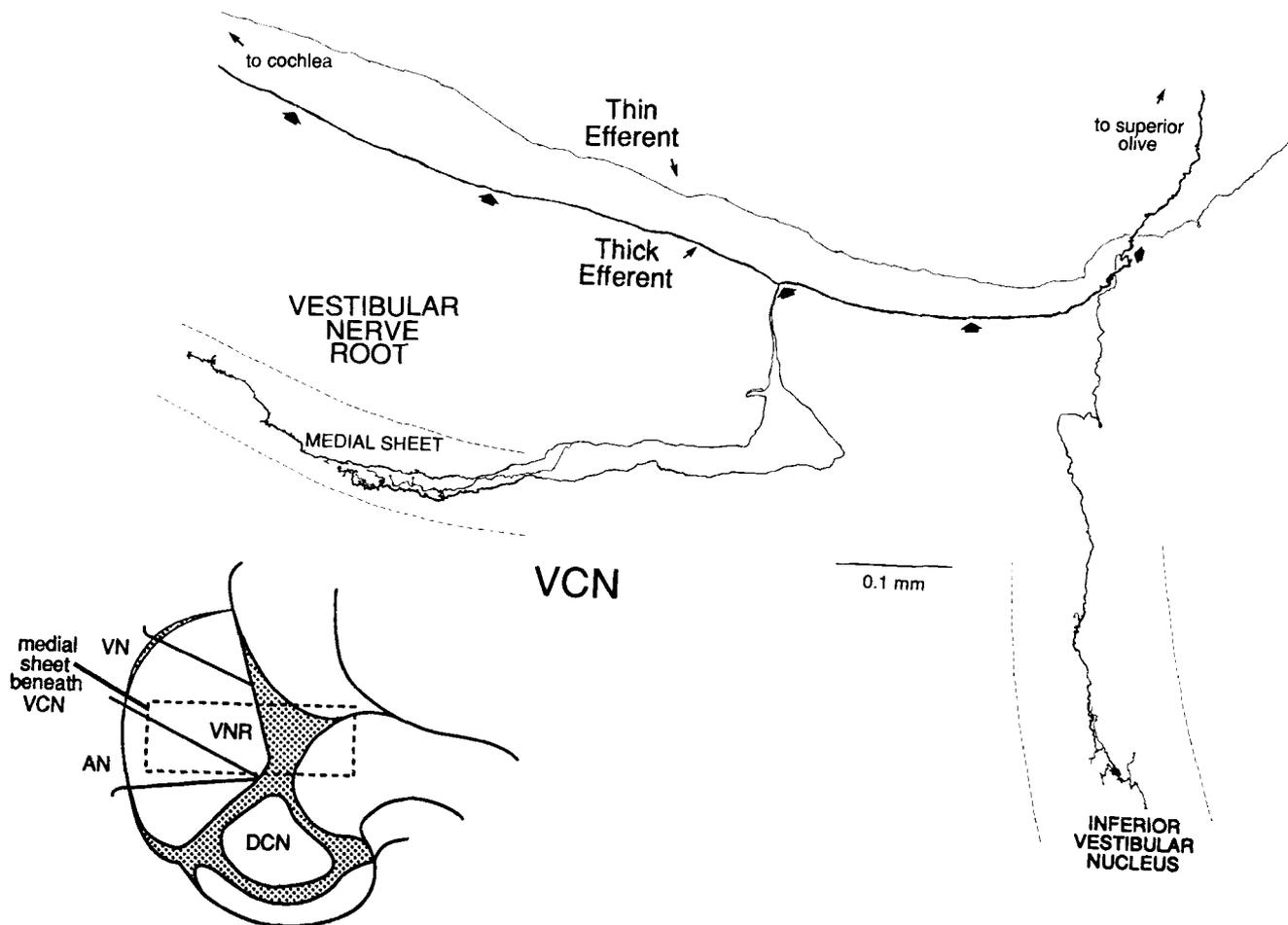


Fig. 5. Camera-lucida reconstruction of a single thick OC axon and a single thin OC axon in the vestibular nerve root of a mouse (modified sagittal plane). The thick axon branches to the medial sheet of granule cells (dashed lines) along the medial border of the ventral cochlear nucleus (VCN)

and to the inferior vestibular nucleus (dashed lines). The thin axon is unbranched. Large arrowheads indicate nodes of Ranvier on the thick axon. Inset: Low-power drawing illustrating the position of the reconstruction that is near the CN.

brainstem. Although it is conceivable that branches were missed at this separation, we saw no indications that MOC fibers branched to the vestibular periphery.

Fibers of the OCB stain positively for acetylcholinesterase (AChE), and this observation has been used to explore the course of the OCB and its endings in the cochlea (Churchill and Schuknecht, '59; Ishii and Balough, '68) as well as the CN (McDonald and Rasmussen, '71; Martin, '81; Osen et al., '84). Our AChE-stained material from cats, gerbils, and mice is consistent with these earlier AChE studies. AChE-positive fibers form a compact bundle within the vestibular nerve root. Some AChE-positive fibers enter the VCN from its medial aspect, and AChE-positive fibers can be found in all regions innervated by HRP-labeled OC collaterals described above. Other AChE-positive fibers however, are also seen in the strial corner of the DCN and to some extent throughout the superficial layer of the DCN proper—regions where HRP-labeled OC branches have not been seen. It is thus likely that these AChE-positive fibers originate from sources other than OC efferents.

Terminal morphology

The brainstem branches of thick OC axons in cats and rodents can be divided into "nonterminal" regions where swellings are uncommon and "terminal" regions where

swellings and branchlets are plentiful. In practice, we defined the beginning of the terminal region as the point at which there were more than five swellings or branch points within a 20- μ m length of the branch. About 60% of all branches appeared to be myelinated (having periodic, darkly labeled constrictions or nodes of Ranvier) in the nonterminal region, but all appeared to be unmyelinated in the terminal region. Branch morphology and length differ according to the region innervated (Figs. 8, 9). Branches within the vestibular nerve root in cats and to the vestibular nuclei in rodents are shorter and thinner than those directed to the lamina and the medial sheet of granule cells in the CN. Vestibular branches, like CN branches, form obvious en passant and terminal swellings in their terminal regions (Fig. 10), and the sizes of the the terminal swellings in vestibular nuclei are not obviously different from those in the CN. Vestibular collaterals, however, tend to form fewer swellings per collateral than those directed to the CN (Fig. 9). In cats, the branches to the CN are longer than in rodents, because in the cat the CN is larger and the branches are directed toward the superficial granule-cell layer in the VCN, away from vestibular nerve root. One branch in cat showed more than 160 en passant and 50 terminal swellings—about four times the number typically found in rodents (Fig. 9). In all species, and in both

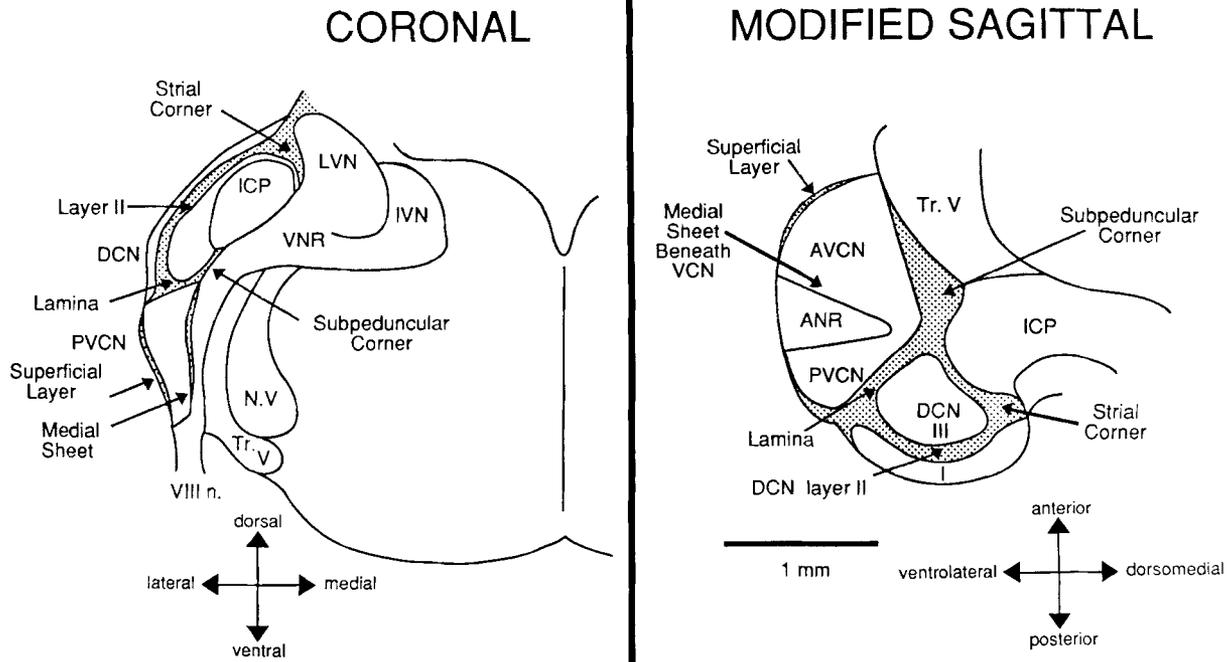


Fig. 6. Schematic of the granule-cell regions of the left cochlear nucleus in a mouse from cresyl-violet-stained sections. The same regions are illustrated as they would appear in two section planes, coronal and modified sagittal. The mammalian CN consists of a layered dorsal cochlear nucleus (DCN) and a non-layered ventral cochlear nucleus (PVCN, AVCN), which are separated by a lamina of granule cells. DCN granule cells are abundant

in the strial corner and in layer II. VCN granule cell regions cover the lateral surface of the nucleus (superficial layer) or delineate the boundary of the CN from surrounding brainstem structures (medial sheet and subpeduncular corner). Some granule cells (not illustrated) are found scattered throughout the main body of the CN.

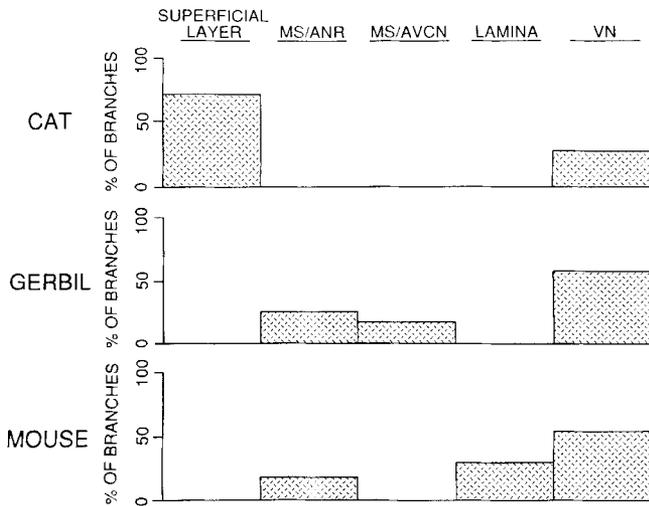


Fig. 7. Graphic distribution of thick OC branches to various subdivisions within the brainstem for axons that could be completely reconstructed. The data are from four axons in cats, seven axons in a gerbil, and eight axons in a mouse. MS/ANR: MS beneath ANR; MS/AVCN: MS beneath AVCN.

the cochlear and vestibular nuclei, OC terminals were usually found in neuropil and only occasionally on somata.

When several fiber types are labeled, as in the CN of our rodent preparations, the distinctive appearance of the OC

TABLE 1. Percentage of Efferent Axons Forming Branches

| | Cochlear nucleus | Vestibular nuclei |
|-----------------------|--------------------------------|--------------------------------|
| Rodents | | |
| Thin efferents | 0% (0 branches/ 15 axons) | 0% (0 branches/ 2 axons) |
| Thick efferents | 67% (10 branches/ 15 axons) | 87% (13 branches/ 15 axons) |
| Cats | | |
| Thick efferents (MOC) | 100% (10 branches/ 4 axons) | 75% (3 branches/ 4 axons) |

branches makes them easily distinguishable from afferent fibers (Fig. 11). OC branches are reminiscent of the branches of "group VI" axons described by Cant and Morest ('78) in Golgi stains of the CN. The thin branches of type II afferents often enter the granule-cell regions but are separable from efferent branches, because they are thinner and their endings are smaller. The smooth terminal branches of the type I axons have rounded swellings, whereas the OC branches are quite varicose in the terminal regions, and the swellings are serrulate and more finely divided.³ For efferent collaterals, the entire terminal region appears to be unmyelinated, since it is uniformly and darkly labeled.

³Cat efferent collaterals, although quite varicose, tend to form smoother swellings than rodent collaterals.

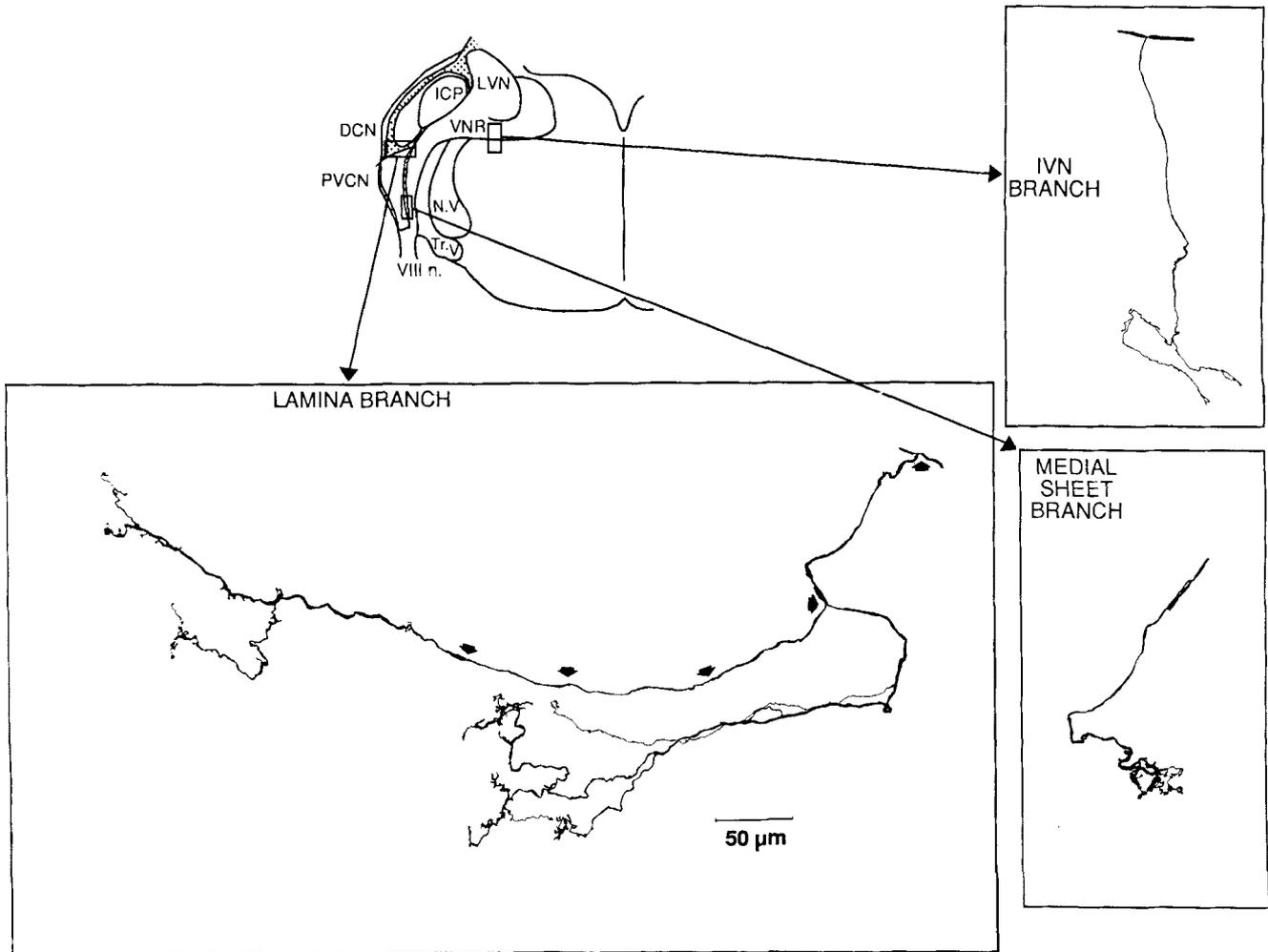


Fig. 8. Camera-lucida tracing of thick OC branches from different axons, directed to three different regions in a mouse. Top diagram of brainstem cross section indicates regions of innervation and stippling represents gran-

ule cell regions. Boxed figures illustrate collateral morphology as seen in the modified sagittal plane. Arrowheads on lower tracing indicate obvious nodes along the nonterminal region of the branch.

This distinctive pattern is reminiscent of the peripheral morphology of OC axons, where thick efferent branches appear to be unmyelinated for long distances in the osseous spiral lamina and in the organ of Corti before innervating the outer hair cells. Type I afferent branches in CN appear to be myelinated to the vicinity of their endings, just as their peripheral processes are myelinated to a point within 50–100 μm of the inner hair cells that they contact (Lieberman, '80; Brown, '87).

DISCUSSION

Branching patterns of efferent and afferent fibers

Results of this study suggest an innervation plan for the CN that maintains separate pathways for the neurons innervating outer hair cells and those innervating inner hair cells (Fig. 12). The afferents contacting outer hair cells originate from type II spiral ganglion cells (Spendlin, '69; Kiang et al., '82), and the efferents to outer hair cells are provided by the thick axons of MOC neurons (Warr and Guinan, '79; Guinan et al., '83; Liberman and Brown, '86; Brown, '87). Within the CN, these afferent and efferent fibers to the outer hair cells have terminal swellings asso-

ciated in part with granule-cell regions. In contrast, the neurons to the inner hair cell region, type I afferents and LOC efferents terminating on the dendrites of type I afferents (Smith and Rasmussen, '63; Liberman, '80), are not obviously associated with granule-cell regions in the CN. This overall regional difference in CN trajectory does not rule out the possibility of overlap in the neural networks associated with the two hair cell groups. For instance, within the main body of the CN, there is significant overlap in axonal trajectory and swelling location from type I and type II afferents, suggesting shared postsynaptic targets (Brown et al., '88a). However, further observations with the electron microscope are required to resolve this issue.

The evidence that MOC neurons produce thick, myelinated axons directed to outer hair cells and that LOC neurons produce thinner, unmyelinated axons directed to inner hair cell regions has been reviewed by Guinan et al. ('83). The present results are consistent with this view and further suggest that the formation of collaterals to the CN is common for MOC axons and is absent for LOC axons. If our small sample is representative, it indicates another fundamental difference between the MOC and LOC efferent systems. However, several other issues need to be considered.

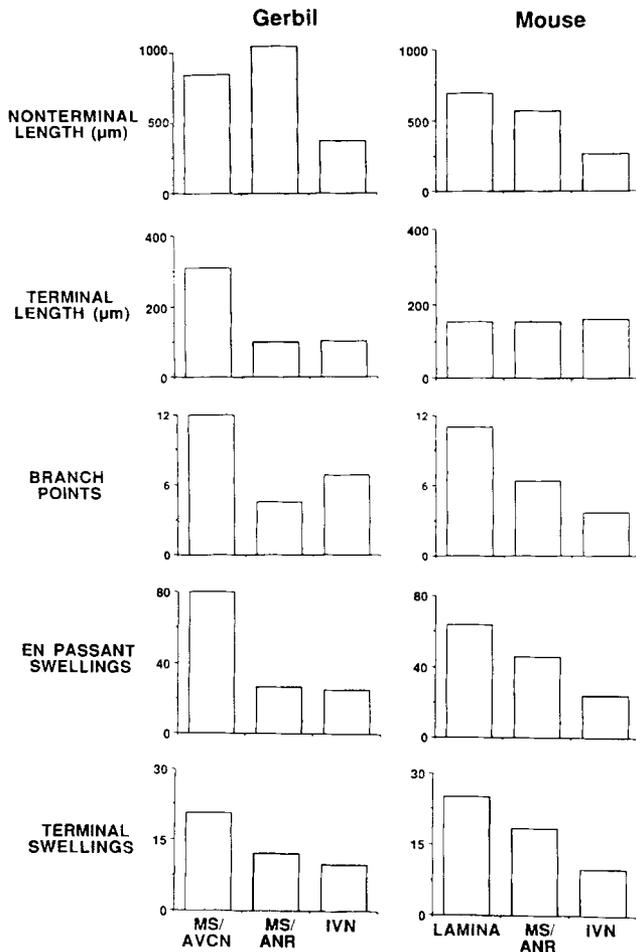


Fig. 9. Quantitative analysis of thick OC branches in gerbils and mice. Each bar graph represents an average value per collateral. Averages are for the following branches in gerbils: two branches to MS/AVCN, two branches to MS/ANR, and seven branches to IVN (inferior vestibular nucleus); and for the following branches in mice: three branches to the lamina, two branches to MS/ANR, and six to IVN. The nonterminal length is the distance from the parent fiber to the point where the terminal region begins, defined as the first point where there are at least five swellings and/or branch points within 20 μm . The terminal length represents the sums of the lengths of all branches in the terminal region. En passant swellings were counted if their diameters were twice the diameters of the parent fibers and terminal swellings were counted if the branches forming them were longer than 3 μm .

First, the thin LOC axons may form fine collaterals that do not transport HRP as well as the thicker collaterals of the MOC axons. This does not seem likely, since the thin axons from type II neurons, labeled in the same injections, give off fine branches that are easily traced within the cochlear nucleus (Brown et al., '88a). An additional consideration is that LOC neurons may form collaterals in the brainstem in a region considerably medial to the cochlear nucleus. It is not possible to address this question with our material, since the reaction product in LOC axons faded before reaching the cell bodies. However, thin efferent fibers labeled in the present study certainly do not branch near the cochlear nucleus. Finally, the efferent fibers labeled in the present study innervate predominantly the basal turn, since the HRP injections are made through the round window. Although it might be suggested that thin efferent fibers in-

nervating the apical turns branch near the CN, preliminary data from other HRP injections made into the apical turn in mice suggest that this is not the case (A.M. Berglund, personal communication).

The branching differences for MOC and LOC axons are consistent with other HRP studies of the projections of the olivary complex to the CN. After HRP injections in the CN, retrogradely labeled neurons were never found (Covey et al., '84; Spangler et al., '87) or rarely found (Adams, '83) in regions known to be the origins of the LOC system, whereas many labeled somata were found in medial periolivary regions (origins of the MOC system), suggesting a prominent descending input to the CN. However, recent autoradiographic studies of OC projection is in the gerbil come to a different conclusion (Ryan et al., '87). Following cochlear incubation with ^3H -D-aspartic acid, LOC (but not MOC) somata were labeled in the brainstem and labeled fibers were seen entering the CN where they terminated in the central region of the VCN, a region where no OC collaterals were seen in the present study. These data were interpreted to indicate that LOC fibers branch to innervate the CN. This conflict is not easily resolved unless the thin-fiber projection to the CN involves a small portion of the LOC neurons. The HRP method has the advantage that axons are continuously stained and can be individually reconstructed, but its disadvantage is that relatively small sample sizes are obtained. Thus the present data certainly do not rule out the possibility that a subset of LOC neurons sends branches to the CN.

Our data on the innervation of the cochlea and CN by thin fibers are most comprehensive in the rodents (mice, gerbils, and guinea pigs). The labeling studies have been most productive in smaller species, because it appears that the thin axons only transport HRP over limited distances (Brown et al., '88a). In cats, the central projections of type II ganglion cells have been only partially reconstructed (Ryugo et al., '86; Benson and Ryugo, '87), and there are virtually no published data on collaterals of LOC axons in cats. Nevertheless, the innervation plan based on data from rodents is not contradicted by the cat material. There might be considerable differences in primates, which apparently lack OC collaterals to the cochlear nucleus. This situation may be related to the observation that the primate CN contains few if any granule cells (Moore, '80).

MOC innervation of the vestibular nuclei

The consistent finding of OC efferent collaterals to vestibular nuclei suggests a relationship between certain portions of the auditory and vestibular system. Although other investigators (Adams and Mugnaini, '87) found collaterals to these regions, their sources were not identified. One potential source of such collaterals is vestibular efferent axons. As conventionally defined, the fibers labeled in the present study are auditory, not vestibular, efferents, because 1) they innervate the organ of Corti, 2) they do not innervate the vestibular periphery, and 3) their cell bodies are located in and around auditory nuclei, in areas reported to be the origin of cochlear efferents. Thus, in mammals, single efferent neurons do not appear to innervate auditory and vestibular endorgans, a situation clearly different from urodele amphibians, in which a common efferent system innervates both the inner ear and lateral line (Fritzsch and Wahnschaffe, '87).

Axon degeneration studies have shown that the inferior vestibular nucleus receives input primarily from the utricle

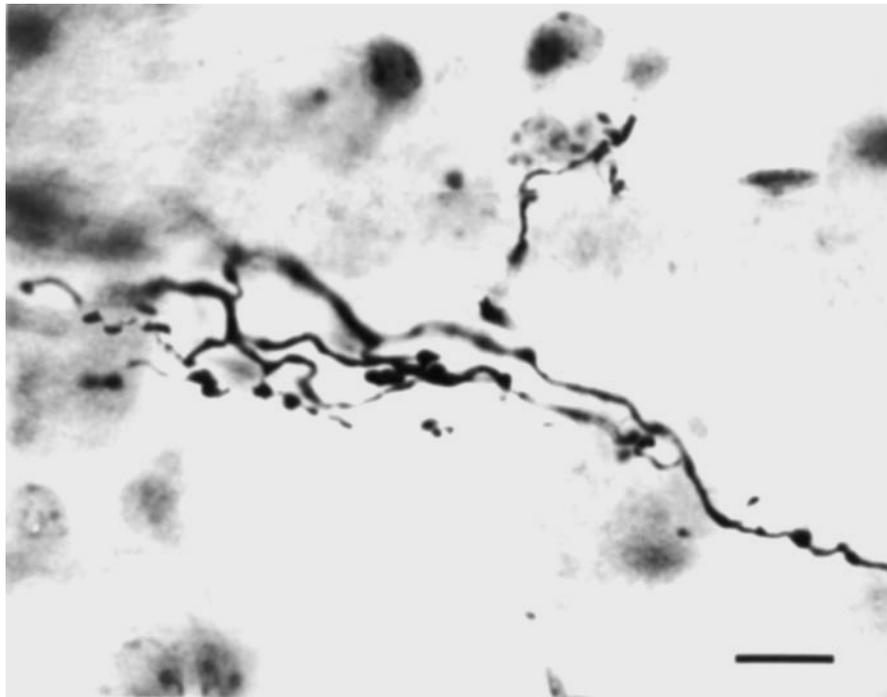


Fig. 10. Montage photomicrograph of an OC collateral in the inferior vestibular nucleus of a mouse. Scale bar represents 10 μ m.

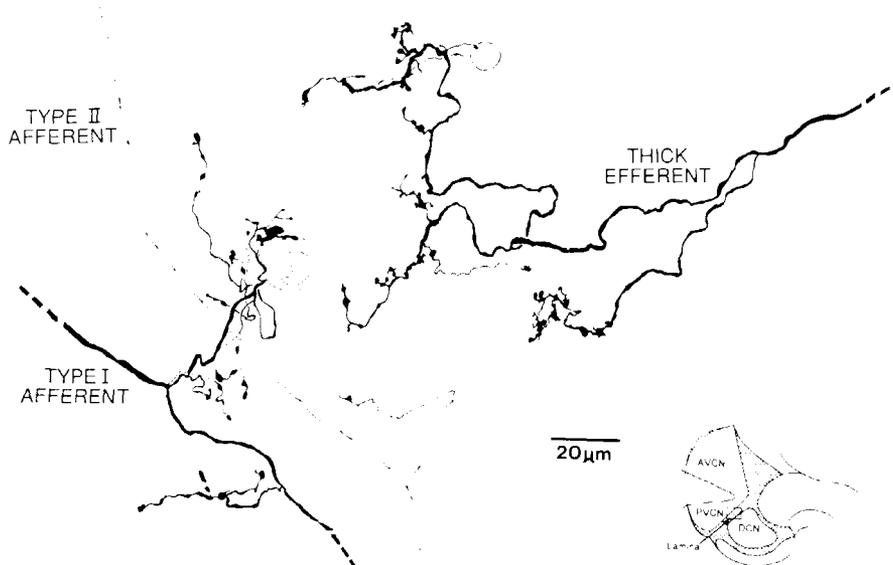


Fig. 11. Camera-lucida tracings of the three classes of labeled fibers that terminate in the CN. These fibers are ending in the same tissue section (80- μ m thickness) near the lamina in the PVCN (see inset) in a mouse. The type I afferent is terminating deep to the lamina near large cells, one of which is

outlined. The thick OC efferent is terminating more superficially in the lamina. (One granule cell is outlined by the small circle.) Inset: Schematic showing the position of the enlarged drawing.

and the saccule (Stein and Carpenter, '67). Saccular projections to the inferior vestibular nucleus and to the interstitial nucleus of the vestibular nerve have subsequently been demonstrated in HRP studies (Kevetter and Perachio, '85; Didier et al., '87). The saccule serves an auditory function in fish (Furukawa and Ishii, '67) and may have an auditory function in mammals as well, at least for sounds of moderate and high levels (Cazals et al., '83). In this context, the

presence of OC collaterals to the vestibular nuclei reinforces the idea that portions of these nuclei also have auditory functions.

MOC innervation of the cochlear nucleus

The mammalian CN has been subdivided according to the projection of the auditory nerve onto the CN and the distribution of cell types within the CN (Brawer et al., '74;

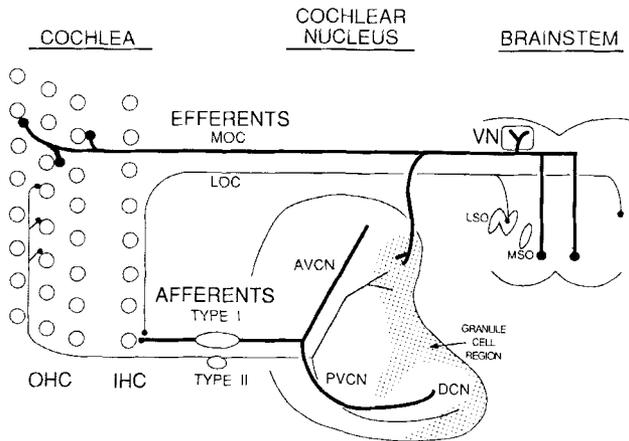


Fig. 12. Schematic diagram of the OC efferent and primary afferent innervation of the cochlea and CN. Thick lines indicate thick axons ($> 0.7 \mu\text{m}$ diameter) and thin lines indicate thin axons ($< 0.7 \mu\text{m}$). In cats, the LOC somata are on the margins of the LSO as drawn (Warr, '75); in rodents, the somata of the LOC neurons are within the LSO (White and Warr, '83).

Lorente de Nó, '81). The granule-cell "regions," where MOC collaterals terminate, form a rind around the main nucleus (Mugnaini et al., '80a). These regions receive other inputs besides MOC efferents, even from nonauditory sources, including neurons in the cuneate nucleus and spinal trigeminal nucleus (Weinberg and Rustioni, '87; Itoh et al., '87). The granule-cell regions contain granule and Golgi cells (Mugnaini et al., '80a), as well as other cell types (McDonald and Rasmussen, '71). The most common cell type, the granule cell, shares many anatomical features with its counterpart in the cerebellum, including its status as the smallest neuron in the nucleus and the participation of its dendrites in neural "glomeruli" clustered around a large mossy ending (Mugnaini et al., '80a,b). The synaptic connections between inputs and cell types within the granule-cell regions of the CN, however, are not well understood.

Our results are consistent with a previous suggestion that MOC efferents are a source of mossy endings in the VCN (McDonald and Rasmussen, '71). Type II afferent terminals are probably too small (Brown et al., '88a), whereas the largest MOC terminals are in the appropriate size range. Some of the mossy endings have been shown to be AChE-positive (McDonald and Rasmussen, '71), which is also consistent with MOC efferents being their source. Mossy endings that are not AChE-positive may also be from the OC system since not all OC efferents stain for AChE (Vetter et al., '86). Many of the en passant and terminal swellings formed by MOC collaterals, however, are probably too small to form mossy endings. These may correspond to the smaller boutons, another class of AChE-positive endings studied by McDonald and Rasmussen ('71). Although mossy fibers clearly innervate granule and Golgi cells, the postsynaptic targets of the bouton endings are unknown.

Type II afferents and MOC efferents, which converge on granule-cell regions, have overlapping distributions in some areas but not in others. Endings from these two fiber types are in close proximity in the lamina, the subpeduncular corner, and the interstitial nucleus of the cochlear nerve. However, only MOC fibers appear to terminate in the medial sheet and only type II neurons appear to end in layer II of the DCN. Light microscopic observations indicate that

the endings of type I neurons do not usually invade granule-cell regions (Fekete et al., '84; Brown et al., '88a).

Although observations with the light microscope are suggestive, further work with the electron microscope is necessary to determine the postsynaptic targets for OC collaterals in the CN. The only physiological studies of the effects of efferent stimulation on responses of single CN cells have shown complex effects, sometimes inhibitory, sometimes excitatory (Starr and Wernick, '68; Comis and Whitfield, '68; Comis, '70). This complexity must reflect interaction between direct effects mediated monosynaptically via OC collaterals or multisynaptically through interneurons and indirect effects mediated by suppression of the responses of inner hair cells (Brown and Nuttall, '84) and type I afferents (Wiederhold and Kiang, '70). The effects of OC stimulation on CN electrophysiology should be reinvestigated by using modern concepts of efferent anatomy and cell classification of single units in the CN. Sound-driven activity in the OC collaterals may provide the CN with information regarding the amount of feedback the MOC neurons are providing to the hair cells, thereby calibrating the nucleus for incoming messages from the auditory nerve or adjusting the gain of the afferent signal relayed to the MOC neurons themselves (Kim, '84; Guinan and Gifford, '88). In this context, the granule-cell regions become important intermediates in the MOC feedback system.

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