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# Central Projections of Cochlear Nerve Fibers in the Alligator Lizard

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ABSTRACT

The auditory (cochlear) ganglion cells of the alligator lizard (*Gerrhonotus multicarinatus*) give rise to two types of peripheral fibers: tectorial fibers, which contact hair cells covered by a tectorial membrane, and free-standing fibers, which contact hair cells without a tectorial membrane. To determine the central projections of these fibers, we applied intracellular and extracellular injections of horseradish peroxidase (HRP) to the peripheral component of the cochlear nerve. After histological processing with diaminobenzidine, individual cochlear nerve fibers could be traced through serial sections with the aid of a light microscope and drawing tube. The projection patterns formed two morphologically distinct groups. Neurons whose peripheral processes contacted tectorial hair cells in the cochlea projected to three divisions of the cochlear nucleus: nucleus magnocellularis lateralis (NML), nucleus magnocellularis medialis (NMM), and nucleus angularis lateralis (NAL). Neurons whose peripheral processes contacted free-standing hair cells projected primarily to the nucleus angularis medialis (NAM), although some also sent a single, thin branch to the NML; these neurons never projected to NAL or NMM. Morphometric comparisons of tectorial and free-standing fibers demonstrate that tectorial fibers have a larger axonal diameter, form a greater number of terminal swellings, and make proportionally more somatic contacts. By correlating the morphologically defined groups with previously reported physiologically defined groups, we conclude that different divisions of the cochlear nucleus are associated with separate frequency ranges and that stimuli in the different frequency ranges may be processed separately in the brain.

**Key words: axon, cochlear nucleus, comparative anatomy, hearing, horseradish peroxidase, primary afferent, reptile, synaptic endings**

The cochlear nerve in lizards conveys auditory information from the hair cells of the basilar papilla to the ipsilateral cochlear nucleus. Although the anatomy and physiology of the peripheral auditory system of lizards have been relatively well described (Mulroy, '74, '83, '86; Weiss et al., '74, '76, '78; Bagger-Sjöbäck, '76, Manley, '77; Holton and Weiss, '78, '83; Wever, '78; Miller, '80, '85; Eatock et al., '81), little is known about the central auditory system. Knowledge of the central projections of reptilian cochlear nerve fibers has been based on anterograde degeneration techniques and whole nerve stains (Beccari, '12; Hamilton, '63; Leake, '74; DeFina and Webster, '74; Foster and Hall, '78; Barbas-Henry and Lohman, '88). These studies describe the distribution of nerve fibers and terminals within the dorsal medulla for the entire nerve fiber population but do not provide information on arborization patterns or morphology of terminals.

In many species of lizards, the basilar papilla has more than one population of hair cells (Mulroy, '68, '74; Baird and Marovitz, '71; Bagger-Sjöbäck, '76; Miller, '80). A common situation is that one population has hair cells all having kinocilia oriented in the same direction and a second population with some hair cells having kinocilia oriented in one direction and some in the opposite direction. In the alligator lizard, the hair cells in the unidirectional region are covered by an overlying tectorial membrane and can be called tectorial hair cells. The bidirectional hair cells in this species are not covered by a tectorial membrane and can be

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called free-standing hair cells. Intracellular recordings show that tectorial and free-standing hair cells respond differently to acoustic stimulation (Weiss et al., '74). Morphologically, the two types of hair cells make different types of contacts with the peripheral processes of cochlear nerve fibers (Miller, '85; Mulroy and Oblak, '85; Mulroy, '86). These differences may be reflected in the physiology of single cochlear nerve fibers with respect to tuning-curve shapes, spontaneous discharge rates, characteristic frequencies (CF; frequency to which the fiber is most sensitive), and other electrophysiological characteristics (Frezza, '76; Weiss et al., '76, '78; Manley, '77; Holton and Weiss, '78; Turner et al., '81; Turner, '87). In the alligator lizard, tectorial fibers (which contact tectorial hair cells) have low CFs (100–800 Hz), low spontaneous discharge rates (<25 s/s), and sharp, asymmetric tuning curves. In contrast, free-standing fibers (which contact free-standing hair cells) have high CFs (900–4,000 Hz), high spontaneous discharge rates (>25 s/s), and broad symmetric tuning curves. These data demonstrate a clear dichotomy between the two populations of hair cells and primary fibers in the auditory periphery and suggest that the two populations of fibers may differ in their central projections. In the present study, horseradish peroxidase (HRP) techniques were applied to stain individual cochlear nerve fibers in order to address this issue. In these preparations, fibers could be traced with the light microscope and subjected to morphometric analyses.

## MATERIALS AND METHODS

A total of 18 adult alligator lizards (*Gerrhonotus multicarinatus*), each weighing between 10 and 40 g, were used in this study. The lizards were anesthetized by intraperitoneal injections of a 25% urethane solution dissolved in distilled water at a dosage of 3.13 mg/g body weight. In the 16 HRP experiments, the middle ear was exposed by a ventral pharyngotomy and the inner ear was exposed by removing the round window membrane and some of the surrounding bone (described in detail by Weiss et al., '74). This exposure made it possible to view the cochlear nerve and the basilar papilla through the scala tympani.

### Protargol staining

One lizard was perfused through the heart with a lizard Ringer's solution containing 1% sodium nitrite (30 ml, pH 7.2), followed by 120 ml of a slightly modified Bouin's fixative (0.9% picric acid, 25% formalin, 5% glacial acetic acid) and postfixed overnight in the same fixative. The next day the brain was dissected out of the head, and the Bouin's fixative was leached out of the brain in a 10% formalin solution over a period of 1 week. The brain was dehydrated, embedded in paraffin, cut on a rotary microtome into 15-$\mu$m-thick horizontal sections, and mounted onto gelatin-coated slides. The brain was stained by using a protargol method (Bodian, '36).

### Cresyl violet staining

One lizard was perfused through the heart with a 20 ml wash of 0.15 M phosphate buffer, pH 7.4, and then with 100 ml of a fixative containing 4% glutaraldehyde, 1% paraformaldehyde, 0.2% picric acid, and 4% sucrose, in a 0.15 M phosphate buffer, pH 7.4, at room temperature (Barbas-Henry and Wouterlood, '88). The head was postfixed overnight in the same fixative. The next day the brain was dissected out of the head, embedded in a gelatin-albumin mixture (Frank et al., '80), and sectioned in the horizontal plane into 50-$\mu$m-thick sections on a vibratome. The sections were mounted onto gelatin-coated slides, air dried overnight, and stained with a 0.5% cresyl violet solution.

### Physiological recording and HRP injection

The technical details of the acoustic stimulus generation and recording have been previously described (Weiss et al., '76; Holton and Weiss, '83). The intracellular injection of HRP into individual cochlear nerve fibers is similar to the method used in studies of the cat cochlear nerve (Liberman, '82). Briefly, the micropipette electrodes for the intracellular labeling experiments were backfilled with a filtered solution containing 10% HRP (Sigma type VI), 0.15 M KCl, and 0.05 M Tris buffer, pH 7.3. In the one successful case, the initial impedance was 60 MΩ and the pipette was bevelled (30° off horizontal) to a final resistance of 38 MΩ.

The extracellular labeling experiments involved procedures similar to those used for the intracellular recording and injection experiments. The micropipettes had tips that were broken to 9–13 $\mu$m I.D. and were backfilled with a solution containing 30% HRP and 0.05 M Tris buffer, pH 7.3. The injection of HRP was made (>40 nA positive current, 50% duty cycle, 15 min duration) into the cochlear nerve peripheral to the intraotic ganglion. To place the micropipette in the nerve, the compound action potential in response to an acoustic click was monitored while the electrode was advanced. When the compound action potential was at its maximum amplitude, the HRP was iontophoretically injected into the nerve.

### HRP histochemistry

Approximately 15–30 hours after the last injection of HRP, each animal was perfused through the heart with a lizard Ringer's solution containing 1% sodium nitrite (30 ml, pH 7.2), followed by 60–100 ml of fixative (2.5% glutaraldehyde, 0.5% paraformaldehyde, in 0.1 M phosphate buffer, pH 7.2). The same fixative was then perfused through the scalae of the inner ear. The animals were decapitated, and the heads were postfixed in the same fixative overnight at 5°C. The next day, the entire hindbrain and most of the eighth nerve were removed from the skull and isolated in a single tissue block. The tissue was embedded in a gelatin–albumin mixture and sectioned with a vibratome in the horizontal plane at a thickness of 50 $\mu$m. This orientation plane minimizes the number of histological sections required to span the cochlear nucleus and nerve.

Sections were maintained in serial order in a 0.1 M solution of Tris buffer, pH 7.6, washed several times in fresh changes of buffer, and then incubated for 1 hour in a solution of 0.1 M Tris buffer, pH 7.6, containing 0.5% $CoCl_2$ and 1% dimethylsulfoxide (DMSO). The tissue was then washed twice for 5 min in Tris buffer and washed twice more for 5 min in 0.1 M phosphate buffer, pH 7.3. The sections were immersed for 15 min in a solution containing 0.05% 3,3′-diaminobenzidine (DAB) and 1% DMSO and then immersed for 60 min in a solution containing 0.05% DAB, 1% DMSO, and 0.01% $H_2O_2$. The processed sections were washed in phosphate buffer, mounted onto gelatin-coated slides, air dried overnight, and counterstained with 0.5% cresyl violet. The basilar papillae were histologically processed en bloc according to the same procedures as for the brain. The papillae were embedded in JB-4 and either whole mounted or sectioned at 20 $\mu$m on a rotary microtome perpendicular to the long axis of the papilla.

## Identification of HRP-labeled fibers

One fiber, recorded from intracellularly, was characterized as a tectorial unit on the basis of the shape of its tuning curve, characteristic frequency (325 Hz), and spontaneous discharge rate (2.5 s/s). The fiber was then labeled by injecting HRP through the same recording micropipette. A continuously negative DC potential plus the similarity of pre- and postinjection tuning curves argues that the labelled fiber is the same fiber that was intracellularly characterized and injected. The identification of labeled fibers following extracellular injections of HRP depended on the presence of labeled peripheral processes under either the free-standing or tectorial hair cells. Labeled peripheral processes were located throughout the tectorial and free-standing hair cell regions. The assumption was that labeled central fibers would be correlated with their labeled peripheral extensions. Typically only one population of fibers was labeled in any nerve; this result is consistent with anatomical and physiological studies that show that the tectorial and free-standing fibers are segregated in the peripheral component of the cochlear nerve (Weiss et al., '76; Mulroy and Oblak, '85).

## Fiber reconstruction and terminology

Labeled fibers were reconstructed from serial sections with the aid of a light microscope and drawing tube (total magnification ×800). The continuity of individual fibers was established by matching the cut ends of labeled fiber segments on adjacent section surfaces. Fibers that could not be unambiguously reconstructed were not included in this study. A fiber was considered to be completely labeled when all segments were uniformly and darkly stained; furthermore, the tips of all terminal branches were usually marked by a distinct swelling. Swellings that occurred at the tips of branches were called *terminal swellings* and those that occurred along the branches were called *en passant swellings*. Thus, for each ganglion cell, the entire axonal arborization and all *en passant* and terminal swellings were available for analysis. All fiber reconstructions are shown as if they were obtained from a left cochlear nucleus; fibers from the right side have been deliberately reversed.

Swellings were operationally defined according to the criteria applied to cochlear nerve fibers in cats (Rouiller et al., '86). Terminal swellings were variable in size and shape, ranging from a simple, continuously convex structure (bouton ending) to a multiply-branched and lobulated formation (resembling the mammalian endbulb of Held). Small endings appeared as simple boutons, as structures having three or fewer lobulations or (rarely) as blunt endings without a distinct swelling; these endings could be preceded by a short string of en passant swellings. An *en passant* swelling was identified when the axon increased abruptly (over a distance of 2 μm) to at least three times its diameter and just as abruptly returned to its original caliber. Blunt endings were included in the counts of terminal number, but they were not used for measurements of terminal area. Small endings were found throughout the nucleus, mostly in the neuropil but sometimes apposed to cell bodies. Large endings were defined by the presence of four or more lobulations from the same branch that appeared to be apposed to a single cell body. Such large axosomatic endings were found only in the NMM. These structures were called *endbulbs* because of their resemblance to mammalian endbulbs of Held. In general, however, the structure of lizard endbulbs is simpler than that of mammalian endbulbs.

All swellings were drawn with the aid of a drawing tube, and their silhouette areas were calculated by using an electronic planimeter. Endings were also analyzed with respect to fiber type (tectorial or free-standing), location in the cochlear nucleus, and termination target (somata or neuropil). Swelling size was represented by the silhouette area of the drawn ending. The sum of the areas of terminal swellings (i.e., excluding *en passant* areas) is defined as the "total terminal area." Because terminals could be found that appeared to appose cell bodies or that appeared to terminate in the neuropil, there are values defined as "total area of somatic terminals" and "total area of neuropil terminals," indicating the relationship between the terminal and its postsynaptic target. It is important to note that in some cases only a portion of a "somatic terminal" appeared to contact a cell body; thus the value "total area of somatic terminals" may be greater than that actually actually contacting cell bodies.

The diameter of the central axon in the cochlear nerve root was determined for each of the reconstructed fibers. A 50 μm segment of the fiber was drawn (total magnification ×800) from the initial branch point of free-standing fibers or from the bifurcation of tectorial fibers in the cochlear nucleus back towards the cell body. The diameter was calculated by dividing the silhouette area of the drawn fiber segment by its length.

## Data analysis

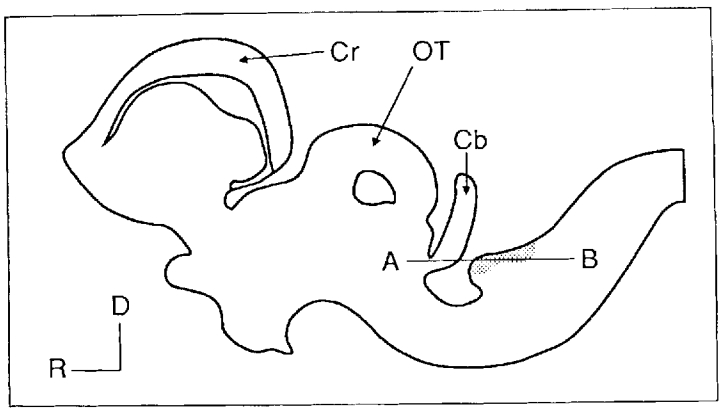
Tectorial and free-standing fibers were compared with respect to central projection pattern; central axon diameter; and distribution, type, size, and number of terminal and *en passant* swellings. The underlying hypothesis is that a constellation of structural differences among separate sets of neurons will establish cellular circuits with definably different functions. The mean, standard deviation, and $P$ values (ANOVA model for repeated measures) are provided where appropriate. This ANOVA model was chosen because it takes into consideration the inherent biases of a statistical comparison where many samples are taken from some animals and not from others. Consequently, it is a more rigorous test of statistical significance (Kleinbaum et al., '88). The cytoarchitectonic criteria and nomenclature of the lizard cochlear nucleus are based on previously reported observations (Miller, '75).

# RESULTS

The present results are based on an analysis of 19 cochlear nuclei from 18 lizards. Sixteen cochlear nuclei involved extracellular injections of HRP into the cochlear nerve; in addition, one cochlear nucleus contained a single fiber intracellularly labeled with HRP, one was prepared with a protargol stain, and one was prepared with a cresyl violet stain.

## General description

Fibers from the basilar papilla enter the dorsolateral medulla from the caudal and dorsal portion of the eighth cranial nerve. At the central end of the cochlear nerve lies an accumulation of cell bodies of second-order neurons upon which primary fibers terminate (Fig. 1). This juncture lies at the dorsal surface of the medulla, forming a slight bulge on the medial edge of the alar elevation, and is called the acoustic tubercle (Holmes, '03) or the cochlear nucleus (Miller, '75). The cochlear nucleus is bordered laterally and



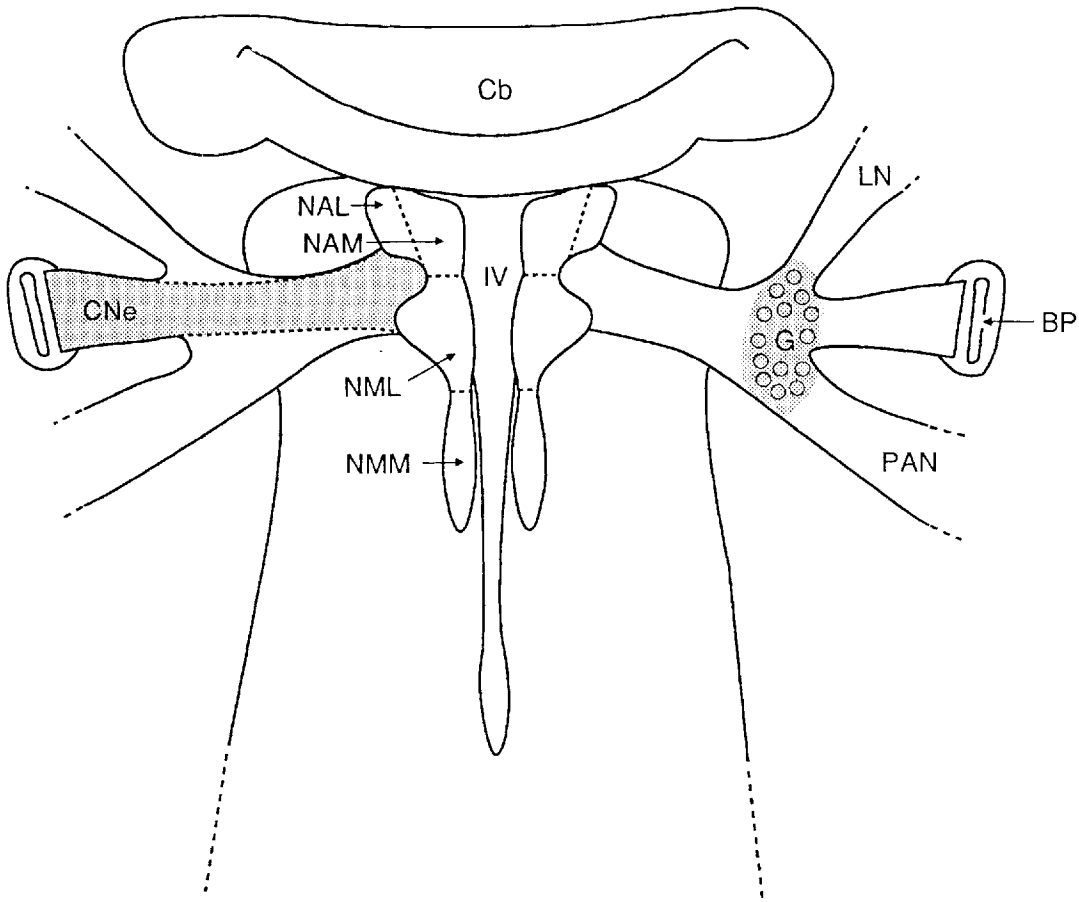




Fig. 1. Diagrammatic horizontal section of the lizard hindbrain. This drawing illustrates the cochlear nerve (shaded area on the left), the posterior division of the eighth nerve ganglion (shaded area on the right), and the cochlear nuclei, site of nerve termination. The inset shows a parasagittal section of the lizard brain and the level of the horizontal section (line AB); the shaded region represents the cochlear nucleus. Abbreviations: BP, basilar papilla; Cb, cerebellum; CNe, cochlear nerve; Cr, cerebrum; D, dorsal; G, ganglion; IV, fourth ventricle; LN, lagenar nerve; NAL, nucleus angularis lateralis; NAM, nucleus angularis medialis; NML, nucleus magnocellularis lateralis; NMM, nucleus magnocellularis medialis; OT, optic tectum; PAN, posterior ampullary nerve; R, rostral.

ventrally by vestibular nuclei and medially by the fourth ventricle.

The cochlear nucleus of the alligator lizard can be cytoarchitecturally parcelled into four subdivisions on the basis of Nissl stains (Fig. 2). Nucleus angularis (NA) occupies the rostral position in the cochlear nucleus and is partially segregated from the nucleus magnocellularis lateralis (NML) by cochlear nerve fibers. The NA is composed of two regions: a lateral region (NAL), containing small (10–15 $\mu$m), spindle-shaped cells, found only within the most dorsolateral part of

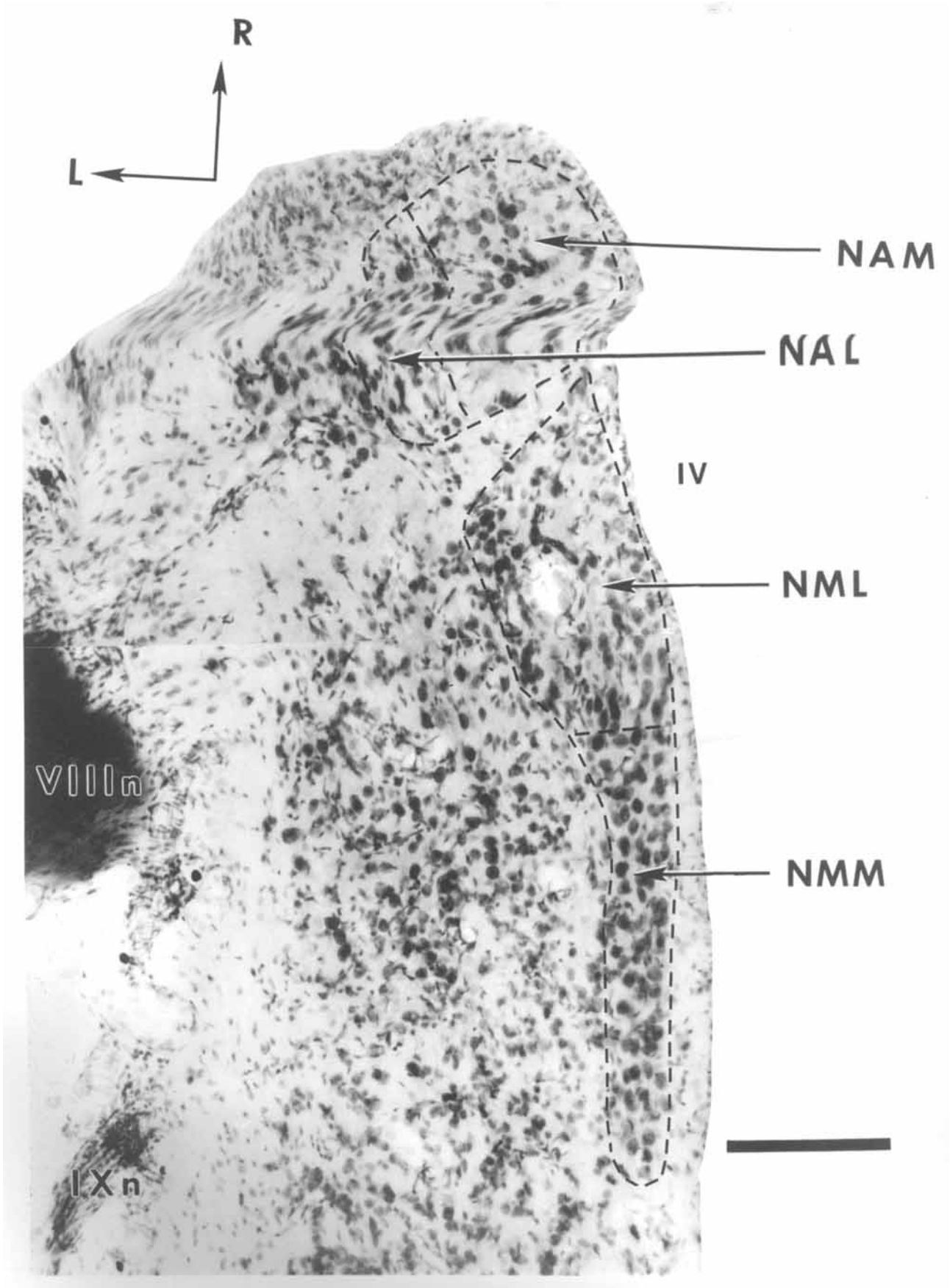



Fig. 2. Photomicrograph of a cresyl violet-stained horizontal section (50 $\mu$m) of the left cochlear nucleus. Dashed lines show the outline of the cochlear nucleus and boundaries between cytoarchitectonic subdivisions. Abbreviations: IV, fourth ventricle; L, lateral; NAL, nucleus angularis lateralis; NAM, nucleus angularis medialis; NML, nucleus magnocellularis lateralis; NMM, nucleus magnocellularis medialis; R, rostral; IXn, ninth cranial nerve; VIIIn, eighth cranial nerve. Bar = 250 $\mu$m.

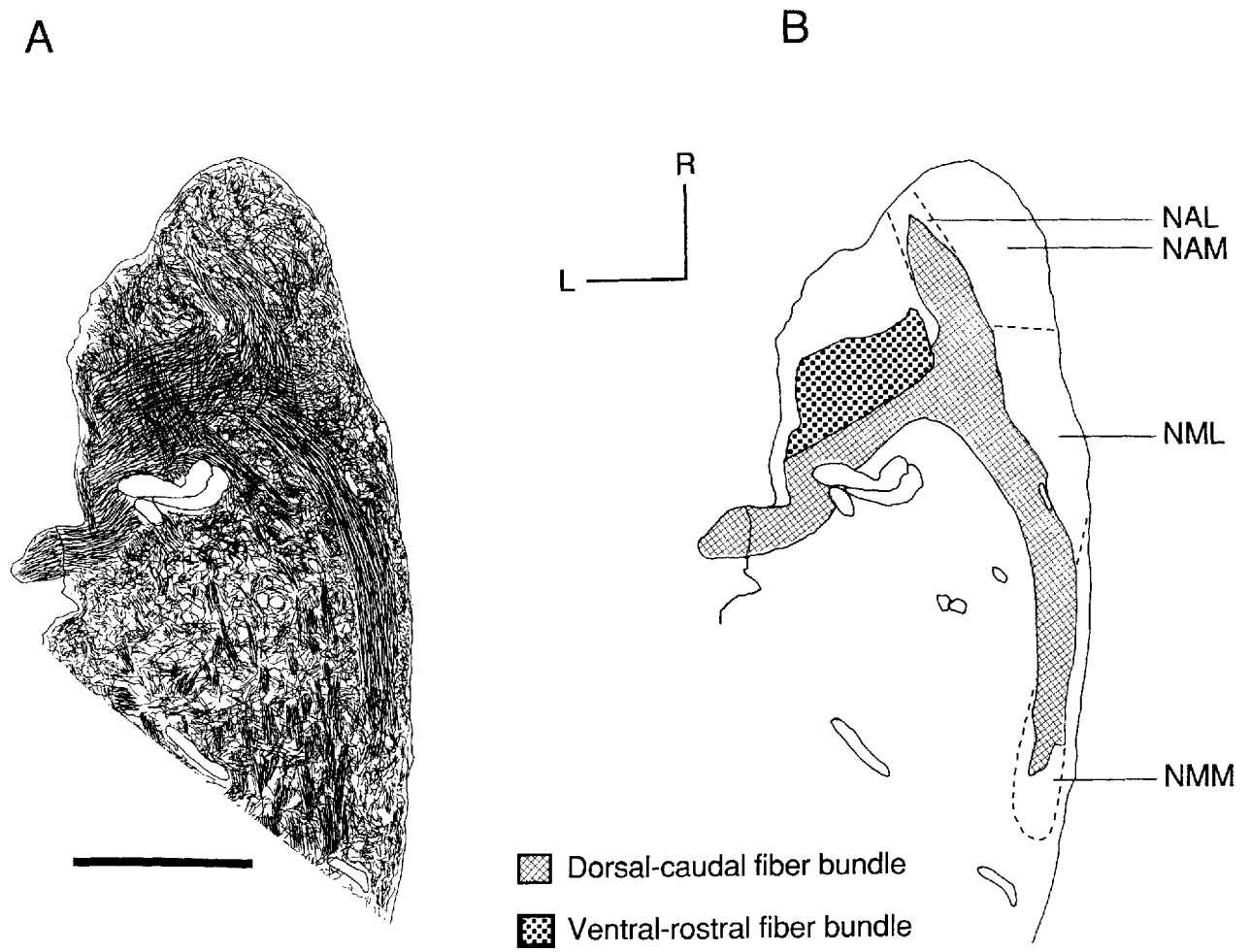



Fig. 3. Fibroarchitecture of the alligator lizard's cochlear nucleus. **A:** Drawing of protargol-stained horizontal section made with the aid of a drawing tube and light microscope. **B:** Corresponding schematic interpretation. This section is through the dorsal part of the nucleus. The dorsal-caudal and ventral-rostral fiber bundles represent two groups of cochlear nerve fibers. The subdivisions of the cochlear nuclei (NAL, NAM, NML, NMM) may be distinguished on the basis of fibroarchitecture. Abbreviations: L, lateral; NAL, nucleus angularis lateralis; NAM, nucleus angularis medialis; NML, nucleus magnocellularis medialis; NMM, nucleus magnocellularis medialis; R, rostral. Bar = 250 $\mu$m.

the NA, and a medial region (NAM), containing cell bodies of various diameters (10–20 $\mu$m). Nucleus magnocellularis medialis (NMM) occupies the caudal position in the tubercle. NMM is an elongated structure, roughly 500 $\mu$m in length but less than 100 $\mu$m in width and depth. NMM contains a single type of cell, 15–20 $\mu$m in somatic diameter, which exhibits a crescent-shaped, eccentrically placed nucleus. Lying between these rostral and caudal extremes of the cochlear nucleus is the nucleus magnocellularis lateralis (NML). The NML contains small (<15 $\mu$m) and large (~20 $\mu$m) cell types. The flanking concavities formed by the bulge of NML correspond to changes in cyto- and fibroarchitecture, and help to define the borders of this subdivision.

## Central projections of the cochlear nerve

A protargol stain of the lizard's hindbrain reveals two distinct bundles of fibers entering the cochlear nucleus from the eighth cranial nerve. One fiber bundle passes dorsally in the medulla and bifurcates upon entering the middle of the cochlear nucleus (Fig. 3). In more ventral sections, a second fiber bundle enters the rostral one-third of the nucleus without bifurcating; this latter bundle partially separates the NA from the NML (Fig. 4). The HRP experiments, as described below, allowed us to identify the origin of these two fiber bundles.

## Population patterns

***Tectorial fibers.*** Extracellular injections of HRP into the cochlear nerve typically labeled axons that were restricted to one or the other of the fiber bundles described above. Labeled fibers streamed away from the injection site, but individual fibers could not be traced through the dark reaction product at this site. We therefore relied on the relationship between the locations of central and peripheral HRP labeling to infer fiber identification. In all cases the location of labeled peripheral processes was correlated with the location of labeled fibers found in the dorsal-caudal or ventral-rostral fiber bundles in the medulla. Labeled fibers of the dorsal-caudal bundle (n = 8) were associated exclusively with peripheral processes that contacted tectorial hair cells of the basilar papilla, whereas fibers of the ventral-rostral bundle (n = 4) were associated only with peripheral processes that contacted free-standing hair cells (Table 1). In four cases, no peripheral labeling was found despite the presence of labeled fibers in the dorsal bundle. Since the

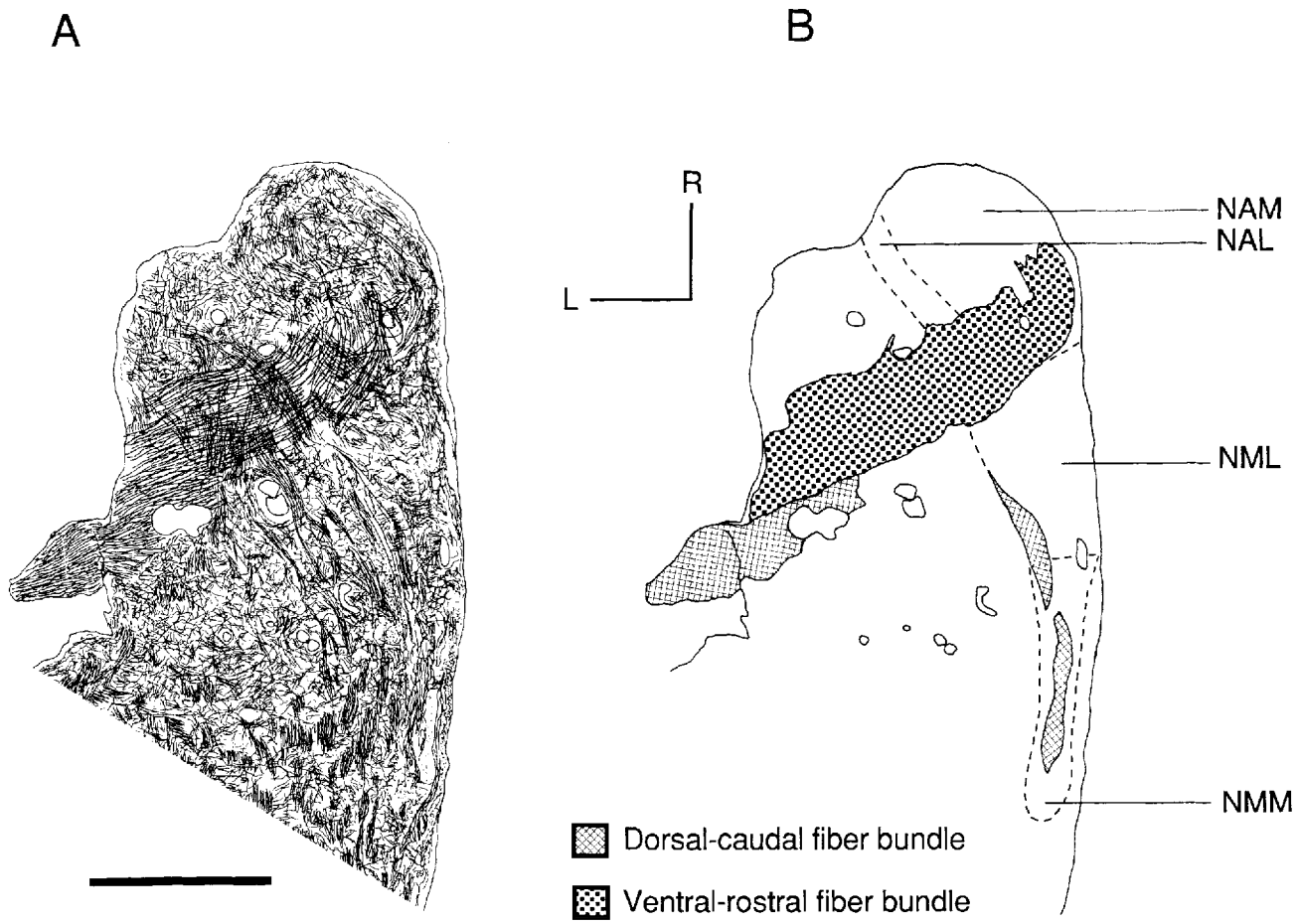



Fig. 4. Fibroarchitecture of the alligator lizard's cochlear nucleus. **A:** Drawing of a protargol-stained horizontal section made with the aid of a drawing tube and light microscope. **B:** Corresponding schematic interpretation. The top of this section is 15 μm ventral to the bottom of the section shown in Figure 3. The dorsal-caudal and ventral-rostral fiber bundles represent two groups of cochlear nerve fibers. The subdivisions of the cochlear nuclei (NAL, NAM, NML, NMM) may be distinguished on the basis of fibroarchitecture. Abbreviations: L, lateral; NAL, nucleus angularis lateralis; NAM, nucleus angularis medialis; NML, nucleus magnocellularis medialis; NMM, nucleus magnocellularis medialis; R, rostral. Bar = 250 μm.

basilar papilla is oriented such that the tectorial hair cell population is located rostral to the free-standing hair cell population, our results imply that the two primary fiber bundles must cross en route to the cochlear nucleus.

In some cases so many fibers were labeled that all the individual arborizations could not be isolated and reconstructed in the cochlear nucleus. Nevertheless, terminal swellings were darkly labeled and their distribution within the cochlear nucleus was distinctly related to whether tectorial or free-standing fibers were labeled in the basilar papilla. The population of fibers associated with the tectorial region of the basilar papilla terminated in NAL, NML, and NMM (Fig. 5A). In contrast, the population associated with free-standing fibers terminated primarily in NAM, but a very few terminals could also be found in the NML (Fig. 5B). On the basis of these observations, central axonal trajectory and distribution of endings provide important clues to the origin of the cochlear fibers.

In 12 cochlear nuclei, terminals of HRP-labeled cochlear fibers were found in the NAL, NML, and NMM, exhibiting the same distribution pattern as illustrated in Figure 5A and suggesting tectorial origin. HRP-labeled fibers were found in the tectorial region in eight of the corresponding basilar papillae, supporting the identification based on central termination pattern. In the other four papillae, no HRP label could be detected, but for one of these cases we collected electrophysiological data indicating that the intracellularly labeled fiber was a tectorial fiber. On the basis of the nine unequivocal cases, we propose that cochlear fibers terminating in NAL, NML, and NMM qualify as tectorial fibers.

***Free-standing fibers.*** In four other cochlear nuclei, terminals of HRP-labeled fibers were found exclusively in the NAM (n = 3), or predominantly in NAM with a few in NML (n = 1). These cases exhibit terminal distribution patterns similar to that illustrated in Figure 5B. In all the cases, HRP-labeled peripheral processes were found in the free-standing region of the basilar papilla. On the basis of these data, we propose that cochlear fibers terminating predominantly or exclusively in NAM qualify as free-standing fibers.

In one lizard, terminals of HRP-labeled fibers were found in all subdivisions of the cochlear nucleus (NAL, NAM, NML, NMM), and labeled peripheral processes were found in both the tectorial and free-standing region of the basilar papilla. This case is consistent with the hypothesis that fibers originating in the different regions of the basilar papilla project to different subdivisions of the cochlear

TABLE 1. Distribution of HRP-Labeled Terminals and Location of Label in the Basilar Papilla[1]

| Case | Physiologically characterized fibers | Location of labeled fibers in the basilar papilla | Location of labeled fibers in the cochlear nucleus | Single fibers reconstructed |
|---|---|---|---|---|
| 37L | | THC | NAL, NML, NMM | — |
| 47L | | THC | NAL, NML, NMM | — |
| 55R | | THC | NAL, NML, NMM | — |
| 56R | | THC | NAL, NML, NMM | — |
| 67R | | THC | NAL, NML, NMM | — |
| 68L | | THC | NAL, NML, NMM | — |
| 46L | | THC | NAL, NML, NMM | 1T |
| 49L | | THC | NAL, NML, NMM | 4T |
| 39L | | — | NAL, NML, NMM | 1T |
| 41L | | — | NAL, NML, NMM | 1T |
| 53L | | — | NAL, NML, NMM | 3T |
| 1L | Tectorial unit | — | NAL, NML, NMM | 1T |
| 68R | | FHC | NAM | — |
| 69L | | FHC | NAM | — |
| 75L | | FHC | NAM | — |
| 44R | | FHC | NAM, (NML) | 10F |
| 48L | | THC, FHC | NAL, NAM, NML, NMM | 1T, 3F |

[1]Abbreviations: F, free-standing fiber; FHC, free-standing hair cell region; L, left; (NML) small projection to NML; R, right; T, tectorial fiber; THC, tectorial hair cell region.

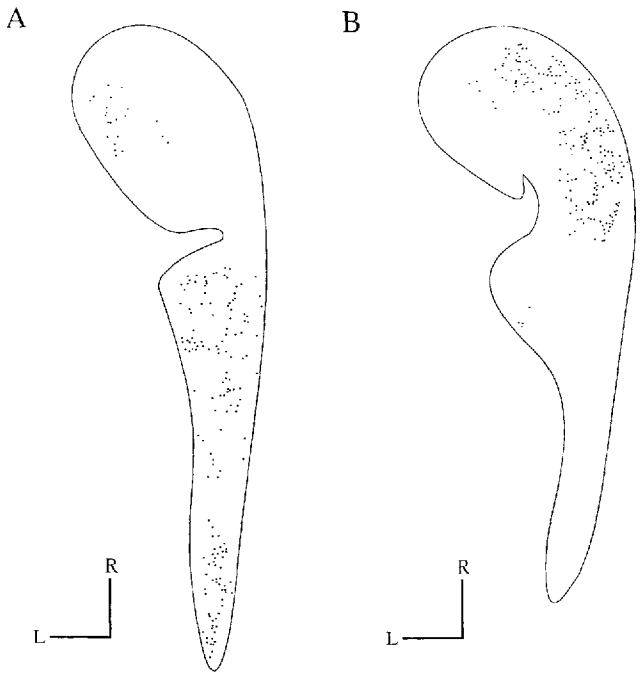



Fig. 5. The distribution of all HRP-labeled terminals of cochlear nerve fibers in the cochlear nucleus. **A:** The central distribution of terminals in a case where labeled peripheral fibers were found only under tectorial hair cells in the basilar papilla. **B:** The central distribution of terminals in a different case where labeled peripheral fibers were found only under free-standing hair cells in the basilar papilla. Both drawings are a planar representation of a three-dimensional reconstruction of a left cochlear nucleus, which was sectioned horizontally. The different silhouette outlines of the nuclei in A and B reflect differences between individual lizards. Each filled circle represents a fiber terminal swelling or *en passant* swelling. Abbreviations: L, lateral; R, rostral.

nucleus. We were able to reconstruct four individual fibers: One fiber projected to NAL, NML, and NMM; two fibers projected exclusively to NAM; and one fiber projected to NAM and NML. The first fiber was classified as a tectorial fiber, and the latter fibers were classified as free-standing fibers.

## Individual arborizations

The next task was to determine the arborization patterns of individual cochlear nerve fibers. Individual tectorial or free-standing fibers might each behave like their overall respective population, or different subtypes might exist whose aggregate trajectories yield the population pattern.

***Tectorial fibers.*** The central arborizations of 12 tectorial fibers were reconstructed in their entirety from the cochlear nerve root to their terminal swellings in the cochlear nucleus. Five of these fibers were correlated with peripheral processes contacting the tectorial region of the basilar papilla, and a sixth was an unambiguous tectorial fiber because of its physiological response properties (Table 1). The other six were hypothesized to be tectorial fibers because of their central termination patterns. The projection patterns of all of these fibers are basically identical, and we have illustrated fibers that exhibit the least (Fig. 6) and the greatest (Fig. 7) degrees of branching. All fibers bifurcate in the cochlear nerve root immediately lateral to the NML. The bifurcation gives rise to two major branches, one that ascends and ramifies in NML and NAL and one that descends and ramifies in NML and NMM. The ascending branch projects across the nerve root lateral to NML and gives rise to a terminal tuft in NAL. The descending branches bundle together and project through the core of NMM. A single descending branch gives rise to one or two primary collaterals in the rostral part of the NMM and then forms a tuft of collaterals in the caudal part of the NMM.

***Free-standing fibers.*** The central arborizations of 13 free-standing fibers were reconstructed in their entirety, ten of which were correlated with peripheral processes contacting the free-standing region of the basilar papilla (Table 1). The other three fibers are hypothesized to be free-standing fibers because of the characteristic distribution of their terminals in the cochlear nucleus. There is one general pattern with an occasional minor variation, both obviously distinct from that of tectorial fibers. Nine of the 13 reconstructed fibers did not bifurcate but gave rise to three to seven primary collaterals that ramified exclusively in the NAM (Fig. 8). Four of the 13 reconstructed fibers ramified primarily in NAM but also sent a single, thin (mean diameter = 0.9 ± 0.3 $\mu$m) collateral to the NML (Fig. 9). In a larger sample of unreconstructed free-standing fibers, less than 5% have a branch that projects to the NML. Because the arborizations of free-standing fibers appeared to represent a relatively uniform population, they were collapsed into a single group for statistical comparisons with tectorial fibers (Table 2).

## Morphometry

***Diameter of central axons.*** The average diameter of tectorial fibers is 4.4 ± 0.8 $\mu$m, with a range of 2.5–6.0 $\mu$m (based on 12 tectorial fibers from seven lizards). In contrast, the average diameter of free-standing fibers is 2.7 ± 0.6 $\mu$m with a range of 1.6–3.6 $\mu$m (based on 13 fibers from two lizards). Although there is some overlap in diameter between the two fiber types, the means are statistically different ($P < 0.05$; Table 2).

***Terminal swellings.*** The tips of nearly all collateral branches are marked by the presence of a distinct swelling. On average, tectorial fibers have 39.1 ± 11.0 swellings per fiber, whereas free-standing fibers have 20.5 ± 5.0 per fiber. These terminal swellings are considered to be synaptic endings on the basis of observations of cochlear nerve fibers in cats (Fekete et al., '84; Rouiller et al., '86). Similarly, our

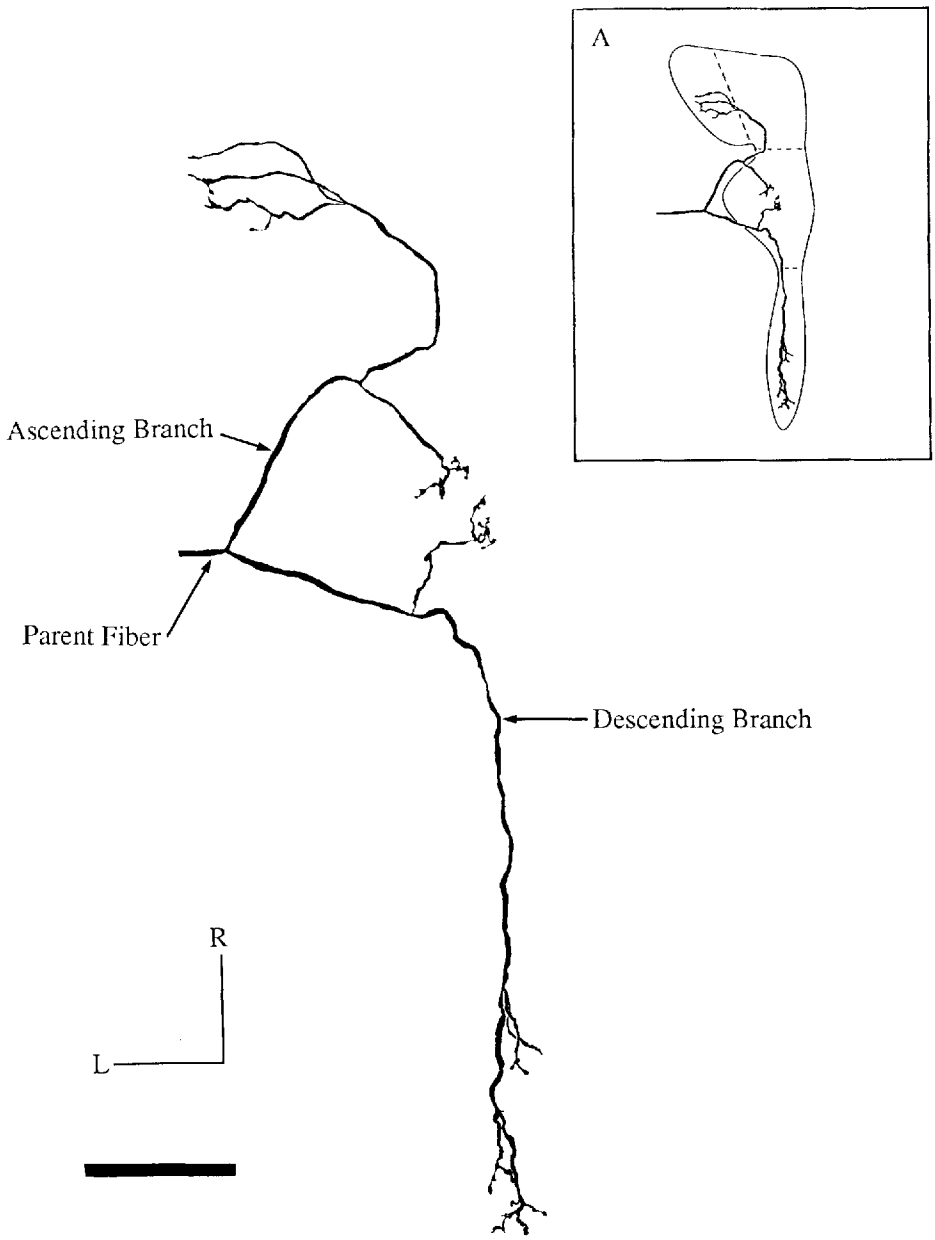



Fig. 6. Drawing tube reconstruction of the central arborization of a tectorial fiber. This fiber was labeled with an extracellular injection of HRP into the auditory nerve. It has 26 terminals, a central axon diameter of 4.6 $\mu$m, and a total terminal area of 309 $\mu m^2$ and forms terminals in the NAL, NML, and NMM. The inset (A) illustrates the location of this relatively simple arborization within the cochlear nucleus. Abbreviations: L, lateral; R, rostral. Bar = 100 $\mu$m.

preliminary electron microscopic observations of HRP-labeled swellings from tectorial and free-standing fibers in the lizard reveal the presence of active zones, characterized by clear round vesicles associated with a regular expansion of the intercellular cleft, and a postsynaptic density.

Generally, the terminals of lizard cochlear nerve fibers fall into two separate classes, much like that reported for mammals (e.g., Held, 1896; Ramón y Cajal, '09; Lorente de Nó, '81). There are small, relatively simple endings and large, relatively complex endings. The small endings are bouton-like and are often preceded by a short string of *en passant* swellings having various sizes and shapes (Figs. 10 A–C, 12 A,B). Small endings are found in all subdivisions of the cochlear nucleus and are usually, but not always, found in neuropil. The large endings appear as prominent swellings having lobulations, branchlets, and filopodia with swellings. These large endings were obviously distinct from the others, and each characteristically formed an extensive axosomatic association with a second-order neuron in the NMM (Figs. 10D, 11). We refer to such endings as endbulbs because of their resemblance to the endbulbs of Held so typical of primary auditory fibers of birds and mammals. Free-standing fibers never exhibit endbulbs, whereas four of the 12 tectorial fibers gave rise to one or two endbulbs in the NMM.

***Distribution of terminals.*** The distribution of terminals within the subdivisions of the cochlear nucleus is qualitatively and quantitatively distinct for the two types of

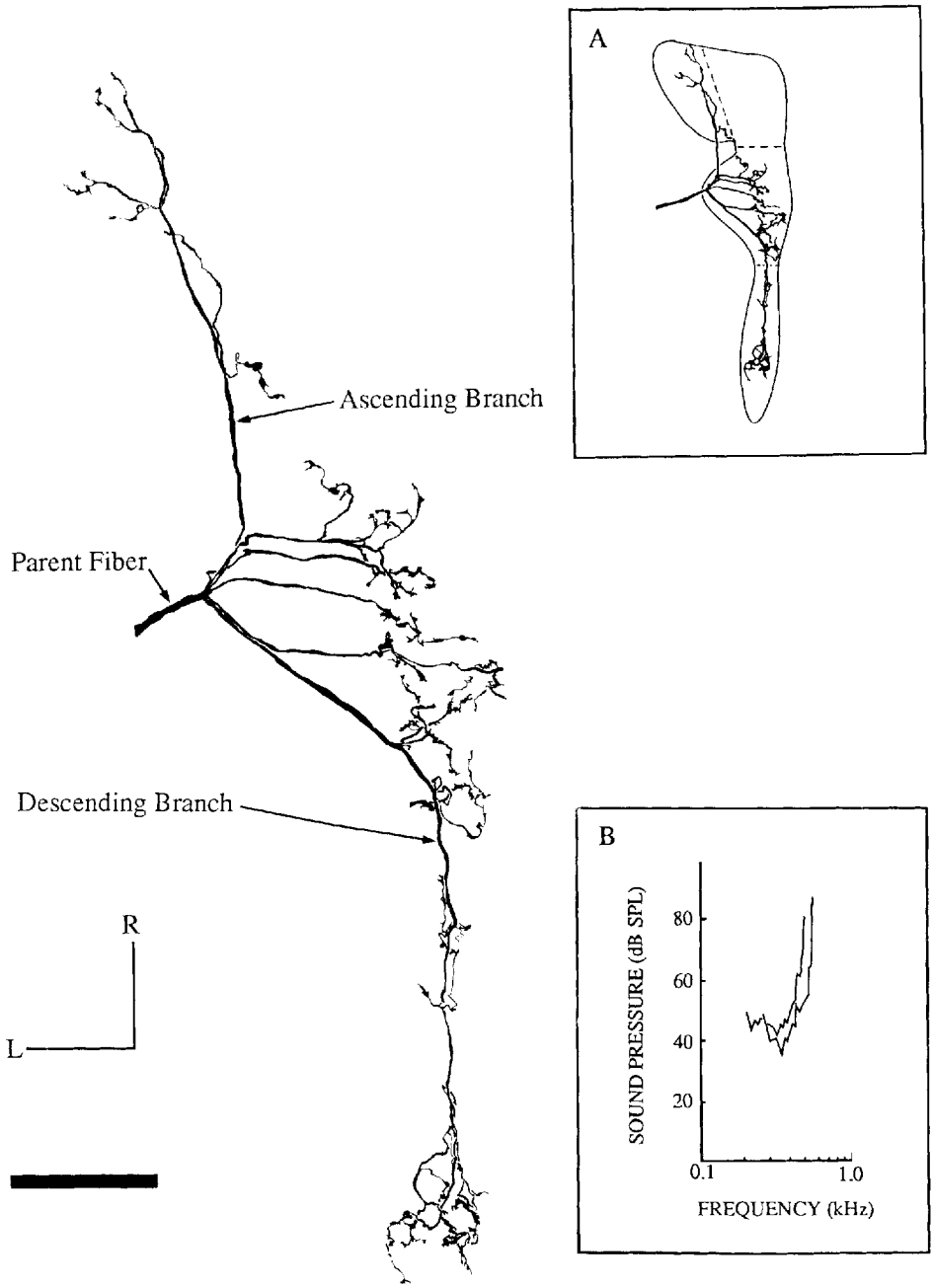



Fig. 7. Drawing tube reconstruction of the central arborization of a tectorial fiber. This fiber was electrophysiologically characterized by using a pipette inserted into the axon and then labeled with an injection of HRP through the same pipette. This fiber has 65 terminals, a central axon diameter of 5.0 $\mu$m, and a total terminal area of 694 $\mu m^2$. Its basic morphology is consistent with that of tectorial fibers labelled with extracellular injections of HRP (see Fig. 6). The upper right inset (**A**) illustrates the location of this relatively complex arborization within the cochlear nucleus. The lower inset (**B**) shows tuning curves measured from this fiber. The HRP was iontophoretically injected with a positive current of 5.0 nA for 2.5 minutes through a microelectrode. A tuning curve was taken before and after the iontophoresis of HRP. The preinjection characteristic frequency (CF; 325 Hz) and spontaneous rate (SR; 2.7 spikes/second) and postinjection CF (350 Hz) and SR (2.5 spikes/second) identify the fiber as a tectorial unit. The DC resting potential was monitored throughout the recording session. Continuity of the intracellular DC potential and similarities in the pre- and postinjection tuning curve indicated that the electrode remained within the same fiber throughout the recording and injection period. The preinjection tuning curve is incomplete below 200 Hz, and the postinjection tuning curve is incomplete below 275 Hz. Abbreviations: dB, decibel; kHz, kiloHertz; L, lateral; R, rostral; SPL, sound pressure level. Bar = 100 $\mu$m.

fibers. The terminals of free-standing fibers are restricted mostly to the NAM. Although four of the 13 fibers also sent a small branch that delivered terminals to the NML, for the entire free-standing sample population, 97% of the terminals are located in the NAM. In contrast, every tectorial fiber gave rise to terminals in the NAL, NML, and NMM. For the population of 12 tectorial fibers, 22.2% of the terminals were located in the NAL, 45.7% were in the NML, and 32.1% were in the NMM.

In the NAM, NAL, and NML, approximately 90% of the

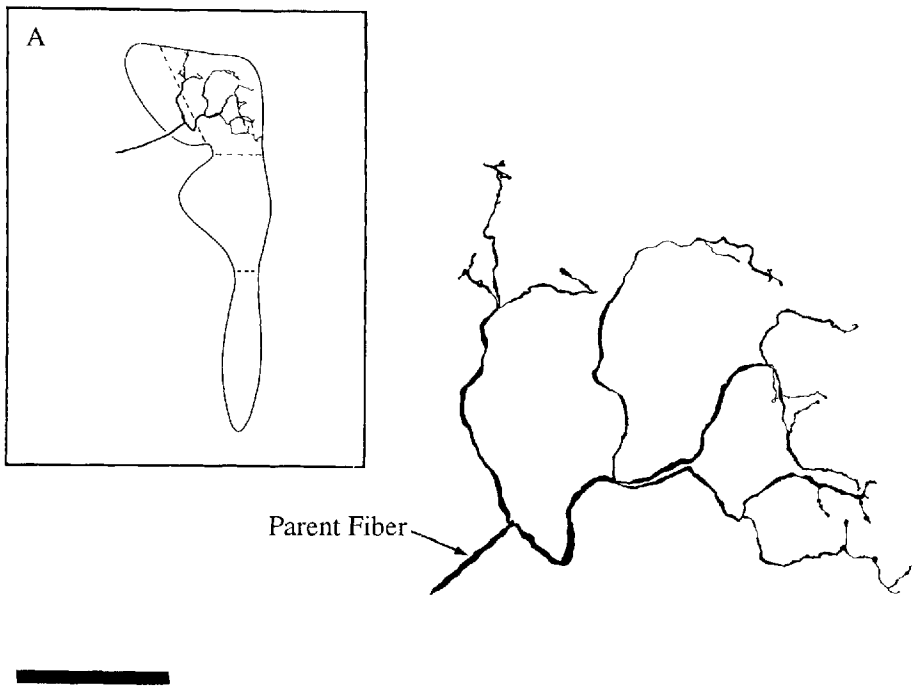



Fig. 8. Drawing-tube reconstruction of the central arborization of a free-standing fiber that was labeled with an extracellular injection of HRP into the cochlear nerve. It has 22 terminals, a central axon diameter of 2.8 μm, and a total terminal area of 178 $\mu m^2$ and forms terminals exclusively in the NAM. The inset (**A**) shows the location of the arborization within the cochlear nucleus. Bar = 100 μm.

terminals of either fiber type are in the neuropil, whereas the remaining terminals were apposed to cell bodies. In the NML, terminals of free-standing fibers tend to make somatic contacts, but these results should not be emphasized because the sample size is small (eight terminals). In the NMM, 64% of the terminals of tectorial fibers are found in the neuropil, and the remainder are apposed to cell bodies.

***Terminal area.*** We use the silhouette area of individual terminal swellings as an approximation of terminal size. Because synaptic active zones are contained within terminals, we assume that larger terminals contain more active zones and so propose that the summed area will reflect the amount of synaptic input to a region. In this context, the total area of the terminals for individual tectorial fibers is 312 ± 159.2 $\mu m^2$, compared to 77.0 ± 40.6 $\mu m^2$ for free-standing fibers. The total area of terminals that are apposed to cell bodies is about 20 times greater for tectorial fibers compared to free-standing fibers, and the total area of terminals in the neuropil is almost three times greater (Table 2). In addition to the greater number of terminals per fiber, tectorial fibers tend to have larger terminals than do free-standing fibers (Table 2). If we use the average total terminal area per fiber as a point of reference, then tectorial fibers have 38% of their terminal area apposed to cell bodies, whereas free-standing fibers have less than 10% apposed to cell bodies. Not only do tectorial and free-standing fibers distribute their terminals to different regions, they also appear to distribute them to different parts of the target neurons in those regions.

***En passant swellings.*** *En passant* swellings are found only on the terminal branches of cochlear nerve fibers. All the *en passant* swellings of the 13 free-standing fibers are located in the NAM. In contrast, the *en passant* swellings of the 12 tectorial fibers are distributed evenly among the other subdivisions of the cochlear nucleus (20 in NAL, 17 in NML, 16 in NMM). Although the tectorial fibers are occasionally seen to pass through the NAM, they were never observed to form *en passant* swellings there. On average, the total number, the total area, and the mean area of individual *en passant* swellings were similar for the two types of fibers (Table 2).

## DISCUSSION

The present study used HRP staining and light microscopic reconstruction of single neurons to analyze the two known types of cochlear nerve fibers in the alligator lizard. These two types of fibers, called tectorial and free-standing, have been shown to differ across a wide variety of peripheral anatomical features (Mulroy, '74, '83, '86) and electrophysiological response properties (e.g., Weiss et al., '74, '76, '78), and we have demonstrated that they have separate projection patterns into the brain. A summary of the two patterns of central projections is schematically illustrated (Fig. 13).

In addition to the qualitative differences in the central projection patterns, the tectorial and free-standing fibers were also markedly different in a number of anatomic parameters, including fiber diameter, number of terminals, total terminal area, and average terminal size. These distinctions between fiber types are consistent with the idea that neural signals conveyed by the two fiber populations are processed in separate ways.

One finds that a greater number of terminals is correlated with a larger axon diameter when comparing tectorial and free-standing fibers. Because the number of terminals equals the number of branch points plus one, the observation is consistent with the notion that large-diameter axons have more branching potential than do small axons (Lasek, '88). The peripheral processes of tectorial fibers are also thicker (2.8 μm) than those of free-standing fibers (2.0 μm; Mulroy

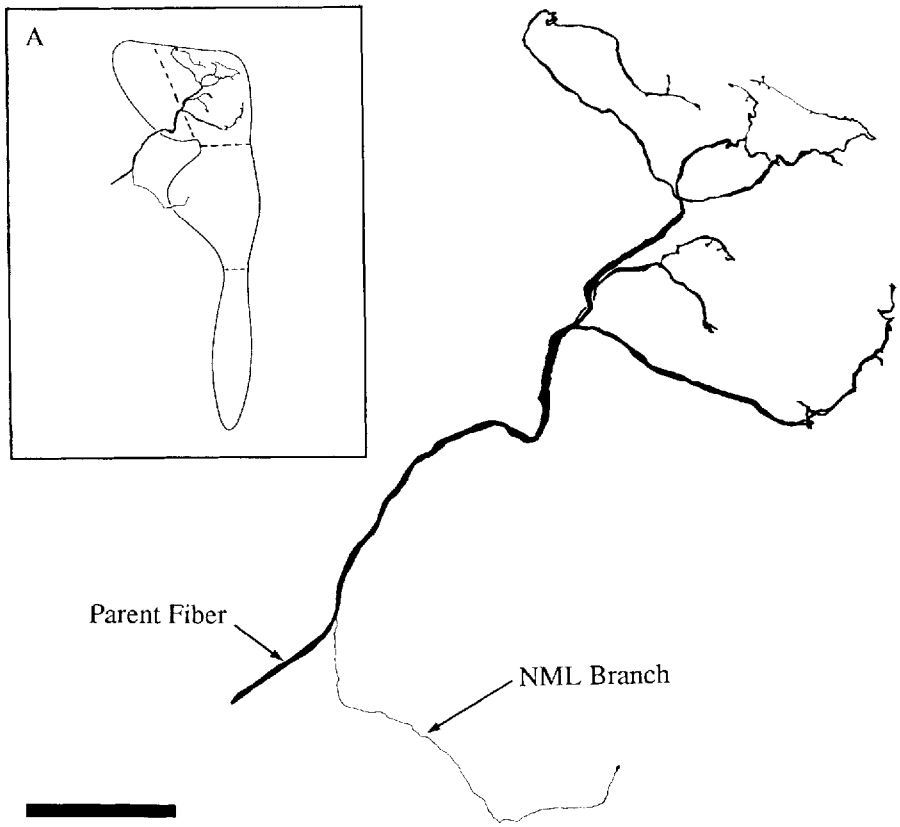



Fig. 9. The central arborization of a free-standing fiber that forms terminals in the NAM but also emits a single, small caliber branch to the NML. The fiber is from the same case as that shown in Figure 8 and has 22 terminals, a central axon diameter of 2.3 $\mu$m, and a total terminal area of 47 $\mu m^2$. The inset (A) shows the location of the arborization within the cochlear nucleus. Abbreviation: NML, nucleus magnocellularis lateralis. Bar = 100 $\mu$m.

TABLE 2. Characteristics of Individual Tectorial and Free-Standing Fibers: Means, Standard Deviations, and Comparisons Between the Two Fiber Populations

| | Tectorial fibers | Free-standing fibers | *P* value |
|---|---|---|---|
| Number of fibers | 12 | 13 | — |
| Mean diameter ($\mu$m) | 4.43 ± 0.8 | 2.71 ± 0.6 | <0.05 |
| Number of terminals | | | |
| Total number of terminals | 39.1 ± 11.0 | 20.5 ± 5.0 | <0.05 |
| Number of endbulbs | 0.4 ± 0.7 | 0 | — |
| Area of terminals ($\mu m^2$) | | | |
| Total terminal area | 312.0 ± 159.2 | 77.0 ± 40.6 | <0.05 |
| Total area of somatic terminals | 111.7 ± 72.6 | 5.5 ± 8.4 | <0.05 |
| Total area of neuropil terminals | 200.3 ± 112.0 | 71.5 ± 41.3 | n.s. |
| Area of individual simple terminals | 11.2 ± 3.3 | 6.6 ± 2.6 | <0.05 |
| Area of individual endbulbs | 47.8 ± 21.2 | — | - |
| Area of individual simple somatic terminals | 23.8 ± 9.9 | 7.0 ± 4.9 | <0.05 |
| Area of individual neuropil terminals | 9.5 ± 3.4 | 6.2 ± 2.4 | n.s. |
| *En passant* swellings | | | |
| Number per fiber | 4.4 ± 4.7 | 3.5 ± 1.8 | n.s. |
| Area of individual swelling ($\mu m^2$) | 10.9 ± 4.1 | 7.4 ± 3.1 | n.s. |
| Total swelling area ($\mu m^2$) | 50.0 ± 62.0 | 24.3 ± 14.5 | n.s. |

and Oblak, '85). One consequence of these diameter differences is that tectorial fibers may have greater conduction velocity (Hursch, '39; Bullock and Horridge, '65; Mountcastle, '74), and the auditory information conveyed by the two fiber populations may be temporally separated in the cochlear nucleus.

The observation that individual tectorial fibers have on average a greater number of terminals and a greater terminal area than do individual free-standing fibers suggests that the former may provide a greater number of synapses to the cochlear nucleus. It has been shown (at least in mammals) that large endings have multiple synaptic active zones (e.g., Lenn and Reese, '66; Ibata and Pappas, '76; Cant and Morest, '79), and it has been hypothesized that large endings are synaptically more efficacious than small endings (e.g., Kuno, '71; Bourk, '76). Consequently, we predict that individual tectorial fibers are more synaptically efficacious than free-standing fibers in the cochlear nucleus.

These data suggest that the alligator lizard has two separate systems for conveying sensory information from the hearing organ to the brain. The two systems may differ with respect to the kind of information conducted from the periphery, the time of arrival of this information, and the postsynaptic neural targets. The functional significance of these differences remains to be determined.

## Tectorial fibers

There are many morphological, connectional, and physiological similarities suggesting that tectorial fibers may correspond to mammalian type I fibers and avian cochlear nerve fibers. All such fibers contact unidirectionally oriented hair cells covered by a tectorial membrane (Weiss et al., '76; Mulroy, '87), an arrangement thought to have been present in the stem reptiles from which modern reptiles, birds, and mammals evolved (Baird, '74; Miller, '80; Manley, '81). In the brain of alligator lizards, tectorial fibers occasionally contact cells in the NMM by way of large, axosomatic endings, which resemble mammalian and avian endbulbs of

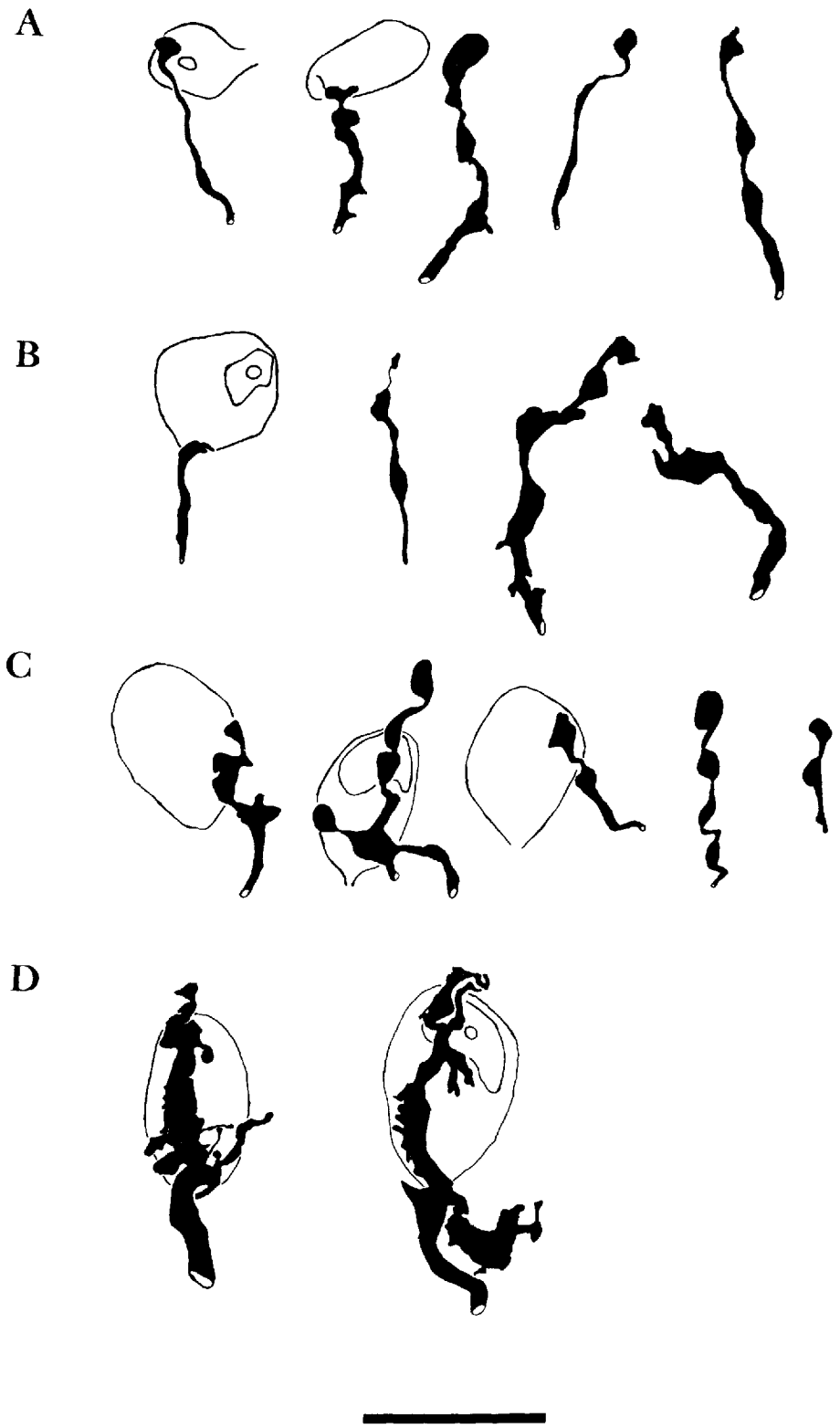



Fig. 10. Examples of terminals of tectorial fibers in the different divisions of the cochlear nucleus. **A:** Simple axosomatic and neuropil terminals in the NAL. **B:** Simple axosomatic and neuropil terminals in the NML. **C:** Simple axosomatic and neuropil terminals in the NMM. **D:** Large axosomatic terminals (endbulbs) in the NMM. Bar = 20 $\mu$m.

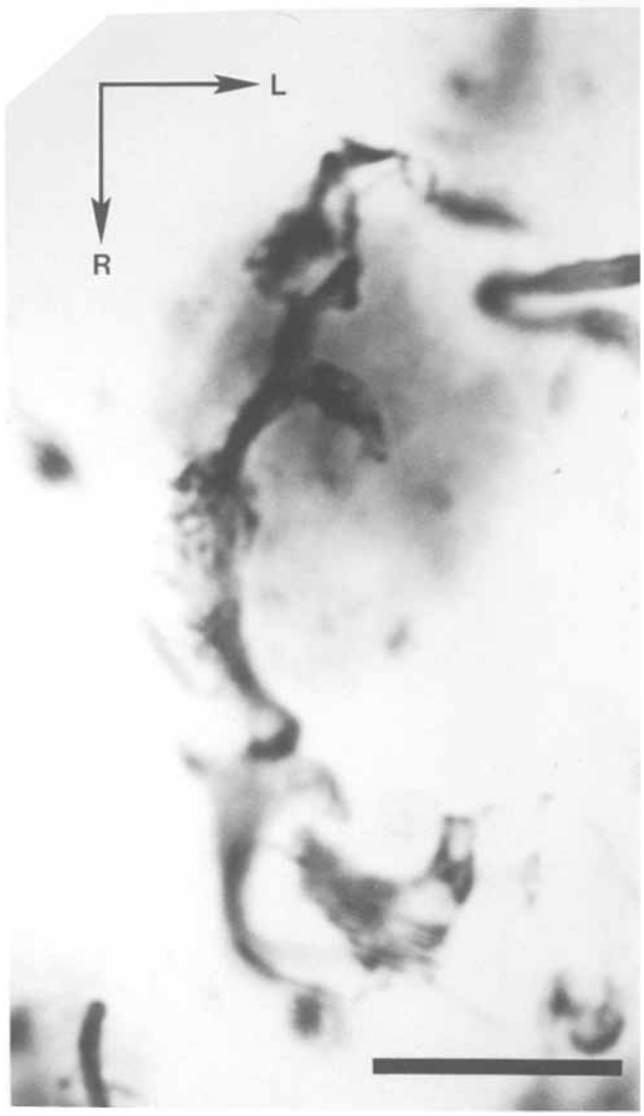



Fig. 11. Photomicrograph of a large axosomatic terminal (endbulb) in the NMM. This photomicrograph corresponds to the second endbulb shown in Figure 10D. Abbreviations: L, lateral; R, rostral. Bar = 10 μm.

Held (Ramón y Cajal, '08; Parks and Rubel, '78). This has been substantiated by electron microscopic examinations of the NMM, which reveal the presence of large axosomatic terminals that appose as much as one-quarter of the target cell circumference (unpublished observations). These target neurons make up a homogeneous population, which may correspond to the bushy/spherical cells in the anteroventral cochlear nucleus (AVCN) of mammals (Brawer et al., '74), the medial NM of birds (Jhaveri and Morest, '82), and the NM of turtles (Browner and Pierz, '86). Consequently, it may be that the relationship between endbulb-like endings and spherical neurons in the auditory pathway is homologous across amniotic vertebrates (Boord and Rasmussen, '63; Miller, '75; Rubel and Parks, '75; Miller and Kasahara, '79; Marbey and Browner, '87; Browner and Marbey, '88). In the NA, terminals of tectorial fibers consist of small bouton endings in the neuropil, and so are reminiscent of terminals of myelinated cochlear nerve fibers distributed to the NA of birds (Takahashi, reference in Sullivan, '85) and the posteroventral cochlear nucleus (PVCN) of mammals (Harrison and Irving, '66; Ryugo and Rouiller, '88). Indeed, certain cells of the avian and reptilian NA may correspond to cells of the mammalian PVCN (Foster and Hall, '78; Sullivan, '85).

Electrophysiological data from the cochlear nerve and nucleus are also consistent with the idea that tectorial fibers, avian cochlear nerve fibers, and mammalian type I fibers may be fulfilling comparable functions. Both tectorial and type I fibers exhibit sharp tuning, asymmetric tuning curves, two-tone rate suppression, and phase-locked responses to low frequency sounds at low intensity levels (Weiss et al., '76; Holton and Weiss, '78; Rose and Weiss, '88). The phenomenon of phase locking to the stimulus waveform is of particular interest in that it allows cochlear nerve fibers to convey faithfully information on the timing of the stimulus. In birds, notably the barn owl, phase-locking neurons of the NM are thought to be key to the timing pathway used for localizing sounds in azimuthal space (Sullivan and Konishi, '84; Takahashi et al., '84; Sullivan, '85). This capacity is presumably ensured by the highly efficacious synapses, the endbulbs of Held, that connect cochlear fibers to the second-order bushy cells (Parks and Rubel, '78; Parks, '81; Jhaveri and Morest, '82) and preserves the primary-like PSTH response patterns across the synapse (Pfeiffer, '66; Manley, '76; Sullivan, '85).

The pathway that permits the use of binaural phase comparisons to localize sound sources may proceed through the NMM of the alligator lizard by way of tectorial fibers, since it is here that we found the largest axosomatic contacts. In the periphery, individual tectorial fibers make a greater number of synapses on their presynaptic hair cells (Mulroy, '86). This increase in active sites per fiber may enhance the ability of tectorial fibers to synchronize (phase lock) to the acoustic stimulus. The ascending projections of the NMM are not known, but in the turtle bushy cells of the NM project to the nucleus laminaris (NL; R. Browner, personal communication). In reptiles, the size of the NL and NMM is correlated with the size of the unidirectional hair cell region (Miller, '75; Miller and Kasahara, '79). Furthermore, the NL of birds, which corresponds to the medial superior olive of mammals, is the first site of binaural convergence from the NM in the time-coding pathway (Young and Rubel, '83; Takahashi and Konishi, '85; Sullivan and Konishi, '86). A similar pathway may be present in the alligator lizard. However, since interaural phase difference is dependent on the distance between the two ears and the wavelength of the incident sound, the small dimension of the alligator lizard's head (1 cm) limits its ability to use binaural time cues. The extent of this limitation can be understood from the following "best case" scenario. For a sound source that is maximally displaced to one side of the head (90° from the midline), the time for the sound to travel between the two ears of the alligator lizard is approximately 30 μsec. Thus a 0.5 kHz[1] sound (a period of approximately 2,000 μsec) will have a maximal interaural phase difference of 1/67 of a wavelength (5° in phase). Since this value approximates the best discriminative performance of humans (Zwislocki and Feldman, '56), the alligator lizard

[1]This is the corner frequency of the synchronization filter-function for tectorial fibers (Rose and Weiss, '88). It was chosen to represent the highest frequency at which the tectorial fibers can phase lock without a significant loss in fidelity.

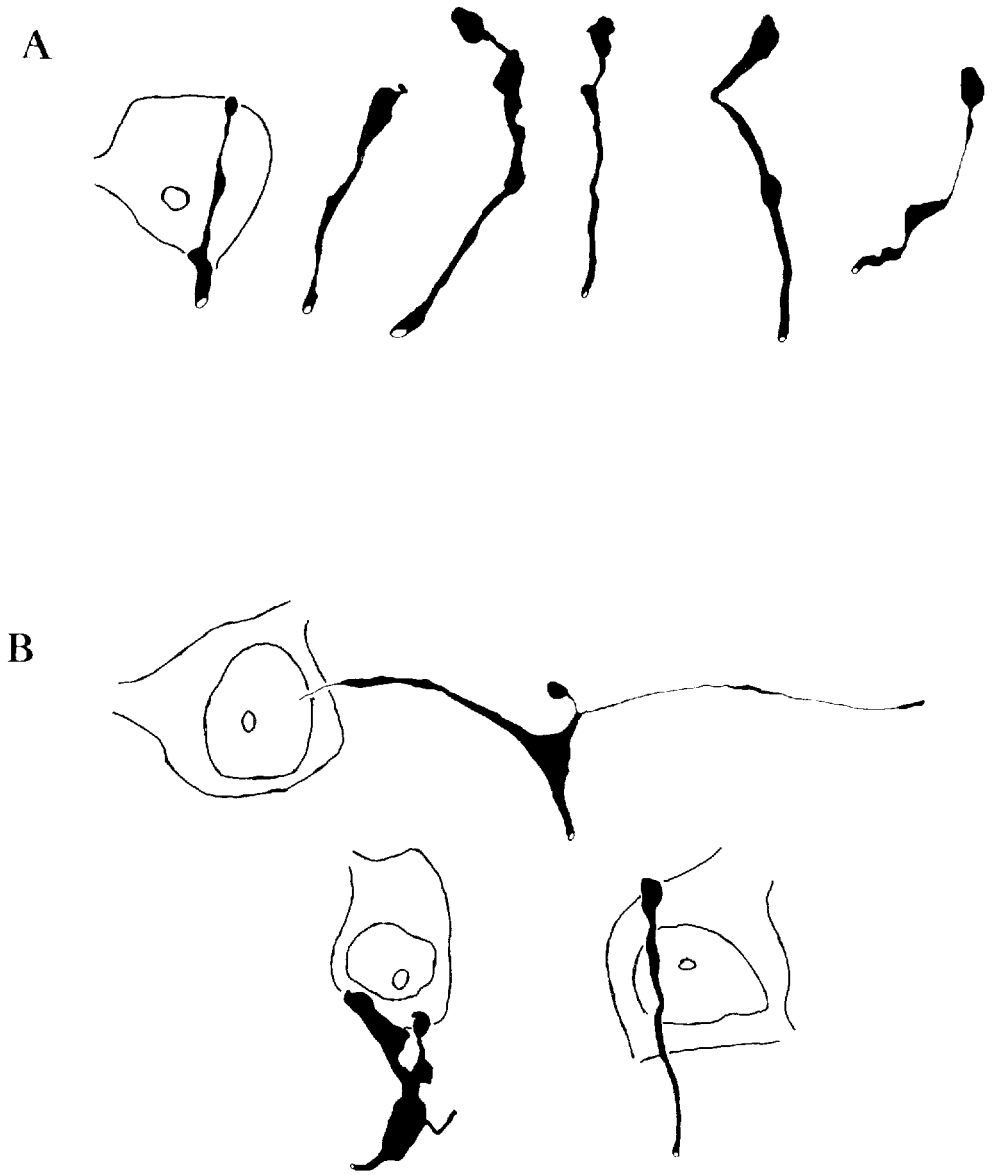



Fig. 12. Examples of terminals of free-standing fibers in the NAM and NML. **A:** Simple axosomatic and neuropil terminals in the NAM. **B:** Simple axosomatic and neuropil terminals in the NML. Bar = 20 $\mu$m.

would require a comparative mechanism for interaural phase differences that was at least as sensitive as that of humans merely to determine whether the source was located to the left or to the right of the midline. Presently, there is no evidence that the alligator lizard has such a capacity.

## Free-standing fibers

The relationship of free-standing fibers to cochlear nerve fibers of other species lacking free-standing, bidirectional hair cells (e.g., birds and mammals) is not clear. Free-standing fibers, mammalian type I fibers, and avian cochlear nerve fibers have myelinated and relatively thick central axons and give rise to small synaptic endings. On the other hand, free-standing fibers differ from avian and mammalian cochlear nerve fibers in many ways: Their tuning curves are broader; their peripheral processes contact hair cells having long stereocilia (9–31 $\mu$m) that are free of an overlying tectorial membrane; they do not phase lock to low-

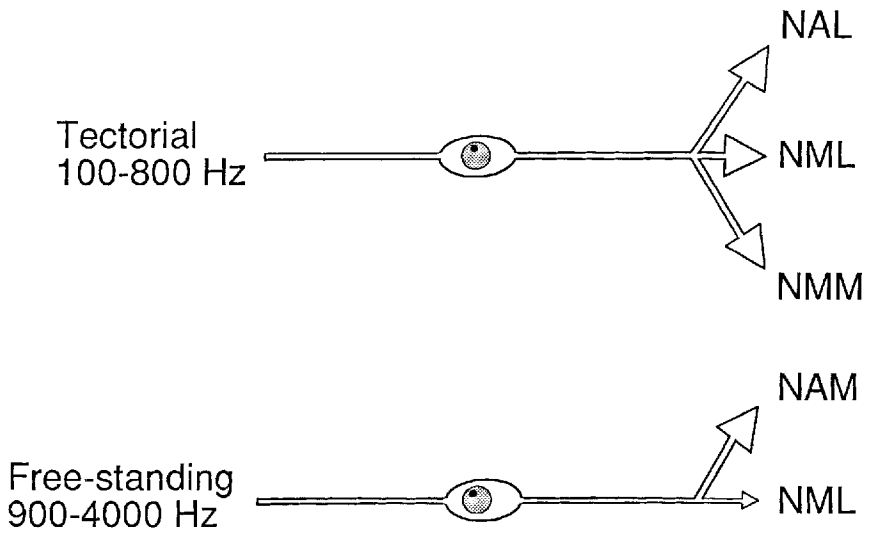



Fig. 13. Summary figure of the central projections of tectorial and free-standing fibers in the alligator lizard. Tectorial fibers project to NAL, NML, and NMM. Free-standing fibers project primarily to NAM, but a small fraction of free-standing fibers also project a fine branch to NML. Abbreviations: NAL, nucleus angularis lateralis; NAM, nucleus angularis medialis; NML, nucleus magnocellularis lateralis; NMM, nucleus magnocellularis medialis.

frequency, low-intensity sounds; they do not exhibit two-tone suppression, neither they nor their presynaptic hair cells are associated with efferent terminals; and their spontaneous discharge rates are all above 25 s/s (Mulroy, '68; Weiss et al., '74; '76; Frezza, '76; Holton and Weiss, '78; Rose and Weiss, '88). Furthermore, we have shown that the central projections of free-standing fibers avoid certain major subdivisions of the cochlear nucleus entirely. This system that uses bidirectional hair cells and free-standing fibers may be a uniquely derived (autapomorphic) feature of lizards; it has not been found in any other vertebrate group.

## CONCLUDING REMARKS

In general, we have confirmed and extended the observations provided by population studies of eighth-nerve projections in the brainstem of lizards (Beccari, '12; Hamilton, '63; DeFina and Webster, '74; Foster and Hall, '78; Barbas-Henry and Lohman, '88). The greatest concentration of primary afferent fibers is found in the NA and the NM. For the tegu and monitor lizard, however, it has been suggested that the nucleus laminaris (which is a second-order nucleus in birds, crocodiles, and turtles) receives primary input (DeFina and Webster, '74; Barbas-Henry and Lohman, '88), whereas for the iguana, it has been explicitly reported that the nucleus laminaris does not receive primary input (Foster and Hall, '78). In the present study, we were unable to identify a distinct nucleus laminaris, a situation common to other lizard species (Beccari, '12; Weston, '36; Miller, '75). The variance in observations among studies of the lizard may be attributable to differences in the following: species examined, methods used, or schemes for partitioning the cochlear nucleus. Regardless, our contribution is in the reconstruction of single cochlear nerve fibers and the demonstration that such fibers may be reliably classified into one of two categories.

Although it was not possible with our material to trace individual fibers continuously from their peripheral termination in the basilar papilla to their central termination in the cochlear nucleus, the labeling patterns were sufficiently consistent for us to be secure in our identification of central fibers as being either tectorial or free-standing. That is, whenever it was possible to recover labeled fibers in the periphery, labeling under tectorial hair cells was always correlated with projections to the NAL, NML, and NMM, and labeling under free-standing hair cells was always correlated with projections to NAM. The poorer recovery of label in the papilla compared to the nerve resembled what has been reported in cats, where labeling in the organ of Corti is always less than that in the auditory nerve (Liberman and Oliver, '84).

In our light microscopic studies of the cochlear nerve, we found no evidence for a population of very thin axons. Our observations are in basic agreement with those of Mulroy ('83). This concern is relevant to comparative issues in auditory neurobiology because of the presence of thin (<0.5 $\mu$m in diameter), unmyelinated axons of type II spiral ganglion neurons in mammals (Ryugo et al., '86; Brown et al., '88). The diameter of tectorial and free-standing fibers is more in accord with measures of the mammalian type I axons (2.5–4.0 $\mu$m in cat, Arnesen and Osen, '78; 2 $\mu$m in guinea pig, Brown, '87) or avian cochlear nerve fibers (1–7 $\mu$m in pigeon; Boord and Rasmussen, '63). The thinnest of the free-standing fibers in our sample (1.6 $\mu$m) is well outside the diameter range of type II axons. An additional line of circumstantial evidence is that mammalian type II fibers are associated with granule cell regions in the cochlear nucleus (Brown et al., '88), whereas we have never seen granule cells in the lizard cochlear nucleus.

The structural organization of the basilar papilla is remarkably variable across lizard families (Miller, '80, '85). There are, however, some features found in all lizard auditory systems described thus far. For example, no lizard species has a papilla with entirely abneural, unidirectional hair cells such as is found in mammals; all lizards have bidirectional hair cells (Miller, '80; Miller and Beck, '88; Wever, '78). Low-frequency reception (<1 kHz) is confined to fibers that innervate unidirectional (tectorial) hair cells (Turner, '80; Manley, '81). Furthermore, those fibers innervating unidirec-

tional hair cells always have greater axon diameters than do fibers that innervate bidirectional hair cells (Miller, '85). The structural variation of the basilar papilla and its representation in the cochlear nucleus by central projections of cochlear nerve fibers raises a number of questions from both evolutionary and functional perspectives. For example, is the diversity in the periphery systematically related to variations in the structure and organization of the central pathways or can a particular set of central neurons accommodate extensive peripheral variation? Are particular neuronal morphologies in the cochlear nucleus consistently associated with certain frequency ranges? We mentioned that the size of the NMM is related to the size of the unidirectionally oriented hair cell region (Miller, '75; Miller and Kasahara, '79); this observation may be explained by our results, which demonstrate that the only primary afferent supply to the NMM is via tectorial fibers arising from unidirectional hair cells. In turn, cells of the NMM may be sensitive exclusively to low-frequency (<1 kHz) sounds. Ultimately, more data on different species are needed before we can understand how form and function are related.

## ACKNOWLEDGMENTS

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