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Central Projections of Intracellularly Labeled Auditory Nerve Fibers in Cats: Morphometric Correlations With Physiological Properties

D.K. RYUGO AND E.M. ROUILLER
Department of Anatomy and Cellular Biology, Harvard Medical School, Boston, Massachusetts 02115; Eaton-Peabody Laboratory, Massachusetts Eye and Ear Infirmary, Boston, Massachusetts 02114

ABSTRACT

The central arborizations and endings of type I spiral ganglion neurons were labeled with intracellular injections of horseradish peroxidase (HRP) after their characteristic frequency (CF) and spontaneous discharge rate (SR) were physiologically determined. A fiber-by-fiber analysis was conducted and the morphological data compared with the fiber's response properties. The total number of branch points was correlated with total fiber length, a relationship that remained relatively constant when analyzing the ascending and descending branches together or separately. On the other hand, the ascending branches of four out of five fibers having CFs below 0.5 kHz bifurcated and gave rise to a pair of terminal endbulbs of Held.

Low- and medium-SR fibers gave rise to more endings than did high-SR fibers, especially on the ascending branch. This difference was accounted for by small endings, a category composed of terminal boutons, string endings, and small complex endings. The categories of modified endbulbs, and endbulbs of Held did not vary in number with respect to fiber SR. The mean area of each ending type within the small ending category was statistically smaller for low- and medium-SR fibers than for high-SR fibers, whereas the mean area of modified endbulbs and endbulbs of Held was not correlated with fiber SR. Total ending area per fiber appeared independent of either CF or SR. These results are discussed in relation to issues of conservation of axon arborizations and terminals, and convergence of input from the different SR groups.

Key words: axon, cochlear nucleus, hearing, horseradish peroxidase, primary afferent, spontaneous discharge rate, synaptic endings

In mammals, acoustic information originating from hair-cell receptors in the cochlea is transmitted to the brain by way of spiral ganglion neurons whose axons form the auditory nerve. Most of our present knowledge is derived from the more plentiful type I neurons. In adult cats, these type I neurons represent 90–95% of the ganglion population (Spoendlin, '71), their peripheral processes contact inner hair cells exclusively (Kiang et al., '82; Liberman and Oliver, '84), their central processes are myelinated (Arnesen and Osen, '78), and they exhibit a cochleotopic projection into the cochlear nucleus (Fekete et al., '84). Golgi studies of this population of auditory nerve fibers established their basic structure (Lorente de Nó, '81; Ramón y Cajal, '09), upon which other anatomical variations are imposed. We are now concerned with studying those variations hypothesized to have functional significance.

The application of intracellular recording and labeling techniques makes possible a comparison of anatomical variables with physiological properties. Type I neurons can be described by their characteristic frequency (CF) and spontaneous discharge rate (SR) using electrophysiological methods. The CF (frequency of tone to which a neuron is most sensitive) is determined from a "tuning curve" (Li-

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Address reprint requests to Dr. David K. Ryugo, Center for Hearing Sciences, Johns Hopkins Univ. Sch. of Medicine, Traylor Research Building, 720 Rutland Avenue, Baltimore, MD 21205.

E.M. Rouiller's present address is Department of Physiology, School of Medicine, 7, rue du Bugnon, 1005 Lausanne, Switzerland.

TABLE 1. Summary of Individual Fiber Characteristics With Respect to SR: Average Values per Fiber (± S.D.) and Their Comparisons Between SR Groups

	High-SR group	Low-medium-SR group	*P*-value
No. of fibers	15	12	—
Total fiber length[1]	12.8 ± 0.5 mm	15.6 ± 1.2 mm	< 0.05
Total No. of endings	58.5 ± 5.2	98.6 ± 44.5	< 0.01
Small endings	53.8 ± 19.5	94.2 ± 41.3	< 0.01
Terminal boutons	40.2 ± 15.1	71.8 ± 25.6	< 0.01
String endings	7.1 ± 4.3	11.3 ± 7.8	< 0.05
Small complex endings	6.5 ± 2.8	11.1 ± 7.5	< 0.05
Modified endbulbs	2.9 ± 0.5	2.8 ± 1.0	n.s.[2]
Endbulbs of Held	1.7 ± 0.2	1.6 ± 0.2	n.s.
Total ending area (μm^2)	853.2 ± 309	853.0 ± 330	n.s.
Mean ending area (μm^2)	15.2 ± 5.1	9.2 ± 3.4	< 0.01
Small endings	6.6 ± 1.6	4.8 ± 3.4	< 0.01
Terminal boutons	4.8 ± 1.1	3.3 ± 1.0	< 0.01
String endings	10.1 ± 3.4	9.0 ± 3.1	< 0.01
Small complex endings	14.9 ± 4.4	11.7 ± 3.6	< 0.05
Modified endbulbs	39.5 ± 17.2	23.0 ± 18.2	n.s.
Endbulbs of Held	225.8 ± 85.7	238.2 ± 129.8	n.s.
En passant swellings			
No. per fiber	34.9 ± 18.8	74.1 ± 42.9	< 0.01
Area of each (μm^2)	4.2 ± 3.0	4.1 ± 3.3	n.s.
Total area (μm^2)	147.2 ± 77.5	302.1 ± 170.9	< 0.05

[1]Values include parent branch and collateral lengths. Root branch data are not included on this line, but are included for all other calculations in this table.
[2]Not significant.

TABLE 2. Summary of Branch Characteristics With Respect to SR: Average Values (± S.D.) for Numbers and Sizes of Endings Computed Separately for the AB and DB[1]

	High SR		Low-medium SR	
	AB	DB	AB	DB
No. of branches	15	15	12	12
Collateral length (mm)[2]	2.8 ± 1.1	3.6 ± 1.5	5.1 ± 2.9	4.0 ± 1.4
Total No. of endings	25.0 ± 11.1	30.2 ± 12.0	53.7 ± 33.1	37.9 ± 16.0
Small endings	21.7 ± 11.7	29.4 ± 11.4	50.6 ± 30.9	37.2 ± 15.0
Terminal boutons	16.6 ± 9.9	21.7 ± 7.8	37.1 ± 20.6	29.8 ± 11.5
String endings	2.7 ± 2.0	4.3 ± 2.9	6.9 ± 5.9	3.4 ± 2.6
Small complex endings	2.3 ± 1.1	3.4 ± 2.6	6.6 ± 5.8	4.0 ± 2.5
Modified endbulbs	1.6 ± 1.5	0.7 ± 0.9	2.0 ± 2.8	0.9 ± 1.1
Endbulbs of Held	1.5 ± 0.6	0	1.3 ± 0.7	0
Total ending area/branch	543.0 ± 227	244.9 ± 130	605.4 ± 215	160.4 ± 67
Mean ending area (μm^2)	24.2 ± 9.9	8.2 ± 5.1	12.6 ± 5.7	4.3 ± 1.4
Small endings	6.6 ± 1.2	6.4 ± 2.3	5.3 ± 1.6	4.0 ± 1.1
Terminal boutons	5.0 ± 1.4	4.6 ± 1.6	3.5 ± 1.2	2.8 ± 0.9
String endings	10.9 ± 3.6	9.5 ± 3.8	9.7 ± 3.7	5.7 ± 1.6
Small complex endings	12.1 ± 4.7	17.6 ± 8.6	11.9 ± 4.8	10.9 ± 4.7
Modified endbulbs[3]	38.3 ± 13.8	38.0 ± 15.0	49.6 ± 28.0	27.9 ± 11.2
Endbulbs of Held	222.7 ± 88.8	—	249.9 ± 124.8	—
En passant swellings				
No. per branch	15.9 ± 10.7	17.2 ± 10.2	41.0 ± 32.9	28.4 ± 12.6
Area of each (μm^2)	4.1 ± 2.8	4.0 ± 2.8	4.0 ± 3.1	4.1 ± 3.5
Total area (μm^2)	64.9 ± 44.1	68.6 ± 42.7	164.9 ± 140.5	115.5 ± 50.8

[1]RB data are not included for any calculations in this table.
[2]Length values include measurements of collaterals only; parent branch lengths are not included.
[3]This sample is small and may not be representative of the population.

berman, '78), corresponds to the longitudinal location of the peripheral terminal along the cochlear duct (Liberman, '82b), and indicates the spatial distribution of the central projections within the cochlear nucleus (Fekete et al., '84). Among units having similar CFs, SR can range from near zero to approximately 100 spikes per second. Current data for single fiber recordings of the auditory nerve suggest that SR has a bimodal distribution and that neurons of the separate SR classes differ significantly in their response properties such as threshold, dynamic range, and representation of sound in terms of discharge rate profiles (e.g., Liberman, '78; Sachs and Young, '79; Evans and Palmer, '80; Miller and Sachs, '83, '84; Costalupes, '85). Furthermore, differences in SR have been shown to correlate with the caliber of the peripheral terminal and the location of the peripheral synapse on the inner hair cell (Liberman, '82a), as well as with features of the axonal arborization (Fekete et al., '84) and average ending size (Rouiller et al., '86) in the cochlear nucleus. These observations are consistent with the idea that the different SR types, present across the entire range of CF values, represent functionally different components for the processing of acoustic information.

Our working hypothesis is that auditory nerve fibers belonging to different functional groups will have different connections with the cochlear nucleus, reflected in the size,

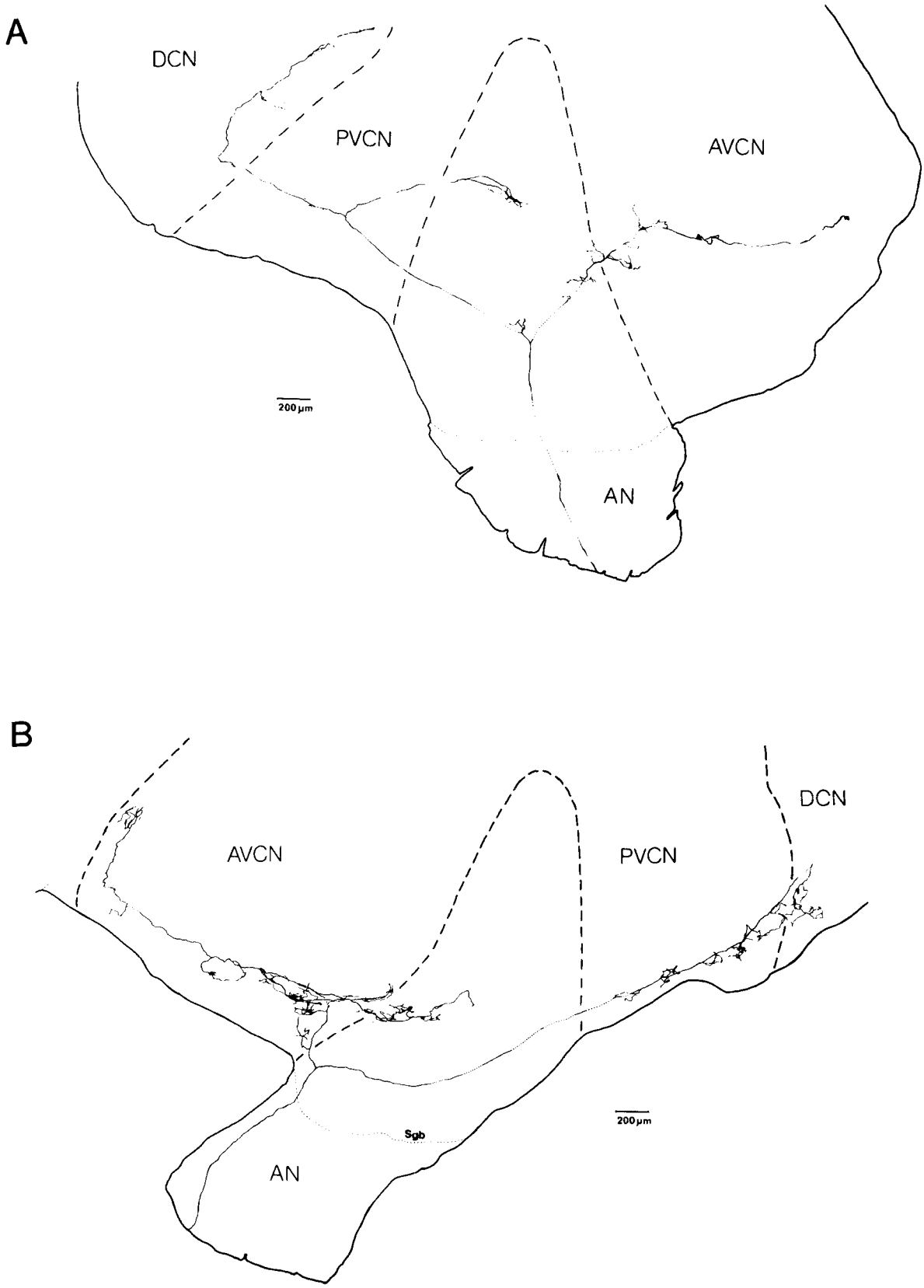

Fig. 1. Drawing-tube reconstructions of a high-SR fiber (A) and a low-medium-SR fiber (B) from the same cat. The high-SR fiber was from the right cochlear nucleus (CF = 1.2 kHz; SR = 85.6 s/s), had total fiber length of 12.5 mm, 58 endings, and a summed ending area of 1,146 μm^2. The low-medium-SR fiber was from the left cochlear nucleus (CF = 0.45 kHz; SR = 1.2 s/s), had a total fiber length of 25.2 mm, 206 endings, and a summed ending area of 1,279 μm^2. These two fibers illustrate features of the high- and low-medium-SR population. Abbreviations: AN, auditory nerve; AVCN, anteroventral cochlear nucleus; DCN, dorsal cochlear nucleus; PVCN, posteroventral cochlear nucleus; Sgb, Schwann-glial border.

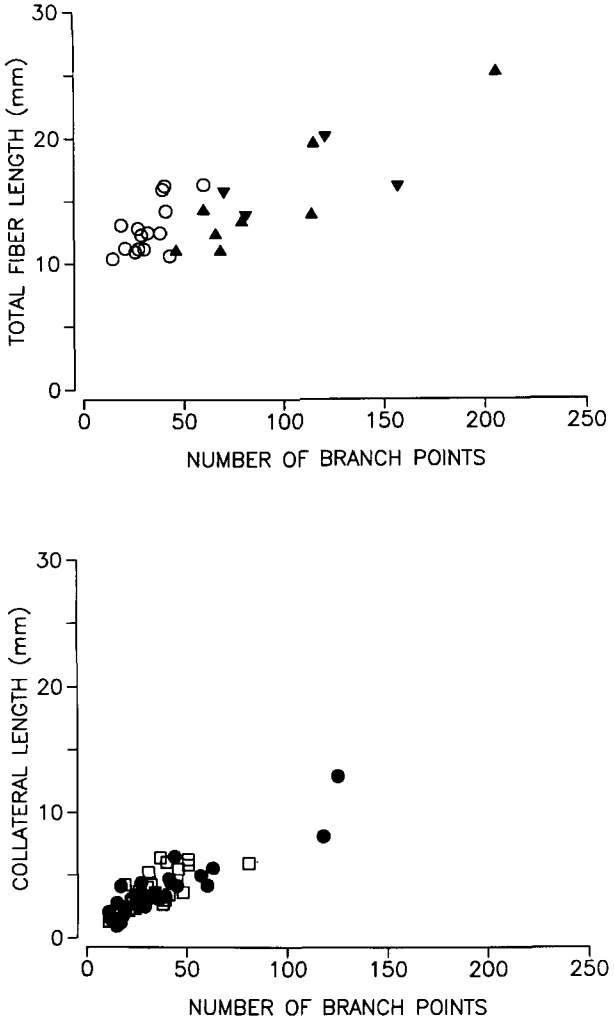

Fig. 2. Scatter plot revealing the relationship between length measures and number of branch points. **Top:** Plot for individual auditory nerve fibers—high-SR fibers (○), medium-SR fibers (▲), low-SR fibers (▼). **Bottom:** Plot for ascending (●) and descending (□) branches.

shape, and distribution of the various types of synaptic endings. Given that fibers of the separate SR groups exhibit differences in the average size of synaptic endings (Rouiller et al., '86), we sought to determine whether this difference in ending morphology extended to the presence of different ending types or proportions of ending types. We also investigated whether these fibers exerted different degrees of "synaptic drive" on the nucleus as indicated by systematic variations in total ending area. In this context, structure-function relationships between auditory nerve fibers and the cochlear nucleus may provide important clues to understanding how neural information is initially distributed to the central nervous system.

MATERIALS AND METHODS

Physiology and histology

In this study, a total of 15 cats was used, each weighing between 1.75 and 3.4 kg and free of middle ear infections. The anesthetic and surgical preparation, the means of presenting acoustic stimuli, and the techniques for recording and processing single unit activity have been previously described (Kiang et al., '65; Liberman, '78). Specific details for intracellular recording and injecting individual auditory nerve fibers, procedures for histological processing, and criteria for recovering labeled fibers from tissue sections have also been described elsewhere (Fekete et al., '84; Rouiller et al., '86). Briefly, animals were anesthetized with intraperitoneal injections of diallyl barbituric acid in urethane solution (75 mg per kg). Supplemental doses were periodically administered in order to maintain areflexia to paw pinches. The bulla and its bony septum were opened to allow for round window recordings and the external meati were cut just peripheral to the tympanic ring to allow insertion of the acoustic system. The skin and muscle layers of the head were removed so that the skull overlying the posterior fossa could be opened with rongeurs. The dura was reflected over the cerebellum, and the cerebellum was retracted revealing the auditory nerve between the internal auditory meatus and the cochlear nucleus. Recording micropipettes were then placed into the nerve under direct visual control. Upon contacting a unit, a threshold tuning curve and a 15- or 30-second sample of spontaneous activity (SR) were obtained before and after the injection of HRP for each unit. The tuning curve was used to determine CF, and SR was defined as spike activity (spikes per second, s/s) in the absence of sound controlled by the experimenter. Individual fibers were labeled by iontophoresing a 10% solution of HRP (Sigma, type VI) in 0.05 M Tris buffer (pH 7.3) containing 0.15 M KCl through micropipettes beveled to a final impedence of 40–60 MΩ. Approximately 24 hours after the HRP injections, the cat was given a lethal dose of barbiturate, artificially respirated, and perfused intracardially with buffered fixatives. The perfusion solutions consisted of 50 ml of isotonic saline (37°C) with 0.1% $NaNO_2$, followed immediately by 500 ml of fixative (37°C) containing 0.5% paraformaldehyde, 1.0% glutaraldehyde, and 0.008% $CaCl_2$ in 0.12 M phosphate buffer (pH 7.4), and then 1.5 liter of a second fixative (37°C) containing 1.25% paraformaldehyde and 2.5% glutaraldehyde, and 0.008% $CaCl_2$ in the same buffer solution. Following perfusion and decapitation, the head was immersed in the second fixative (5°C) with enough bone and tissue removed to expose the auditory nerve and cochlear nucleus to the fixative. After 12–24 hours, the brain was removed from the skull and the nerve and nucleus isolated in a single tissue block. Each block was embedded in gelatin-albumin (Frank et al., '80), sectioned at 40-μm thickness using a Vibratome, and kept in serial order. The sections were rinsed several times in 0.1 M tris buffer (pH 7.6) and then incubated for 1 hour in a solution of 0.5% $CoCl_2$ in Tris buffer. These sections were washed in Tris buffer, washed in 0.1 M phosphate buffer (pH 7.3), and then incubated for 1 hour in a solution of 0.05% 3,3′-diaminobenzidine and 1% dimethylsulfoxide in phosphate buffer (pH 7.3). Sections were washed again and then mounted on glass microscope slides and counterstained with cresyl violet, or postfixed with 0.1% OsO_4 for 15 minutes, stained en bloc with 1% uranyl acetate (overnight), dehydrated, infiltrated with Epon, and flat-embedded between two sheets of Aclar (Allied Engineered Plastics, Pottsville, PA). Tissue was studied using light microscopes and, in a few cases, was thin sectioned and examined with an electron microscope.

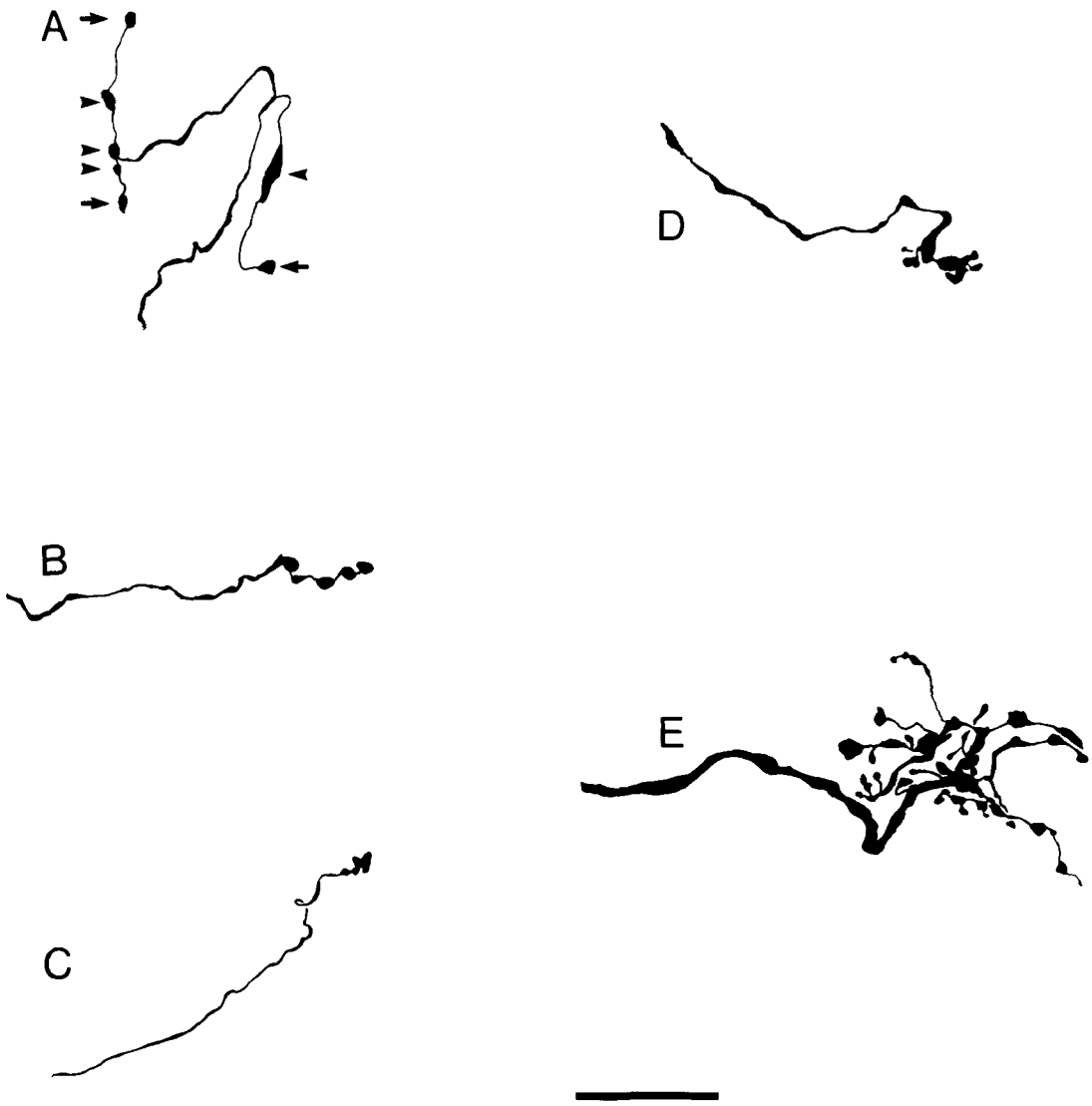

Fig. 3. Drawing-tube reconstructions of endings representative of the different ending categories: A) Terminal boutons (arrows) and en passant swellings (arrowheads); B) string ending; C) small complex ending; D) modified endbulb; E) endbulb of Held. Scale bar equals 20 μm.

Fiber reconstructions

Single fibers were reconstructed from serial sections with the aid of a light microscope and drawing tube at a total magnification of $\times$312. Well-labeled fibers appeared black against the pale tissue of the cochlear nucleus. Each fiber bifurcates into two "parent" branches; the ascending parent branch projects anteriorly through the anteroventral cochlear nucleus and the descending parent branch projects posteriorly through the posteroventral cochlear nucleus and usually into the dorsal cochler nucleus. The lengths of the parent axons (ascending branch, AB, and descending branch, DB) were determined from these low-magnification drawings. "Primary collaterals" arising from these parent axons, including their ramifications and endings, were then drawn at a magnification of $\times$1,250. For each primary collateral, the length of every axon segment (whose ends were marked by a branch point or a terminal swelling) was measured. All length determinations were made with the aid of a computerized planimeter. The sum of the parent lengths (AB and DB) and corresponding collateral lengths yielded a value defined as the "total fiber length." When the values for parent branch lengths were excluded, the measures were termed the "collateral lengths." The length values for that portion of the axon prior to the bifurcation (the root branch and its collaterals) were omitted from portions of the present analysis because they co-varied with fiber CF (Table 2) but were included in other analyses (Table 1).

Endings were classified (according to the criteria of Rouiller et al., '86) and counted for each fiber. The category of small endings consisted of terminal boutons, string endings, and small complex endings. The category of medium-sized endings (modified endbulbs) contacted the perikarya of spherical and globular cells and occasionally the perikarya of octopus cells. The category of large endings (endbulbs of Held) typically made axosomatic contacts with spherical cells in the anterior division of the anteroventral

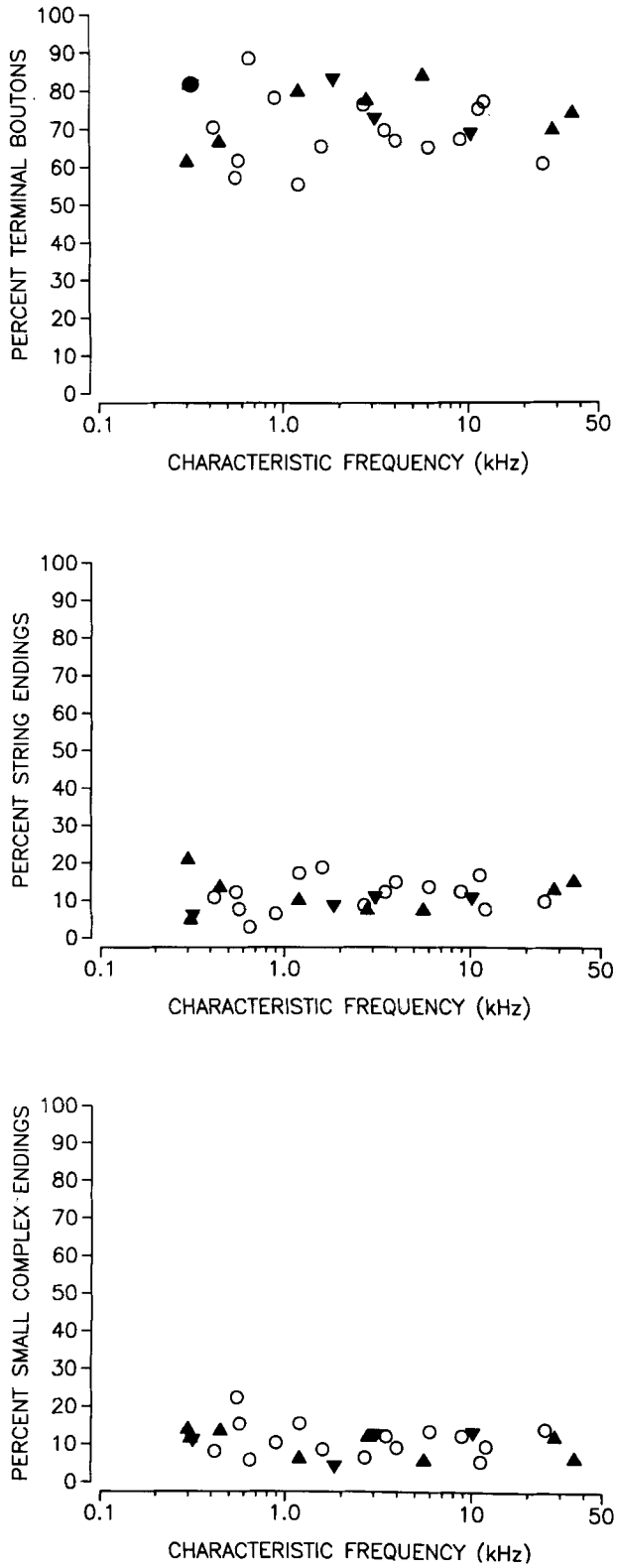

Fig. 4. Scatter plots illustrating the proportional representation of the different types of small endings. Note that the relative number of endings for a particular type is fairly constant. These data demonstrate that the increased number of endings on low-medium-SR fibers includes all the subcategories of small endings rather than a single type. High-SR fibers (○), medium-SR fibers (▲), low-SR fibers (▼).

cochlear nucleus (AVCN) and occasionally with globular cells in the posterior division of the AVCN. This kind of single fiber analysis yielded the absolute and proportional representation of the separate ending categories.

Ending size was represented by ending silhouette area; each drawing was photographically enlarged to a final magnification of ×2,500 and retraced with the aid of a computerized planimeter. For every fiber, the sum of all ending areas yielded a value defined as the "total ending area." Summed ending area was calculated for the separate ascending and descending branches. We also calculated the mean area for the different ending categories, determined for the ascending and descending branches separately and together. Nomenclature and criteria for defining the different regions of the cochlear nucleus are the same as previously reported (Fekete et al., '84; Rouiller et al., '86).

Data analysis

The observations in this report are the third part of a series of studies based on analyses of 19 cochlear nuclei containing the HRP-labeled axons of 27 type I spiral ganglion neurons, ranging in CF from 0.3–36 kHz and in SR from 0–87.7 s/s (Fekete et al., '84; Rouiller et al., '86). These fibers were well characterized physiologically and were recovered with high confidence. Furthermore, they exhibited a distinct swelling at the tip of every terminal branch, thereby providing light microscopic evidence that the entire fiber was completely stained. Units were assigned to SR groups using the criteria of Liberman ('78): Low SR = < 0.5 s/s; medium SR = 0.5–18 s/s; high SR = > 18 s/s. The sample population consists of 15 fibers in the high SR group, 8 fibers in the medium SR group, and 4 fibers in the low SR group. For purposes of statistical comparisons to high-SR fibers, low- and medium-SR fibers have been grouped together because they share a number of electrophysiological (Liberman, '78; Evans and Palmer, '80; Costalupes, '85) and anatomical characteristics (Liberman, '82a; Fekete et al., '84; Rouiller et al., '86). Otherwise, data points for individual fibers have been represented by separate symbols in the figures according to their SR. The means, standard deviations, and Smirnov's *P*-values (nonparametric, two-tailed test) are provided where appropriate.

RESULTS

The basic structure of individual type I auditory nerve fibers in the cochlear nucleus has been previously described (e.g., Lorente de Nó, '81; Fekete et al., '84). In brief, each fiber enters the cochlear nucleus as the root branch and bifurcates. The bifurcation gives rise to an ascending branch that projects anteriorly through the anteroventral cochlear nucleus and a descending branch that projects posteriorly through the posteroventral cochlear nucleus and usually (85%) into the dorsal cochlear nucleus. The average length of the ascending ($x = 2.33 \pm 0.5$mm) and descending ($x = 3.41 \pm 0.7$ mm) branch is relatively constant across fibers and independent of CF and SR. The average number of primary collaterals ($x = 20.8 \pm 4.5$) is also similar across fibers. These features of the axon establish its basic form upon which variations are imposed, depending on CF or SR.

There are certain morphological features of the fiber which reliably correlate with the fiber's physiological properties. Some of these relate to fiber CF. First, the root branch, defined as that segment from the Schwann-glial border to the bifurcation, exhibits a length that systematically varies with fiber CF ($r = 0.95$). Since the trajectory and

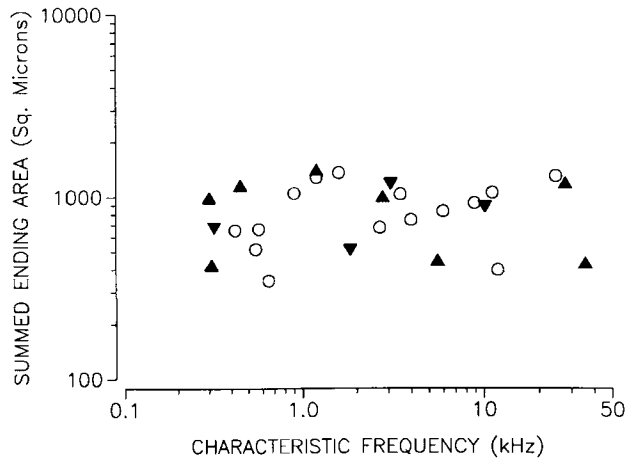

Fig. 5. Scatter plot illustrating the summed ending area for all fibers. Note that this value does not correlate with either CF or SR. Symbols: high-SR fibers (○), medium-SR fibers (▲), low-SR fibers (▼).

arborization of the ascending and descending branches maintain their relative spatial position within the nucleus, the length of the root branch determines the cochleotopic position of each fiber. Second, four out of five fibers having CF's below 0.5 kHz had ascending branches that bifurcated and gave rise to a pair of ascending branches (see Fig. 8B). As is the case for single ascending branches, each of the pair of ascending branches also terminated with an endbulb of Held in the anterior division of the anteroventral cochlear nucleus. Fibers having two terminal endbulbs, however, did not have collateral endbulbs. These paired ascending branches also maintained their CF-appropriate dorsoventral position while diverging in the mediolateral dimension. No fiber having a CF above 0.5 kHz exhibited a pair of ascending branches. Thus, it seems that fibers of any CF can have a single ascending branch, but only fibers having very low CFs can have a pair of ascending branches.

There are also morphological features related to fiber SR. On average, low- and medium-SR fibers have more elaborate arborizations than do high-SR fibers (Fekete et al., '84). This difference, however, was more apparent when fibers from the same cat rather than across cats were compared. In order to emphasize the morphological characteristics that were related to fiber SR, pairs of units from the same cat (n=7) were labeled which were roughly similar in CF but different in SR (see Fig. 1). In one particular cat, a fiber of high-SR (SR=85.9 s/s; CF=1.2 kHz) was stained in the right auditory nerve and cochlear nucleus (Fig. 1A). On the left side, a fiber of low-medium SR (SR=1.2 s/s; CF = 0.45 kHz) was stained (Fig. 1B). This case illustrates the difference in axonal branching and measured lengths of the axonal arborization with respect to fiber SR.

We sought to determine the possible relationship between axon branching and axon length. There is a strong correlation (r=0.90; slope of regression line=0.07) between the values for total fiber length and the number of branch points (Fig. 2, top). We can safely ignore the length values for the separate ascending and descending parent branches because they remain constant independent of fiber CF or SR. Since differences in axonal arborizations are primarily confined to the ascending branch, we hypothesized that a length-to-branching value for the collaterals might quantitatively distinguish the ascending from the descending branch. Such is not the case, however, because the correlation for collateral length and collateral branching for the ascending (r=0.91, slope=0.07) and descending (r=0.71, slope=0.08) branches were similar (Fig. 2, bottom). These correlation coefficients ($P < 0.01$) and the similarity in the slopes of the regression lines suggest a common quantitative relationship with respect to axon elongation and axon branching.

Numbers and types of endings

Fiber analysis. There is an obvious difference in the total number of endings per fiber between the groups of high- and low-medium-SR fibers (Table 1). On average, each high-SR fiber gave rise to roughly 60 endings, whereas each low- or medium-SR fiber had nearly 100. This difference was accounted for by the category of small endings, represented by the subcategories of terminal boutons (Fig. 3A), string endings (Fig. 3B), and small complex endings (Fig. 3C). Irrespective of differences in the total number of endings per fiber, the proportions of these subcategories remained relatively stable across fibers having different SRs (Fig. 4). Therefore, all subcategories of small endings contributed to this absolute numerical difference.

The average number of modified endbulbs (Fig. 3D) and endbulbs of Held (Fig. 3E) per fiber did not vary with respect to fiber CF or SR (Table 1). Modified endbulbs arose from all parent branches, whereas endbulbs of Held arose from only ascending and root branches. Individual fibers from the high-SR group had on average 2.9 ± 0.5 modified endbulbs, whereas those from the low-medium-SR group had an average of 2.8 ± 1.0. In addition, fibers of the high-SR group had on average 1.7 ± 0.2 endbulbs of Held and those of the low- and medium-SR group had an average of 1.6 ± 0.2 endbulbs. Each fiber gave rise to one terminal endbulb and usually one collateral endbulb. The terminal endbulb was found at the anterior tip of the ascending branch, whereas the collateral endbulb arose from a primary collateral.

Parent branch analysis. The average number of the different ending types was computed separately for the ascending and descending branches (Table 2). This analysis focused on the small ending category since endbulbs of Held did not arise from the descending branch, and modified endbulbs rarely arose from the descending branch. In the high-SR group, more small endings were emitted by the descending branch than by the ascending branch; on the other hand, in the low-medium-SR group, more small endings were emitted by the ascending branch than by the descending branch (Table 2). The ascending branch provides for most of the difference in total number of small endings between the two SR groups.

Size analysis for ending categories

The area for each ending category was calculated for each fiber. Individual endings were then grouped according to the separate ending categories. Those small endings (terminal boutons, string endings, and small complex endings) arising from low-medium-SR fibers were smaller on average than those arising from high-SR fibers ($P < 0.01$). There was no statistical difference in the size (silhouette area) of modified endbulbs or endbulbs of Held with respect to fiber SR (Table 1).

Ending area was also examined with respect to origin from ascending or descending branches. For most cate-

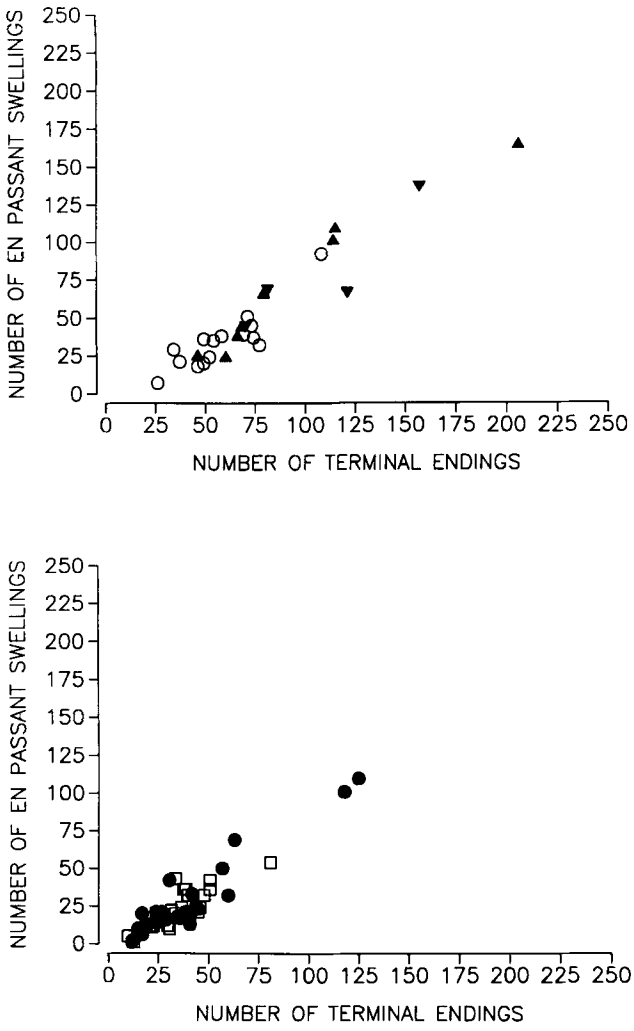

Fig. 6. Scatter plot illustrating the relationship between the number of terminal endings and the number of en passant swellings. **Top:** Plot for entire fiber (includes collaterals of ascending, descending, and root branches)—high-SR fibers (○), medium-SR fibers (▲), low-SR fibers (▼). **Bottom:** Plot for ascending (●) and descending (□) branches taken separately. The proportion of en passant swellings to synaptic endings remains constant throughout the arbor.

gories, endings of the ascending branch were larger than those of the descending branch. The observed size difference was similar for the two SR groups (Table 2).

Total ending area per fiber

Given the differences in the numbers and sizes of endings for the different fiber groups, we calculated the total ending area per fiber. For each fiber, we added the areas for all of its individual endings. The resultant values revealed that the total ending area per fiber was not systematically related to fiber CF or SR (Fig. 5). The SR distribution in our fiber sample is 15% low-SR fibers, 30% medium-SR fibers, and 55% high-SR fibers. This SR representation is consistent with much larger samples of auditory nerve fibers in cats (Liberman, '78), and so our normalized ending values should be fairly representative. Analysis of ending area reveals that 75% of the total ending area arises from the ascending and root branches and is delivered to the anteroventral cochlear nucleus. Furthermore, contributions to the total ending area from fibers of the separate SR groups for the anteroventral cochlear nucleus is roughly proportional to their representation within the fiber population. Such is not the case for the descending branch, of which 17% is from high-SR fibers and 8% is from low-medium-SR fibers.

En passant swellings

Along the length of many but not all terminal branches, distinct swellings could be observed. When the diameter of the fiber expanded at least threefold over a distance of less than 2 μm and then returned to its original diameter in an equally abrupt fashion, the expansion was called an en passant swelling (Fig. 3A). A gradual expansion that might represent a thickening or twisting of the axon was not considered. Our interest in these swellings is derived from the observation that they do not appear to be uniformly distributed throughout the fiber and from the possibility that they might be the presynaptic element of synapses (e.g., Peters et al., '76).

The absolute number of en passant swellings was related to fiber SR. On average, there were fewer en passant swellings on high-SR fibers (34.9 $\pm$ 18.8) than there were on low-medium-SR fibers (74.1 $\pm$ 42.9, $P < 0.01$). For individual fibers, there was a strong correlation (r=0.96; slope=0.92) between the number of terminal endings and the number of en passant swellings (Fig. 6, top). This correlation was also evident for the separate ascending (r=0.95; slope 0.91) and descending (r=0.84; slope=0.74) branches (Fig. 6, bottom). The average ratio of the number of en passant swellings to the number of endings was 0.64 $\pm$ 0.2 for all fibers. These relationships were independent of fiber CF or SR, although there was a tendency (not statistically significant) for this ratio to be higher for fibers of low-medium-SR (0.71 $\pm$ 0.2) than for fibers of high SR (0.58 $\pm$ 0.2).

En passant swellings were, however, uniform in size, regardless of parent branch of origin, fiber CF, or fiber SR. En passant swellings for high-SR fibers were on average 4.2 $\pm$ 3.0 μm^2, whereas those for low- and medium-SR fibers were 4.1 $\pm$ 3.3 μm^2. Consequently, the total area of en passant swellings per fiber is, on average, significantly larger for low-medium-SR fibers than for high-SR fibers (Table 1).

Serial thin sections through five en passant swellings were examined with the aid of an electron microscope. Each swelling was ultrastructurally distinct from the axon (Fig. 7). Four of five swellings contained mitochondria and numerous clear, round vesicles approximately 50 nm in diameter. Two of these swellings each formed a single asymmetric synapse upon a dendritic profile. The other two swellings did not appear to form synapses, but our ultrathin sections were parallel to the pre- and postsynaptic membranes, so we might have missed synapses because of the orientation. The one swelling without vesicles was thinly myelinated and contained a number of mitochondria. En passant swellings do not appear to be a homogeneous population when viewed with the resolving power of an electron microscope.

DISCUSSION

Morphological differences related to CF and SR groupings have been quantitatively demonstrated for the arborizations of type I spiral ganglion neurons. An attempt is made

to illustrate in diagrammatic form the main structural themes of this axon (Fig. 8). In general, the appearance of auditory nerve fibers is qualitatively similar to that described by Golgi studies (e.g., Ramón y Cajal, '09; Brawer and Morest, '75; Lorente de Nó, '81) despite differences in the ages of the animals studied (Ryugo and Fekete, '82) and the more sensitive intracellular HRP method (Brown, '81). It is also worth noting, however, that HRP labeling greatly improved the circumstances for detailed analyses at the single cell level.

In the summary diagram (Fig. 8), the upper panels (A,B) illustrate morphological differences related to fiber CF, and the lower panels (C,D) illustrate differences related to fiber SR. With respect to CF differences, it is of interest that only fibers of very low frequency (<0.5 kHz) gave rise to 2 terminal endbulbs of Held; otherwise, fibers of all higher frequencies gave rise to only a single terminal endbulb. The total number of endbulbs remained stable owing to the compensating presence or absence of collateral endbulbs, but the total number of terminal endbulbs are increased for these low-CF fibers. This low-frequency specialization may result in more low-CF spherical bushy cells and thus, serve to enhance phase-locking in the anteroventral cochlear nucleus, a feature presumed useful for the extraction of timing information in a stimulus (Young and Sachs, '79; Konishi, '85).

With respect to fiber SR, single fibers (ascending and descending branch arbors) were essentially identical for a particular SR group. CF (and thus the length of root branch) determined the position of these arbors within the nucleus. There were marked differences, however, in fiber morphology between the SR groupings. These differences were confined to the ascending branch and further emphasized the basic distinction between ascending and descending branches. On average, the ascending branch of low- and medium-SR fibers has longer collateral lengths, more branch points, and more but smaller synaptic endings when compared to high-SR fibers (Fig. 8C,D). This characteristic was especially prominent for fibers of the different SR groups in the same cat.

Axon characteristics

In the cochlear nucleus, total axon length may be used to indicate the spatial distribution of primary endings, thus providing information on locations of synapses. The number of axonal branchings was used to indicate arbor complexity. The combination of numerous axon branches and relatively short total fiber length would indicate a localized and dense innervation. In contrast, axons having few branches and great fiber length would indicate a widespread and sparse innervation. For auditory nerve fibers, length is correlated with fiber branching within the nu-

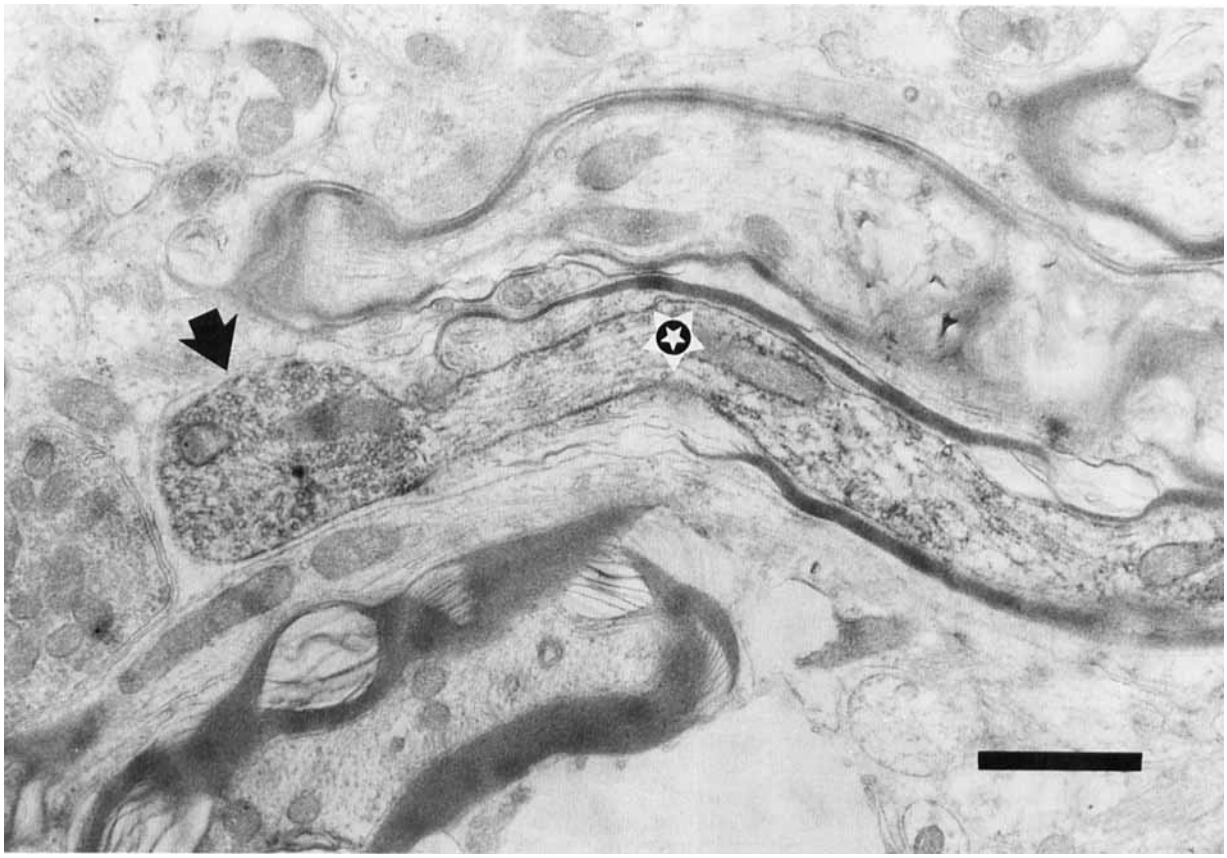

Fig. 7. Electronmicrograph of a labeled unmyelinated en passant swelling. This swelling (arrow) was typical of our population and characterized by the presence of mitochondria and clear round vesicles. Its appearance was clearly distinct from the axon (star). Although this particular swelling was not associated with a postsynaptic density, its appearance was not obviously different from those that were. Scale bar equals 1 micrometer.

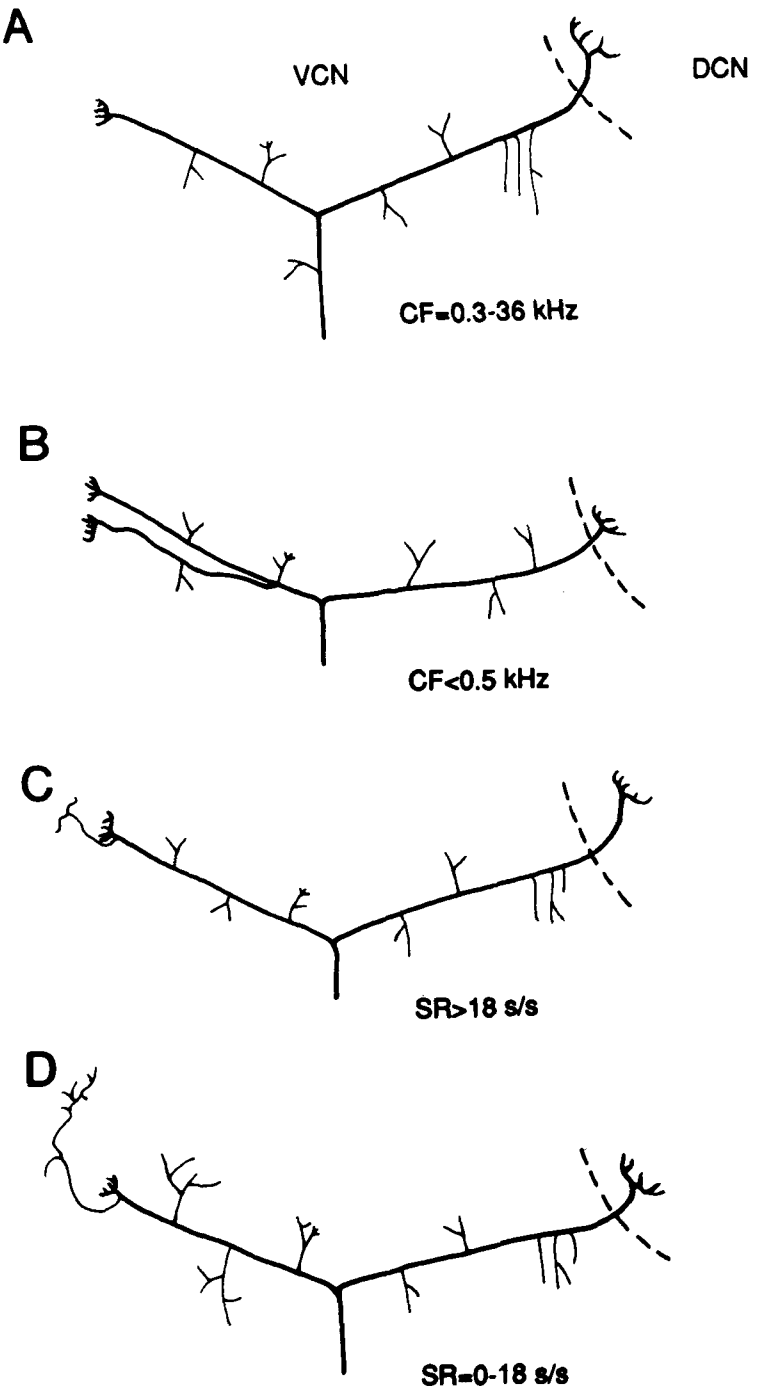

Fig. 8. Schematic illustrations of typical arborizations of auditory nerve fibers. **A,B:** Structural variations related to frequency. Fibers of all CF's typically have a single ascending branch, but only fibers having low CF's exhibit two ascending branches. **C,D:** Structural variations related to SR. Fibers having low-medium SR have roughly twice as many endings on their ascending branches compared to those having high SR.

cleus, revealing a constant ratio of length to branching. This observation may have some relevance regarding rules that determine how growing axons branch: In the case of auditory nerve fibers, a simple rule might be that axons must branch at some "more-or-less" fixed interval.

Since the average number of primary collaterals per fiber is relatively constant, arborization differences with respect to fiber SR are mediated by higher-order branching. Despite arborization differences between the ascending and descending branches, there is a common feature that describes their branching: The ratio of collateral length to collateral branching for ascending and descending branches is similar. Thus, the same rule governing length-to-branching ratios might be in effect for both the ascending and descending branches, but the ascending branch of low-medium-SR fibers exhibits more growth. One question emerges

which concerns the nature of the signal to the ascending branch that results in this branch difference being expressed.

Ending characteristics

The distribution and/or size of endings helped to distinguish the ascending and descending branches (Table 2). First, endbulbs of Held arose only from the ascending branch. Their size did not systematically vary with respect to fiber CF or SR. Second, those endings (of each category) arising from the ascending branch were generally larger than their counterparts of the descending branch. This size relationship was maintained across the SR groups. Third, differences with respect to SR groupings in the numbers and sizes of members in the small ending category are greater for the ascending than for the descending branch. Specifically, there are more but smaller endings for low-medium-SR fibers compared to fewer but larger endings for high-SR fibers.

The features of the large endings were less variable with respect to fiber CF or SR. Specifically, the number of endbulbs of Held and modified endbulbs did not vary systematically from fiber to fiber. This observation on endbulbs of Held has direct relevance to the numerical disparity between SR distribution of the auditory nerve and SR distribution of "primarylike" units having prepotentials in the cochlear nucleus (Bourk, '76). If a single spherical cell is contacted by a single endbulb, then the synaptic drive by the endbulb should endow the postsynaptic neuron with primarylike properties. Such a one-to-one relationship would tend to conserve the SR distribution from nerve to nucleus. Because the percentage of primarylike units in the cochlear nucleus having low SR is unexpectedly smaller than that of the nerve (Bourk, '76), alternative structural substrates must be considered. For example, the proportion of high SR units in the cochlear nucleus would be greater than that in the nerve if high-SR fibers typically gave rise to more endbulbs of Held than did low-SR fibers. Our results, however, argue against such an effect.

Another consideration relates to the nature of the convergence by endbulbs from separate fibers onto the same spherical cell. Multiple endbulbs are known to contact a single neuron (Ramón y Cajal, '09; Lorente de Nó, '81; Ryugo and Fekete, '82). One consequence of this convergence could be to raise the overall average SR for spherical cells (primarylike units) compared to that of auditory nerve fibers, if the output of the spherical cell represents the sum of the inputs by endbulbs of Held. The distribution of SR, however, would obviously be different depending on whether there was a segregation or a mixing of SR inputs onto individual spherical cells. One pattern could mix high- and low-SR inputs by way of separate endbulbs, endowing all spherical cells with high-SR and thereby eliminating low-SR units. This pattern can not be the case because low-SR units are present in the nucleus. Another pattern might be where endbulbs of fibers having similar SR converge in the nucleus. This pattern also does not appear viable because it would tend to preserve the SR distribution from nerve to nucleus. Alternatively, endbulbs from several low-medium-SR fibers might converge onto a single neuron, whereas high-SR fibers might tend to maintain their one-to-one connections. This situation of preferential convergence by low-medium-SR fibers would increase the representation of high-SR primarylike units in the nucleus relative to low-SR units. At present, this last relationship is still plausible because in a general way, it accounts for the electrophysicological data and maintains separate SR "input lines" to the nucleus. The preservation of separate SR input lines in the cochlear nucleus is of some interest because of the exquisite morphological distinction among SR types around the inner hair cells in the auditory periphery (Liberman, '82a).

En passant swellings

En passant swellings emerged as structures more mysterious than we had originally expected. They occur along the unmyelinated and thinly myelinated lengths of terminal branches, sometimes at the sites of branch points. Our criteria for light microscopic detection systematically omit the smallest en passant swellings, thereby underestimating the total number of such swellings and reducing the overall range in size. Nevertheless, the criteria seemed adequate because there were very few instances where the decision to include or exclude a swelling was difficult. What is striking about the en passant swellings is their relatively constant numerical relationship to terminal swellings across the fibers. The result is that the ascending branches of low-medium-SR fibers have many more en passant swellings than do those of high-SR fibers. The other striking feature of en passant swellings is that while they vary systematically in number with respect to fiber SR, they do not vary in size. They are roughly 4 μm^2 whether arising from the ascending or descending branch. In this regard, they exhibit some but not all features of synaptic terminals as viewed in light microscopy. On the other hand, en passant swellings do not always form synapses. A similar conclusion was reached on the basis of serial section electron microscopic studies of varicosities in *Aplysia* sensory neurons (Bailey and Chen, '83). It could be that some of the nonsynaptic swellings represent a bolus of organelles being transported to or from the cell body: At present, it remains to be determined exactly how to treat these entities.

Functional implications

One consequence of the differences in axon morphology is that an individual low- or medium-SR fiber provides an increased and more widespread innervation within the anteroventral cochlear nucleus compared to individual high-SR fibers. This situation may be related to the idea that the perceived loudness of a sound stimulus is proportional to the number of active neurons (Stevens and Davis, '38). In this context, the number of endings per fiber could represent the extent to which the activity of one neuron is transmitted to other neurons, providing that separate endings contact separate neurons. Since the low-medium-SR fibers have relatively high thresholds and exhibit a "divergent" projection, their activation by intense sounds would tend to produce a spread of activity to additional neurons in the cochlear nucleus. This kind of extra "recruitment" at high stimulus levels might be functionally important, since for many auditory nerve fibers, further increases in stimulus level produce a saturation of their evoked discharge rate; it would also tend to exaggerate the already large number of high SR neurons responding.

Two types of axon projections, diffuse and restricted, and having distinct physiological properties, can also be found in the visual system. In a general way, one can compare the more diffuse projections of low-medium-SR fibers to the similarly diffuse projections of the so-called Y-cells of the lateral geniculate nucleus, and the more restricted projec-

tions of high-SR fibers to the circumscribed terminal zones of X-cells of the lateral geniculate nucleus (Sur and Sherman, '82). It has been suggested that Y-cells are related to the detection of large objects and movements, whereas X-cells are concerned with the resolution of fine visual details (Sherman and Spear, '82). In a somewhat analogous way, low-medium-SR fibers might be related to analyses of acoustic information at high sound intensities or in noisy environments, whereas the high-SR fibers could be concerned with the resolution of sounds at low sound levels or in quiet environments.

Summed ending area: On a fiber-by-fiber basis, the total ending area has a fixed range and individual values are not correlated with CF or SR. If ending silhouette area is an accurate indicator of terminal volume and if synaptic sites are uniformly distributed throughout the terminal cytoplasm, then the number of synapses per neuron appears to be conserved. One implication is that the amount of synaptic "drive" delivered by fibers of the separate SR groups to the cochlear nucleus is proportional to their representation within the nerve population. Another implication is that the mechanisms that establish total synaptic sites appear different from those that determine axon branching.

A similar "conservational" phenomenon is evident in the axon arbor for sensory neuron X of the cricket (Murphy, '86). In this case, the number of varicosities in the adult arbor was constant, even under conditions of experimental manipulation. Since varicosities were relative uniform in size and their presence closely paralleled synaptic efficacy, it could be hypothesized that synaptic volume is conserved.

Our data are not, however, consistent with the general notion regarding the conservation of axonal arbors (e.g., Schneider, '73). Although the conditions are significantly different, Schneider proposed that certain neuron populations tend to maintain a fixed axon arbor. If the hypothesis is generally valid for our system and if type I spiral ganglion neurons represent a single population, there should be a compensatory reduction in branching along the descending branch which accompanied the increased branching in the ascending branch for low- and medium-SR fibers. Since there is no compensatory adjustments in branching, axonal arbors in this system are not strictly conserved.

In conclusion, the present data enhance previous observations that type I spiral ganglion neurons exhibit clear morphometric correlations with physiological properties. That is, structural features of the peripheral processes (Liberman, '82a) as well as the central projections (Fekete et al., '84; Rouiller et al., '86) are systematically related to spontaneous discharge rate, threshold, frequency sensitivity, and dynamic range. The data further suggest that the categories of auditory nerve fibers based on these physiological response properties have important functional relevance with respect to the processing of acoustic information. Moreover, the structural characteristics of auditory nerve fibers set important limits with respect to how this information is distributed to second-order neurons in the cochlear nucleus. Ultimately, it is the output of these nuclear neurons--in terms of "coded" spike trains and synaptic connections—which will define the functional pathways of the auditory brainstem.

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