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Mapping functionally identified auditory afferents from their peripheral origins to their central terminations

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An afferent fiber in the VIIIth cranial nerve of a terrestrial vertebrate exhibits exquisitely sensitive and selective response to one of the following stimuli to the ear. linear acceleration or tilt with respect to the gravitational vector; angular acceleration; air-borne sound; and in some lower vertebrates at least, substrate-borne vibration[1,16]. Among afferents responding to a given mode (e.g. angular acceleration), the stimulus–response properties vary markedly from fiber to fiber. The degrees of freedom include the regularity of the response spike train, the axis intercept (i.e. either the threshold level for response to weak stimuli or the zero-input firing rate), the constant of proportionality (gain) between stimulus and response at higher stimulus levels, the degree of adaptation to sustained stimuli, and the dynamics of adaptation[3,6,17]. Fibers exhibiting auditory or vibratory responses have frequency-dependent sensitivities (tuning) and non-linearities (two-tone suppression, difference tone stimulation, and the like), with additional associated degrees of freedom. e.g. the sharpness of tuning (Q); frequency of greatest sensitivity (center frequency); presence or absence of non-linearities[2,4]. Large-scale morphological distinctions generally are presumed to account for the major stimulus–response divisions: e.g. presence of semicircular canals for sensitivity to angular acceleration; presence of otoconial membranes for sensitivity to linear acceleration; tilt or vibration; and presence of intimate connection to the sound-conducting apparatus of the middle ear for sensitivity to air-borne sound. The morphological variations that account for the degrees of freedom within the major divisions, on the other hand, are not known. Candidates include morphological variations known to exist among the receptor cells (hair cells)[16], morphological variations known to exist among the acellular, gelatinous structures (tectorial membranes, otoconial membranes, cupulae) lying over the

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luminal surfaces of the hair cells[10-15], variations in coupling between hair cells and the gelatinous structures, and variations in the patterns of peripheral branching and hair-cell innervation by VIIIth nerve afferent fibers.

To test these various hypotheses, we have penetrated afferent fibers in the VIIIth nerve, determined the response properties of individual fibers, and then traced those fibers precisely to their peripheral origins with iontophoretically injected, fluorescent dye (Lucifer yellow)[18]. Because the dye spreads in both directions from the point of injection, we are also able for the first time to trace individually identified auditory or vestibular fibers precisely to their terminations in the central nervous system. Thus we are on the threshold of considerable refinement not only in our knowledge of the peripheral auditory and vestibular systems, but also in our knowledge of the primary projections of those systems to the brain. To illustrate the method, we present here two auditory fibers from the frog, along with their functional identifications, their peripheral origins, and their central terminations.

With their tips filled with a 5% solution of Lucifer yellow in water and their shafts filled with a 5% solution of Procion red, our glass microelectrodes exhibit DC resistances in the range of 50–200 MΩ. Applying a backing voltage of − 40 mV to its tip, we advance each electrode from a ventral approach (through the roof of the mouth) into the VIIIth nerve as it lies in the cranial cavity after emergence from the intact otic capsule with intact circulation. To identify the response properties of auditory afferent fibers, we use an open-field source (Sennheiser HD 424) to apply stimuli, and we monitor the stimuli with a calibrated microphone (Sennheiser MKH 104). Following identification of the response properties of a penetrated fiber, we inject dye into it by applying 0.5 sec, − 3 nA current pulses at a rate of 1 per sec. Usually within 20 min of dye injection, we immerse the frog in concentrated anesthetic for 20 min, then decapitate it, open the otic capsule, and immerse the head in 4% formalin for 2–4 days. Then we dissect the inner ear and brain for whole-mount observation, clear the tissue with methyl salicylate, and examine it under a fluorescence microscope.

Case 1: an amphibian-papilla afferent. We drove a 74 MΩ electrode into the posterior branch of the VIIIth nerve of a 240 g bullfrog, penetrating and briefly examining several vestibular fibers as we progressed. Finally, at a depth of approximately 370 μm, we penetrated an auditory fiber exhibiting non-adapting responses to tone bursts and to steady tones, with a center frequency at threshold of 400 ± 10 Hz and a bandwidth of 180 ± 10 Hz at a sound level 10 dB above the center-frequency threshold. After thus identifying the response properties of the fiber, we injected dye into it for 10 min. Owing to the low resistance of the electrode, in spite of the − 40 mV backing voltage, subsequent observation revealed spots of fluorescence from the dye in several fibers that had been penetrated but not filled. In a few cases, the dye had spread for short distances along the fibers, leaving very faint fluorescent traces. Those that we could follow joined either the nerve branchlet going to the lagena (a vestibular organ in the frog and other lower vertebrates) or that going to the posterior semicircular canal, but none was sufficiently intense to allow tracing to the sensory organ itself. One fiber had been filled, it fluoresced brilliantly, even at magnifications under which the faintly fluorescent fibers were invisible. We traced this fiber peripherally through

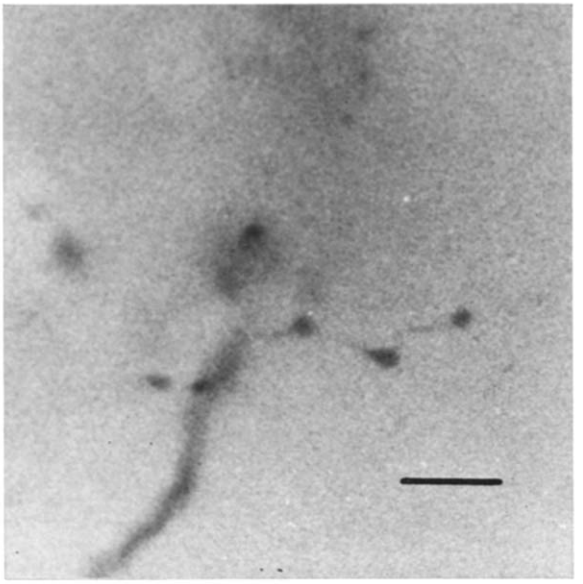

Fig 1 Terminal branch of the 400 Hz amphibian papillar fiber described in the text, near the lateral side of the dorsal medullary lobe Because this micrograph was printed from a color transparency, the fluorescent neural processes appear as dark images on a light background The main branch of the fiber crosses the figure from lower left toward upper right and is slightly out of the plane of focus The terminal branch emerges toward the right and exhibits 3 beads which we take to be synaptic boutons The fact that every branch of this type could be traced to a terminal bead we take to be evidence of complete fill of the terminal arborization of the fiber in the brain Bar equals 10 μm

its soma in the posterior VIIIth nerve ganglion and on to the sensory surface of the amphibian papilla. We also traced it centrally to the ipsilateral dorsal crest of the medulla, where it ascended into the region generally designated as the dorsal acoustical nucleus[7]. We were easily able to trace both central and peripheral arborizations of the fiber. Although we could barely resolve the finest branchlets at our highest working magnification (640), each one ended in a bead of fluorescence that was clearly visible (Fig. 1) We took these beads to be dye-filled synaptic structures (i.e. boutons). Some of the fine branchlets in the medulla appeared to be chains with several beads, which we took to be en-passant synaptic terminals. We found no branches of the fiber that could not be traced to a brightly fluorescing terminal bead. We took this to be strong evidence that the dye filled the cell completely. All of the fluorescing terminal beads in the medulla resided in a single 0 5 mm coronal section taken at the level of the VIIIth nerve; and all could be seen under epi-illumination from either side of that section.

As it ascended into the medulla, the fiber formed 3 disjoint terminal arborizations on the lateral side of the dorsal acoustical nucleus (Fig. 2a). Golgi studies by Larsell[8] indicate that the dendritic fields of single neurons in this region are not sufficiently extensive to span the 3 arborizations. Therefore, the arborizations apparently serve different (perhaps somewhat overlapping) populations of secondary neurons. At the periphery, the fiber's terminals concentrated about a single hair cell in the fourth row from the lateral edge of the papilla (the first row of large-diameter hair

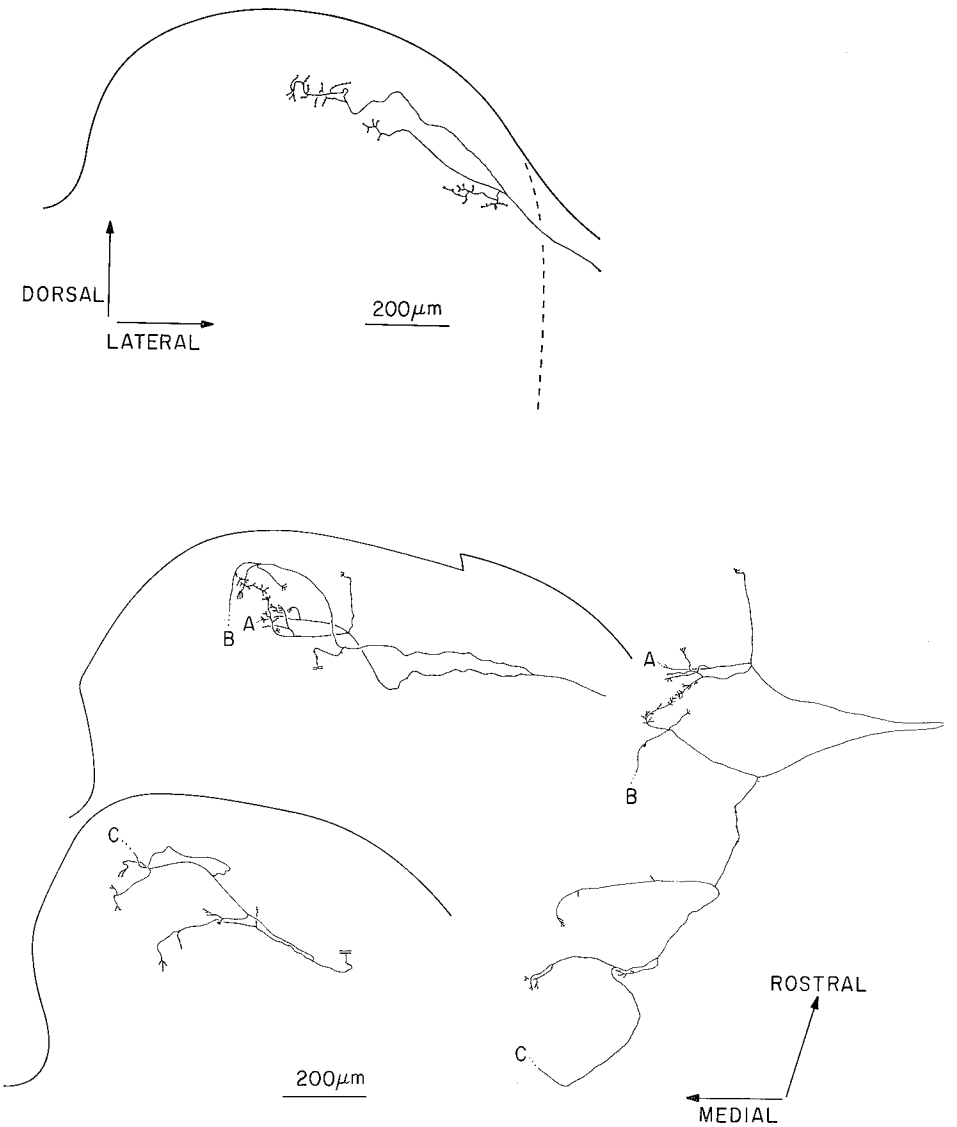

Fig. 2 Drawings of the central arborizations of two primary auditory afferent fibers in the dorsal lobe of the frog medulla. a. coronal projection (viewed from posterior side) of the amphibian-papillar fiber with center frequency at 400 Hz The drawing was made from a single 500 μm coronal section at the level of the VIIIth nerve; all branches terminated well within the section Dashed line on right shows approximate position of lateral margin of medulla, medial termination of VIIIth nerve. The dorsal medullary lobe is bordered on its medial side by the IVth ventricle. b the basilar-papillar fiber with center frequency at 1500 Hz. The two coronal projections on the left were made directly from a series of 3 coronal sections, and are shown as viewed from the posterior side With readings taken directly from the calibrated fine-focus dial, these coronal projections were translated to the horizontal projection on the right, which shows the terminal arborization of the fiber viewed from above The 200 μm marker applies to all 3 axes in b. In both drawings the magnification has been corrected to compensate for approximately 20% shrinkage observed to occur as the tissue was carried from the fixed state through dehydration and clearing. Our procedure did not allow observation of shrinkage during fixation; therefore the magnification has not been corrected for any such shrinkage that may have taken place.

cells), with a very thin collateral of the fiber going to each of two immediately adjacent hair cells, one in the same row and one in the fifth row. From previous S.E.M. studies we know that the hair cells in the fourth and fifth rows exhibit bulbed kinocilia (the more lateral, small-diameter hair cells do not), that they are taller than the more medial hair cells with bulbed kinocilia, that they project into a highly fenestrated region of the tectorial membrane (whereas the more medial hair cells project into discrete pores in the membrane), and that adjacent hair cells here exhibit opposite morphological polarization along a line parallel to the edge of the papilla[10,14]. The location of the peripheral termination at the posterior end of the second bend of the papilla corroborates our previous estimates of the tonotopic organization of the papilla[13].

Case II. a basilar-papilla afferent. Approximately 294 μm from the ventral surface of the posterior branch of the VIIIth nerve of a 310 g bullfrog, with an electrode whose resistance was greater then 100 MΩ, we penetrated an auditory fiber exhibiting a center frequency at threshold of 1500$\pm$50 Hz and a bandwidth of 470$\pm$50 Hz at a sound level 10 dB above center-frequency threshold level. After identification, the fiber was held for 3.5 min of iontophoretic dye injection. Subsequent observation revealed fluorescent dye in only one fiber, which we traced peripherally past its soma to its termination at a single hair cell in the second row (from the medial side) of the basilar papilla. From previous S.E.M. studies we know that the hair cells of this row have bulbed kinocilia and are connected to the tectorial membrane (whereas the more lateral hair cells have unbulbed kinocilia and are free-standing in endolymph), and that all hair cells are polarized in the medial direction[14]. Centrally, we traced the fiber into the ipsilateral dorsal crest of the medulla As it entered the medulla it formed two branches, one rostral and one caudal (Fig 2b). Collaterals from these two branches converged to form contiguous terminal arborizations on the dorsomedial side of the dorsal acoustical nucleus (top of Fig. 2b). The caudal branch continued through 3 thick coronal sections, forming additional arborizations on the medial side of the acoustical nucleus Since 3 of the smallest, faintest branches (at points A, B, C in Fig. 2b) could not be traced to arborizations with terminal beads, we conclude that we were unable to observe the entire central termination pattern. The traceable rostrocaudal extent of that pattern was approximately 1300 μm, between 2 and 3 times that of the amphibian papilla fiber. The terminations actually observed along with those indicated by the fine branches lost at points B and C suggest a sequence of 6 discrete sites of termination spaced more-or-less uniformly along the rostrocaudal axis of the dorsal medullary lobe, with the most extensive arborization occurring at the second-most rostral site. Comparing the positions of the various non-contiguous terminal arborizations of this fiber with previous Golgi studies, we are convinced that it innervates at least 4 (and possibly 6) disjoint populations of secondary neurons

The peripheral termination, which was much closer to the point of dye injection, fluoresced brilliantly and gave absolutely no evidence of incompletely filled branches. Furthermore, the single hair cell on which the fiber terminated exhibited fluorescence markedly greater than the autofluorescence of its neighbors, indicating dye coupling between the fiber and the hair cell. We have observed dye coupling of this type in

approximately 10% of the basilar-papilla and amphibian-papilla fibers that we have filled. It is conventionally believed to be evidence of electrical junctions between cells[18]

To date, we have identified and filled approximately 70 auditory or vestibular fibers and thus have begun definitively to relate structural variations to functional variations within the individual peripheral sensory organs of the ear[9,12,13] Only recently have we begun to direct our attention centrally as well The results, as exemplified by the two cases presented here, promise to provide high-resolution complements to the projection studies of auditory and vestibular fiber populations, such as those carried out in the frog and other vertebrates by degeneration methods[7] For example, using horseradish peroxidase in fiber populations, Fuzessery and Feng[5] demonstrated that the frog amphibian-papilla fibers project generally to the lateral side of the dorsal acoustic nucleus, while basilar-papilla fibers project generally to the dorsomedial side. The results presented here corroborate those findings and extend their resolution to the level of single, functionally identified fibers

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