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Central Trajectories of Type II Spiral Ganglion Neurons

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ABSTRACT

Previous attempts to trace the central pathways of the thin axons from type II spiral ganglion neurons have been hampered by technical difficulties such as fading of the reaction product as distance increases from the injection site (Ryugo et al.: *Soc. Neurosci. Abstr. 12:*779, '86; Brown: *J. Comp. Neurol. 260:*591-604, '87). By using small rodents (gerbils and mice), which have short auditory nerves, we have succeeded in filling the entire central axon and terminals of type II neurons after peripheral injections of horseradish peroxidase. The general course of the type II fibers within the auditory nerve and cochlear nucleus is similar to that of type I fibers except that terminals from type II neurons are often found in regions of the cochlear nucleus that have high densities of granule cells.

Key words: cochlear nucleus granule cell, auditory nerve, outer hair cell, unmyelinated afferent

In mammals, primary auditory neurons can be classified as type I or type II neurons (Spoendlin, '71). The more numerous (90–95%) type I neurons contact inner hair cells, which constitute fewer than 25% of the receptor cells. Type I neurons send axons centrally to innervate neurons in the cochlear nucleus (CN), and their physiological responses to simple acoustic stimuli have been extensively studied (e.g., Kiang et al., '65; Liberman, '82). In contrast, the type II neurons number only 5–10% of the auditory neuron population and contact outer hair cells, which constitute more than 75% of the receptor cell population (Spoendlin, '72; Kiang et al., '82; Berglund and Ryugo, '87; Brown, '87; Simmons and Liberman, '88). The central trajectories of type II neurons within the brain have not been previously described, and their physiological responses remain virtually unknown.

The role of outer hair cells in hearing cannot be completely understood until the central connections and responses of type II neurons are known. Previous failures to trace type II axons into the central nervous system have led some workers to suggest that these neurons have no role in transmitting information centrally (Spoendlin, '79). Other studies, however, have retrogradely labeled type II cells after injections into the CN (Ruggero et al., '82; Leake-Jones and Snyder, '82; Jones et al., '84). By labeling type II neurons with horseradish peroxidase (HRP), the present study not only confirms that their axons enter the CN but also describes their course and terminations within the nucleus.

MATERIALS AND METHODS

In previous studies (Ryugo et al., '86; Brown, '87) on cats and guinea pigs, the reaction product in HRP-labeled axons faded before reaching the termini. In an attempt to reduce the distance that the HRP had to travel to reach the terminations, we switched to smaller animals: gerbils (*Meriones unguiculatus*, 45–60 g) and CD-1 mice (*Mus musculus*, 25–45 g). A total of seven gerbil and ten mouse CNs were examined in detail after HRP injections. Gerbils were anesthetized with ketamine hydrochloride (340 mg/kg, i.m.), and mice were anesthetized with Avertin (5 g of 2,2,2-tribromoethanol dissolved in 3 ml of amylene hydrate and diluted 1:20 in physiological saline) at a dose of 0.2 cc per 10g body weight, i.p. HRP injections and histological pro-

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cessing were conducted as described by Brown ('87). A postauricular incision was made in the skin and in the bulla, and the round window membrane was removed. A small hole was made in the bone overlying the spiral ganglion, and a 30% solution of HRP was deposited by diffusion from pipettes with tips 15–40 μm in diameter. Every injection labeled afferent fibers. Injections placed into the intraganglionic spiral bundle at the peripheral edge of the spiral ganglion also labeled efferent fibers (Brown et al., '88). Animals were injected unilaterally, except for one gerbil in which injections were bilateral. The injections in both mice and gerbils were made directly through the round window and thus were somewhat more basal than those made in a previous study of guinea pigs (Brown, '87). In two mice, injections were made into the auditory nerve rather than the spiral ganglion. After the injections, the skin incisions were sutured, and, after 24 hours, the animals were reanesthetized before intracardial perfusion with fixative. The darkest labeling occurred when the fixation time was short (10–60 minutes) and when the fixative was 2.5% glutaraldehyde in 0.065 M phosphate buffer (pH 7.3). Cochleas were decalcified and the tissue was embedded in a gelatin-albumin mixture (Frank et al., '80). The cochlea and brainstem were either left attached or processed separately. The tissue was Vibratome-sectioned in a modified sagittal plane (Fekete et al., '84) in order to produce sections with long lengths of the afferent central axons. This plane cuts the cochlea into approximately radial sections. Sections were preincubated with $CoCl_2$, treated with diaminobenzidine (Adams, '77), and counterstained with cresyl violet. Morphometric measurements were made by applying computerized planimetry to camera-lucida drawings (magnification 2,000×) made with a 100× objective. Cell-body areas were measured with techniques described by Kiang et al. ('82), and average axonal diameters (as in Fig. 2) were computed from drawings by measuring the area of each segment and dividing by the length of the segment.

RESULTS

Classification of ganglion cells

When injections were made into the peripheral edge of the spiral ganglion, it was possible to identify individual somata of spiral ganglion cells and trace their axons centrally into the auditory nerve and CN. This approach was taken to demonstrate that the axons we traced originated from spiral ganglion cell bodies. Furthermore, by examining the cell bodies, it was possible to classify them as either type I or type II neurons. Previous work (Kiang et al., '82) showed that in cats, plotting the ratios of central process diameter to peripheral process diameter vs. cell body area yields two separate clusters of data points (Fig. 1, top plot). The triangles represent type I neurons that were traced to inner hair cells, and the squares represent type II neurons that were traced to outer hair cells. These morphometric characteristics that clearly separate type I and type II cell bodies in large species such as cats or guinea pigs are much less useful for predicting cell type in the smaller gerbil and mouse (Fig. 1). This situation results from the fact that type I cell bodies are smaller in the smaller mammals, whereas type II cell bodies are nearly the same size across species. Also, in smaller mammals, the processes near the cell body are more similar in diameter. For these reasons, another criterion for identifying type I and type II neurons was adopted for use in gerbils and mice.

We use a method based on the observation that within the auditory nerve, axons of type II cells are much thinner than those of type I cells (Kiang et al., '82; Ryugo et al., '86; Brown, '87). In gerbils and mice, the central axons in the vicinity of the cell body have approximately the same diameters for type I and type II neurons, but the axons of type II neurons gradually taper as they project into the auditory nerve (Fig. 2A). There is clearly dichotomy in the diameters of the axons 100 μm from the cell body: axons of type I neurons are thicker than 1 μm in diameter, whereas axons of type II neurons are thinner than 1 μm. Thus the central axon diameter can be used as the key variable of separation in morphometric plots of spiral ganglion neurons (Fig. 2). In fact, when it is possible to trace their processes peripherally, type II neurons contact outer hair cells and type I neurons contact inner hair cells (squares and triangles, respectively, on Fig. 2).

Central axons from type I and type II cells differ in appearance as well as in diameter. Under the light microscope, type I axons appear to have constrictions at periodic intervals along their course. These are interpreted as nodes of Ranvier (Fekete et al., '84; Liberman and Oliver, '84). In the internodal regions, these axons are more faintly labeled and sometimes the reaction product fades in midsection. In contrast, type II axons are labeled more uniformly and lack axonal constrictions, having instead numerous en passant swellings or varicosities along their course. These differences are consistent with ultrastructural observations that the thick axons of type I neurons are myelinated and the thin axons of type II axons are unmyelinated (Ryugo et al., '86; Benson and Ryugo, '87). Cell bodies of type I and type II neurons also differ in density of labeling, with the most darkly labeled somata being type II neurons. Type II somata, like their axons, are reported to lack a myelin covering (Spoendlin, '71; Romand and Romand, '87). It is likely that the HRP chromogens applied during tissue processing penetrate more easily into unmyelinated somata, resulting in darker labeling.

Central trajectories of axons

Twenty-eight type II ganglion cells and their central axons were reconstructed from seven gerbils with the aid of a drawing tube attached to a light microscope. We will use the term "identified" for central axons traced from identified spiral ganglion cells and the term "presumed" for central axons classified as type II on the basis of fiber diameter and appearance alone. Presumed type II fibers could not be traced from spiral ganglion cells either because the extracellular reaction product of the injection site obscured the ganglion cells or because the cochlea and CN were divided and processed separately. The intracellular reaction product in 24 of the 28 identified central axons faded before the axon could be traced to all endings. Complete reconstructions were available for only four identified type II axons (Table 1). Reconstructions are considered complete if 1) the reaction product did not fade in any branch and 2) each branch was traceable to a terminal swelling. Fading of reaction product was more frequent in type II axons than in type I axons.

Focal injections of HRP always labeled a tight cluster of ganglion cells in the spiral ganglion. The central axons of labeled type I and type II neurons from such injections travel together through the auditory nerve and most of the CN (Fig. 3). Usually, axons of both type I and type II neurons bifurcate in the nucleus, although examples of

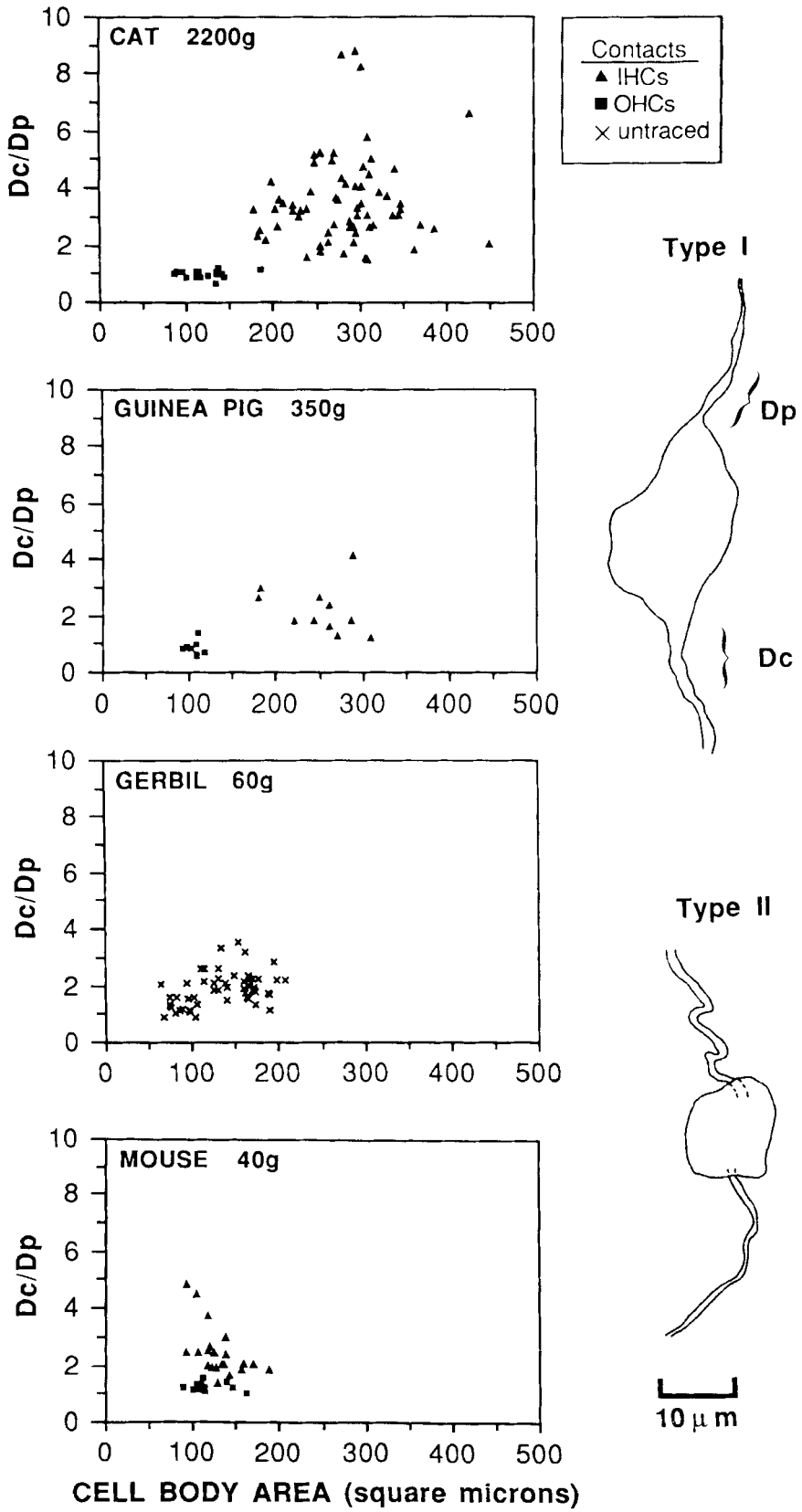

Fig. 1. Dimensions of HRP-labeled ganglion cells from four species. The diameters of the central (Dc) and peripheral (Dp) processes are plotted as a ratio against cell body area. Right: Camera-lucida sketches of labeled ganglion cells showing the segments over which Dc and Dp were measured. The drawings are from a type I neuron traced peripherally to an inner hair cell (IHC) and a type II neuron traced peripherally to outer hair cells (OHCs) in a guinea pig. Data are from cats (Kiang et al., '82), guinea pigs (Brown, '87), gerbils (present study), and mice (Berglund and Ryugo, '87).

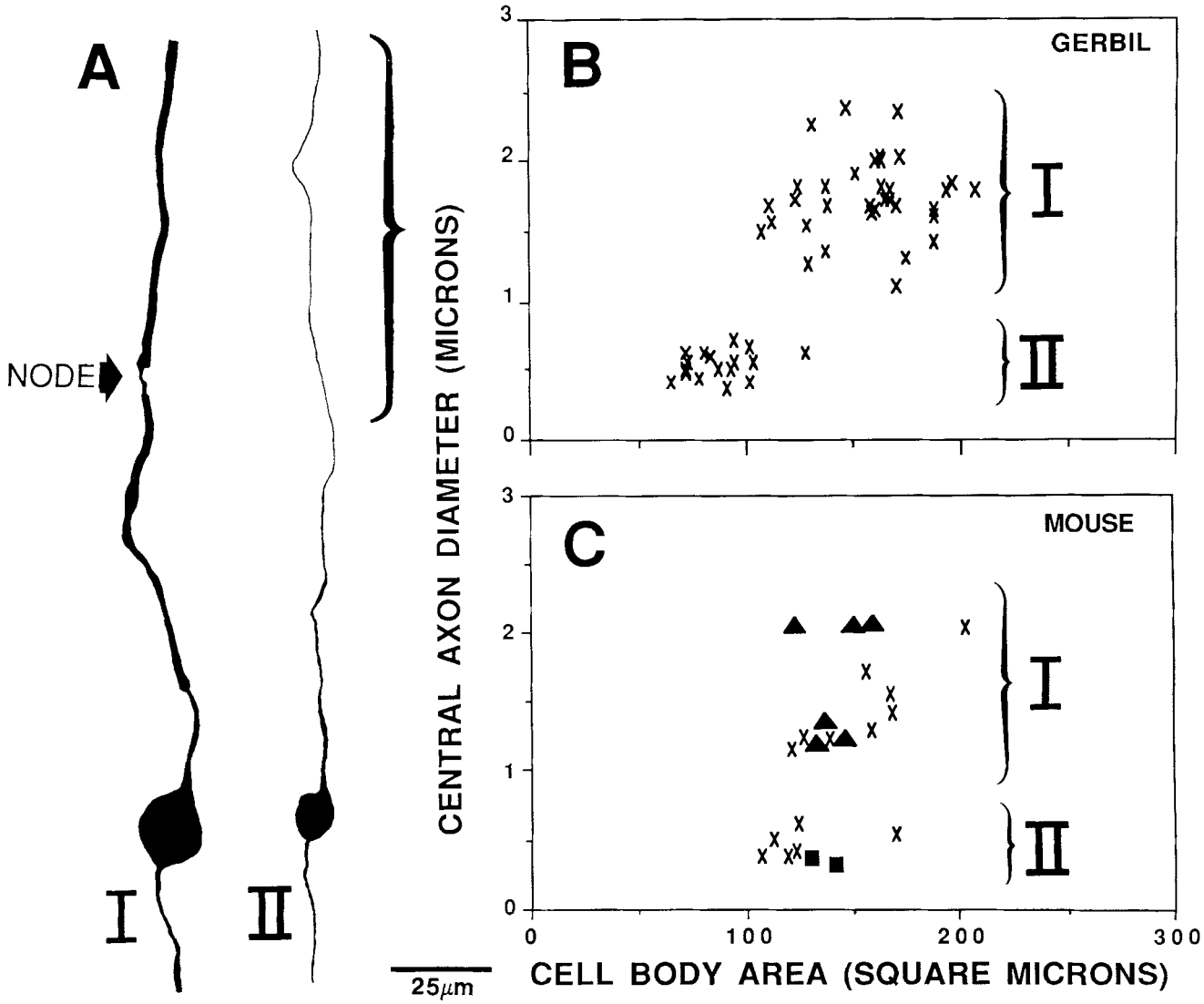

Fig. 2. A: Camera-lucida drawings of labeled type I and type II spiral ganglion cells and their central axons in a gerbil. A node of Ranvier on the type I axon is marked by an arrow. The bracket indicates the region of the central axons used for diameter measurements: Average diameter was calculated for a 100-μm segment length starting 100 μm from the cell body. B,C: Scatter plots of central axon diameter vs. cell body area for three gerbils and for two mice. Triangles: neurons traced to inner hair cells; squares: neurons traced to outer hair cells; X's: untraced neurons.

both types of axons were found that did not bifurcate. These "atypical" type I axons have been encountered previously (Feldman and Harrison, '69; Fekete et al., '84). The bifurcation of each axon results in an ascending branch that travels within the anteroventral cochlear nucleus (AVCN) and a descending branch that travels through the posteroventral cochlear nucleus (PVCN) to reach the dorsal cochlear nucleus (DCN). The axons, originating from the labeled cluster of ganglion cells in the lower basal turn of the cochlea, bifurcate within a restricted region of the extreme dorsal portion of the auditory nerve root, and the branches traverse the most dorsomedial portions of the AVCN, PVCN, and DCN. These observations are consistent with the cochleotopic trajectories of type I neurons into the CN (Ramón y Cajal, '09; Lorente de Nó, '81; Fekete et al., '84) and indicate that the topography of the type II axons is similar. For type II neurons in the gerbil, typical distances measured from the cell body were 2.6 mm to the bifurcation point, 3.3 mm to the AVCN endings, and 4.7 mm to the DCN endings.

Other reconstructed axons followed the trajectory described above but could not be traced to cell bodies (Fig. 4). These axons are "presumed" to be afferent since they travel with the "identified" afferent axons in the auditory nerve, bifurcating in the cochlear nerve root to form ascending and descending branches that eventually terminate in the CN. Afferent axons can be distinctly separated into two groups as described above (presumed type I and presumed type II axons). In addition to the four identified type II axons, five axons in gerbils and six axons in mice that were presumed to be type II were completely reconstructed (Table 1). All of these axons could be traced from the auditory nerve. They bifurcate in a restricted region of the cochlear nerve root, and they form a substantial proportion of their

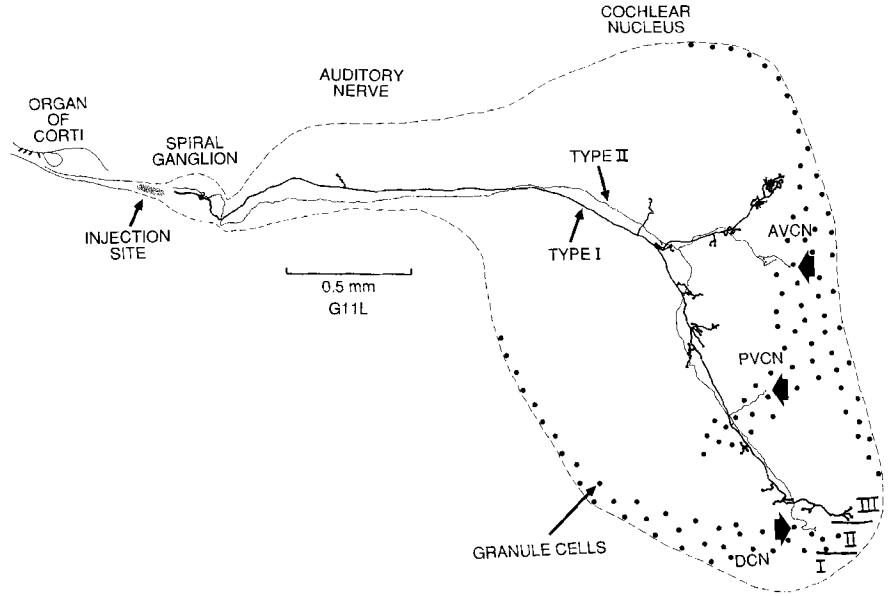

Fig. 3. Camera-lucida reconstruction of central axons from an identified type I cell (thick line) and an identified type II cell (thin line) into the auditory nerve and cochlear nucleus (CN) of a gerbil. Thick arrowheads indicate regions of axon terminations of type II neurons that are consistently separated from the terminations of type I neurons. Dots indicate granule-cell regions. The other axons (one type II and 45 type Is) that were labeled in this injection took a similar course to those shown and are not illustrated. The drawing is a two-dimensional projection of 21 sections, each 80 μm in thickness. AVCN, anteroventral cochlear nucleus; PVCN, posteroventral cochlear nucleus; DCN, dorsal cochlear nucleus. Roman numerals indicate the layers of the DCN.

TABLE 1. Morphometry of Axons

Axons	Traced to cell body?	Collaterals[1]			Term. swellings			Term. swellings in granule-cell regions			
		AB	DB	Total	AB	DB	Total	AB	DB	Total	(Total %)
Type I											
G11L.1	Yes	9	10	19	17	27	44	0	0	0	(0)
G11L.2	No	12	29	41	36	80	116	0	0	0	(0)
G11L.X	No	6	23	29	26	78	104	0	0	0	(0)
G11L.Y	No	17	22	39	34	45	79	0	0	0	(0)
G11L.Z	No	12	29	41	36	80	116	0	0	0	(0)
G2	No	6	12	18	10	25	35	0	0	0	(0)
M3.1	No	5	?	?	26	?	?	0	?	?	?
M3.2	No	7	?	?	24	?	?	0	?	?	?
M34	No	12	13		45	73	0	0	0	0	(0)
MJVL7	No	10	13	23	29	32	61	0	0	0	(0)
Type II											
G11L.2	Yes	2	1	3	5	2	7	2	2	4	(57)
G11L.6	Yes	3	7	10	4	8	12	4	5	9	(75)
G12.1	Yes	0	1	1	1	2	3	0	2	2	(67)
G12.2	Yes	0	2	2	0	3	3	0	2	2	(67)
G2.1	No	3	4	7	6	4	10	2	2	4	(40)
G2.2	No	1	2	3	7	3	10	6	2	8	(80)
G2.3	No	1	2	3	3	3	6	2	1	3	(50)
G2.4	No	2	2	4	4	4	8	1	3	4	(50)
G3.1	No	4	2	6	5	3	8	5	3	8	(100)
M3.1	No	2	1	3	3	2	5	0	1	1	(20)
M3.2	No	1	1	2	2	2	4	2	2	4	(100)
M3.4	No	1	2	3	2	3	5	2	2	4	(80)
M23.1	No	1	2	3	3	2	5	3	2	5	(100)
M23.2	No	1	1	2	2	2	4	2	0	2	(50)
M34.1	No	3	2	5	7	3	10	4	2	6	(60)

[1]Collaterals were counted if they were longer than 5 μm and arose directly from the primary ascending branch (AB) or the descending branch (DB). None of the type I endings was in granule-cell regions.

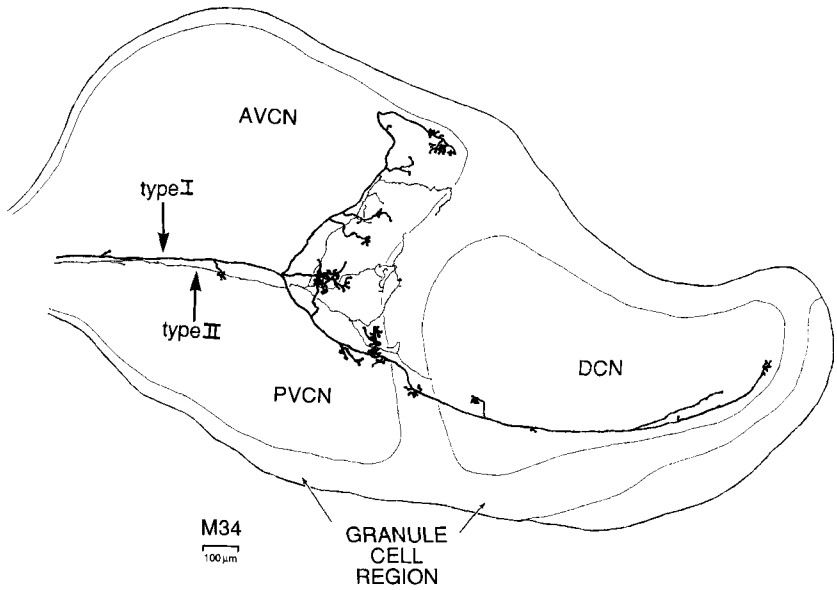

Fig. 4. Camera-lucida tracings of trajectories of presumed type I and type II axons in a mouse. As in the gerbil, the terminal swellings of type II axons are located mostly in granule cell regions.

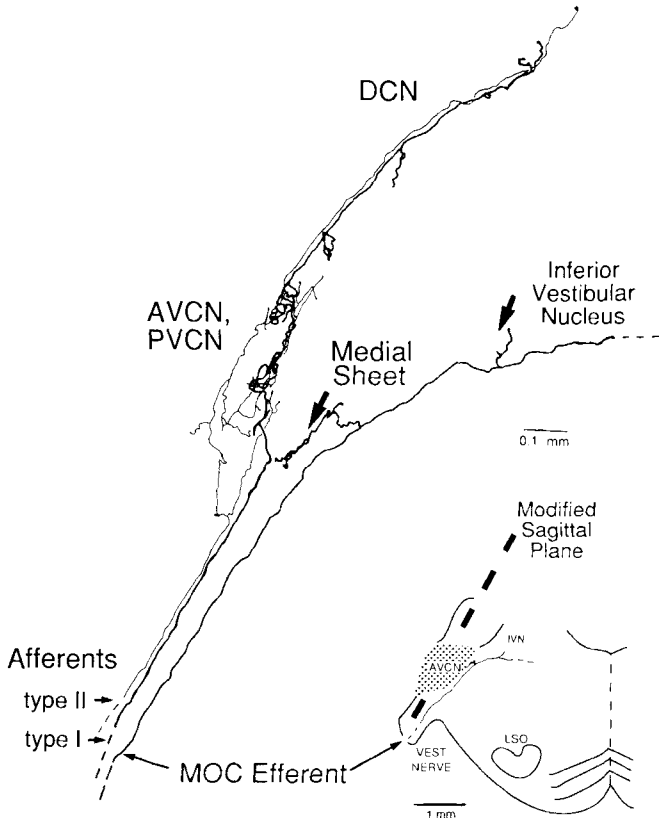

Fig. 5. Comparison of the courses of afferents and efferents in the cochlear nucleus. Single-axon reconstructions from a gerbil, originally traced in the modified sagittal plane in which the tissue was sectioned (heavy dashed line at bottom), and rotated by a computer-aided anatomy system (Neurotrace System, Cambridge, MA) to form a coronal view. The **inset** at lower right is a sketch of a coronal section of a gerbil brainstem on which the medial olivocochlear (MOC) efferent fiber tracing is superimposed. The efferent fiber gives off collaterals to the medial sheet of granule cells and to the inferior vestibular nucleus (IVN). Axons were identified as afferent because of their course in the auditory nerve root and as efferent because of their course in the vestibular nerve root. LSO: lateral superior olive.

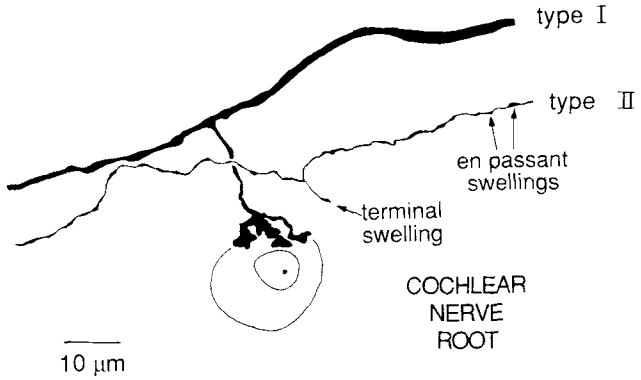

Fig. 6. Collateral branches from axons of presumed type I and type II neurons in the cochlear nerve root of a mouse.

terminal swellings in granule-cell regions. Their trajectories were similar to those of identified type II axons.

There were some indications of interspecies differences: in gerbils all nine reconstructed type II axons were traced into the DCN, but this was true for only two out of six reconstructed axons in mice. In addition, none of the gerbil type II axons formed collateral branches before reaching the main bifurcation area, whereas five of the six axons from mice formed short, simple branches directed to the most ventral region of the cochlear nerve root (leftmost branches on Fig. 4). Collaterals from type II axons in mice were also seen in the vicinity of second-order neurons in the auditory nerve, the "acoustic nerve nucleus" (Harrison et al., '62). Frequently, collaterals from type I axons in both gerbils and mice were seen in the auditory nerve (Fig. 3); these ended on or near neurons in the acoustic nerve nucleus.

The trajectory of afferent fibers contrasts with that of olivocochlear efferent fibers (Fig. 5), which were often labeled in the same injections. The efferent fibers originate from cells in the superior olivary complex, pass medial to the CN in the vestibular nerve root, and cross to the auditory nerve at the inferior vestibular ganglion (in the vesti-

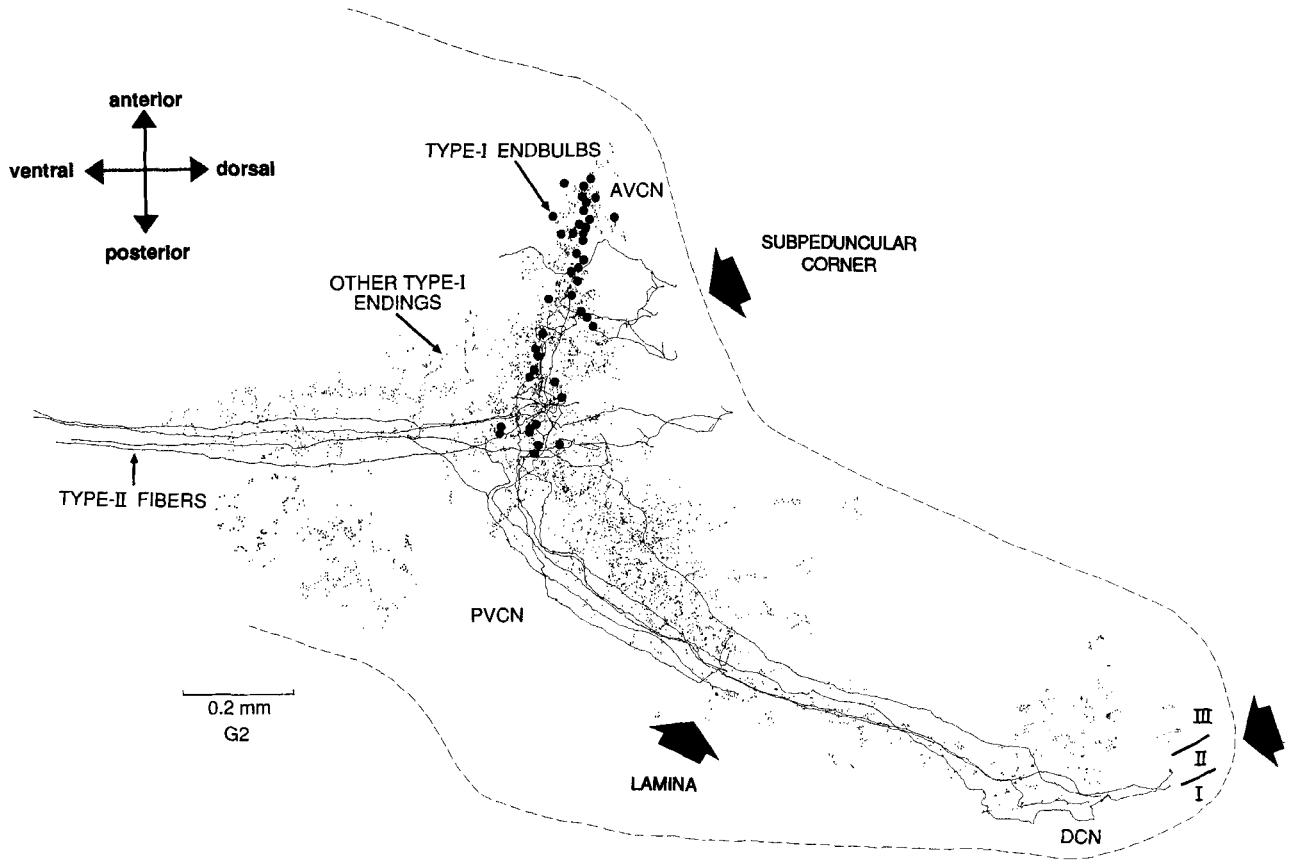

Fig. 7. A modified sagittal view of a gerbil CN, showing four axons (thin lines) presumed to be from type II neurons. Also plotted are endings of thicker fibers presumed to originate from type I neurons, with large filled circles showing the position of endbulbs and smaller symbols indicating other endings. Granule-cell regions where axons of type II neurons terminate are indicated by arrowheads.

bulocochlear anastomosis). They then spiral in the intraganglionic spiral bundle before innervating the organ of Corti. Collaterals of efferent axons directed to the CN are described in the accompanying paper (Brown et al., '88).

En passant and terminal swellings

There are two general types of swellings found on the axons of type II neurons: en passant and terminal swellings (Fig. 6). En passant swellings were usually ovoid in shape or polygonal at branch points. They were located along the length of the axons and along the collateral branches, whereas terminals swellings marked the tips of collateral branches. Our criterion for identifying en passant swellings was that their diameters had to exceed twice that of the axon as judged visually. (Such counts are not influenced by changing the criteria from 1.5 to 2.5 times the axonal diameter.) The vast majority (about 95%) of the swellings were of the en passant type. For all completely reconstructed axons, the average number ($\pm$ s.d.) of en passant swellings occurring central to the cell body was 128 $\pm$ 62. The average number of terminal swellings was 6.9 $\pm$ 2.9. Near the cell body, the central axon lacked en passant swellings, but they appeared when the axon was deep in the nerve, approaching the Schwann-glial border. For the four identified axons traced from type II cells, en passant swellings appeared between 0.5 and 0.9 mm central to the cell body. Central to the Schwann-glial border, en passant swellings were found over the entire course of the type II axon. This was true throughout the main body of the CN as well as the surrounding "granule-cell regions." The relative frequencies of the en passant swellings do not appear to change as the axons enter the granule-cell regions. For type II axons from three gerbils and two mice, we measured the intervals between en passant swellings. The distributions of the intervals between en passant swellings were usually unimodal, with the modes between 5 and 15 μm (range of intervals 2–75 μm).

Most of the course of type II axons is through the main body of the CN, and most of the en passant swellings are in this main body. Since the general course of type I and type II axons is similar, most of the type II en passant swellings are near the axons of type I neurons and also near the endings formed by the short collaterals of type I axons. Occasionally, even the terminal swellings of the two types of axons are in close proximity, so terminal convergence onto one neuron is a distinct possibility. This situation is frequently found in the cochlear nerve root of the mouse (Fig. 6). In this region, as elsewhere, the type II swellings are found in neuropil and are usually not in contact with somata.

Unlike the swellings from type I axons and most of the en passant swellings from type II axons, a significant proportion of the terminal swellings of type II axons is in regions of the CN with high densities of granule cells (Fig. 3, Table 1). For the four identified type II axons, the proportion of terminal swellings in granule-cell regions ranged from 57 to 75%, with an average of 67%. For the presumed and identified axons combined, the range was 20 to 100%, with an average of 64% of the terminal swellings in granule-cell regions. In contrast, endings of type I axons are rarely found in granule-cell regions of either rodents or cats (Fekete et al., '84). The distribution of terminal swellings of type II neurons relative to type I neurons is illustrated for the gerbil CN (Fig. 3, arrowheads). Three regions of

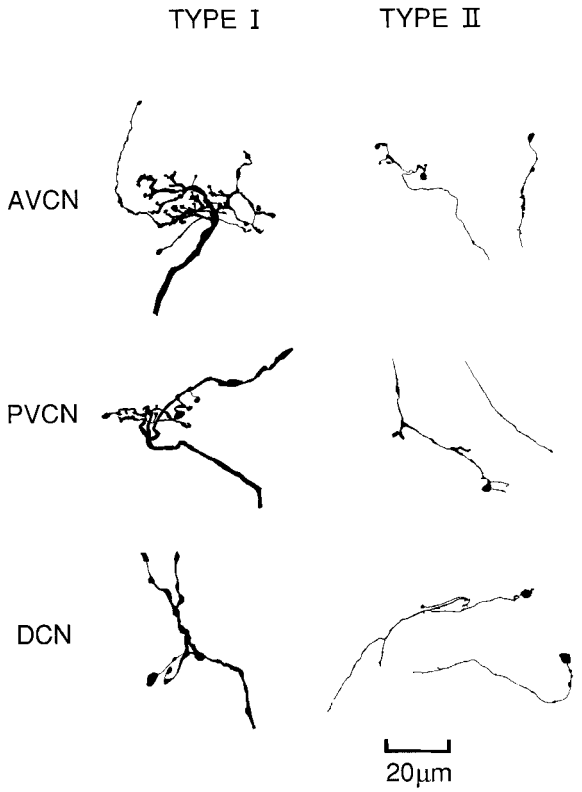

Fig. 8. Camera-lucida tracings of terminal swellings typical for type I and type II neurons in the mouse. The axon diameters for the two types are distinctly different. In the upper left, the ending in AVCN is an "endbulb of Held," the largest and most complex terminal formed by auditory nerve fibers (Ryugo and Fekete, '82).

granule cells in the CN typically receive endings from type II neurons, although not every type II neuron projects to all three regions.[1] These regions are 1) the subpeduncular corner of granule cells in the AVCN, dorsomedial to the endings of type I neurons (11 of 15 completely reconstructed type II axons formed terminals in this region); 2) the lamina of granule cells separating the VCN from the DCN (11 of 15 reconstructed axons); and 3) the granule or pyramidal cell layer (layer II) of the DCN and the subjacent region bordering on layer III (11 of 15 reconstructed axons). In one case, a branch of a type II neuron (G3.1) formed terminal swellings in yet another granule-cell region, the superficial granule-cell layer along the dorsolateral edge of the AVCN.

The relative locations of type I and type II endings can be illustrated by plotting all the terminal and en passant swellings labeled in an HRP injection in one animal (Fig. 7). Most of the swellings are from type I axons. The laminar arrangement of the type I endings is obvious for the large, axosomatic enbulbs of Held (Ryugo and Fekete, '82), which are indicated in Figure 7 by bold circles. The line of endbulbs presumably marks an isofrequency lamina, since the type I afferent input is from a very restricted cluster of labeled neurons from the basal turn. The trajectories of four presumed type II axons are shown by thin lines in Figure 7. Many of the type II terminal swellings are in granule-cell regions (arrow heads). In the AVCN, the type II axons run dorsomedially away from the endbulbs of Held and take a course through a region of presumably higher characteristic frequency before entering the subpeduncular corner of granule cells. This relationship may be significant because peripherally, the processes of type II neurons (outer spiral fibers) are offset from those of type I neurons, terminating in a somewhat higher-frequency region of the organ of Corti (Perkins and Morest, '75; Brown, '87; Simmons and Liberman, '88). Corresponding offsets of the peripheral and central terminations of type I and type II axons may serve to link regions of similar characteristic frequencies.

The axons of type I neurons give rise to terminal structures ranging from small boutons to more massive and complex arborizations known as endbulbs of Held (Rouiller et al., '86). The endings of type II neurons, by contrast, are fewer in number and smaller (< 3 μm in diameter). Drawings of endings for the two types of neurons from comparable subdivisions of the CN are shown in Figure 8. A salient difference is the caliber of the axon branch. Furthermore, the en passant and terminal swellings of type II neurons are most often found in the neuropil, whereas a distinct subset of endings of type I neurons contact cell bodies (Fig. 6). The ending shape of type II neurons did not show obvious regional dependence, unlike that of type I neurons (Ramón y Cajal, '09; Brawer and Morest, '75; Rouiller et al., '86). A species difference was noted in that the shapes of type II terminal swellings were usually spherical in gerbils but more complex in mice.

DISCUSSION

The successful labeling of the entire central axon of type II neurons is a direct result of our use of small animals such as gerbils and mice for the experimental preparation. Attempts to label these neurons in larger mammals such as guinea pigs (Brown, '87) and cats (Ryugo et al., '86) failed to produce complete labeling much beyond the cochlear nerve root region. Apparently, axon labeling experiments in larger animals are compromised by the limited transport of HRP in thin axons, since the reaction product fades within 5 mm from the injection site. The idea to use small animals to more completely label fine axons has been successfully applied to the somatosensory system (Sugiura et al., '86) and may be generally applicable in other situations where there has been difficulty obtaining complete filling of extensive axonal arborizations.

The bulk of available evidence now overwhelmingly indicates that type II ganglion cells send axons to the CN (Leake-Jones and Snyder, '82; Ruggero et al., '82; Jones et al., '84; Ryugo et al., '86; Brown, '87). Our present results not only offer direct evidence but are also consistent with earlier studies (Ryugo et al., '86) that argued that HRP-labeled thin axons in the auditory nerve originated from type II neurons. The only other known source of thin axons might be adrenergic fibers from the sympathetic ganglia (Spoendlin and Lichtensteiger, '67), but their morphological characteristics are distinctly different from those of axons from type II neurons. The fine adrenergic fibers are highly branched to form a network in the auditory nerve and cochlea, and often follow blood vessels (Spoendlin and Lichtensteiger, '67; Hozawa and Kimura, '88), whereas by comparison, our labeled afferent fibers branch infrequently in the auditory nerve. Possibly the adrenergic fibers are not recognized because the HRP reaction product that they contain fades quite near the injection site (Brown, '87). The fact that all primary afferent fibers have similar trajecto-

[1]The granule-cell regions are diagrammed in Figure 6 of the accompanying paper (Brown et al., '88).

ries lends support to our conclusion that HRP-labeled afferent thin axons originate from type II ganglion cells.

Our observations of axons of type II neurons fit descriptions of some axons seen in material stained with the Golgi method (Feldman and Harrison, '69). These axons are described as thin, beaded, and lacking endbulbs of Held. Such Golgi-labeled axons are uncommon, probably since the Golgi method stains only 1–2% of all neurons and type II neurons represent only 5% of the total ganglion cell population. Other Golgi studies do not specifically report fibers that resemble our descriptions for type II neurons. Based on their infrequent occurrence, a group of axons was suggested by Lorente de Nó ('76) to represent the central axons of neurons innervating outer hair cells, but such axons give rise to small endbulbs in the vicinity of the nerve root. We never found endbulbs on our HRP-labeled axons of type II neurons, but the effect of maturation on axon morphology of type II neurons is unknown. This caveat applies limits to our comparisons of Golgi material, which uses young animals, with the present results, which are from adult animals.

Our observations that type II neurons project to the granule-cell regions, whereas type I neurons usually do not explain why some previous studies of cochlear projections have concluded that primary afferents end in the granule-cell regions (Cohen et al., '72; Morest and Bohne, '83); others, however, have suggested that primary afferents are restricted to the magnocellular regions (Osen, '70). All these studies used degeneration techniques, and it may be that the different time courses for the degeneration of thick and thin axons produced the conflicting results. It should be recognized that present observations are limited to neurons from the basal turn of the cochlea. An unresolved issue is where type II neurons from more apical locations terminate in the CN. It is conceivable that there are significant differences in the central morphology of basal vs. apical type II neurons because differences in the morphology of their peripheral processes have been reported (Smith, '75; Simmons and Liberman, '88). Compared to outer spiral fibers from the basal turn, those from the apical turn branch more frequently and more often innervate multiple rows of outer hair cells. The present study showed that type II axons from the basal turn ascend and travel in close proximity to the subpeduncular corner of granule cells (Fig. 3). Ascending branches from more apically situated cell bodies of type II neurons might be expected to traverse the AVCN in a more ventral position, farther from the subpeduncular corner. Whether such apical projections terminate in the subpenduncular corner, in the closer superficial region of granule cells, or near granule cells at all awaits future investigations.

The similar course taken by axons of basal-turn type I and type II neurons in the nerve and nerve root is consistent with previous studies of cats and guinea pigs (Ryugo et al., '86; Brown, '87; Simmons and Liberman, '88). It is now obvious that the course throughout almost all of the CN is overlapping for the two types of axons. This situation results in a regional overlap of the type I endings with most of the en passant swellings and some of the terminal swellings from type II axons and establishes the possibility that some endings from type I and II neurons might converge on the same postsynaptic neurons. Although this overlap exists, the extension of type II axons beyond the main body of the CN to form swellings in the granule-cell regions is also striking. The fact that two-thirds of the terminal swellings of type II neurons lie in the granule-cell region suggests that granule cells and the associated Golgi cells could be postsynaptic targets. Although the most prominent granule-cell regions surround the main body of the CN, granule cells are to some extent scattered throughout the CN (Mugnaini et al., '80). Thus, the remaining one-third of the terminal swellings as well as the en passant swellings might actually contact these scattered granule cells. Therefore, the hypothesis that all endings of type II neurons are on granule cells cannot be disproved on the basis of existing data. Ultrastructural studies on axons of type II neurons in the cat indicate that some of the en passant swellings seen in the light microscope have synaptic specializations (Benson and Ryugo, '87). The fact that other en passant swellings do not have associated synaptic specializations (Benson and Ryugo, '87) suggests that some swellings may have other functions, possibly related to the transport of materials along the axon. The proportion of en passant swellings that might be involved in different functions is presently unknown.

Comparisons of type I and type II fibers in the CN show that the type II axons are much thinner, form fewer collateral branches, and have endings that are less complex than the massive endbulbs of Held found on every type I axon. These morphological features correlate with the usually smaller size of type II ganglion cells, as compared with type I cells. Highly arborized type I terminals such as endbulbs of Held are expected to exert a profound if not dominating influence on the activity of the single postsynaptic cell that receives the endbulb. The smaller endings of the type II neurons, which are rarely clustered about any one cell in the CN, might individually have less influence on postsynaptic elements. However, the large number of en passant swellings and their wide distribution throughout the CN suggests that type II neurons could have a broad influence on CN function, possibly modulating the activity of granule cells as well as other cells.

At a more general level, one can view the transmission of auditory sensory information to the brain in the following terms: a main input channel, consisting of a system of inner hair cells transmitting through the numerous thick axons of type I neurons, which conducts information rapidly and primarily to large cells in the CN; a second input channel involving a system of outer hair cells transmitting through the less numerous type II neurons. Presumably this second channel transmits information much more slowly because of the slow conduction velocities of thin, unmyelinated axons. Conduction times from the organ of Corti to endings in the CN may be greater than 10 msec (Kiang et al., '83; Brown, '87). This slower information may be delivered to some large cells but would also be delivered to smaller cells within the granule-cell regions. Branches from medial olivocochlear efferent axons are also associated with granule-cell regions (Brown et al,. '88). These efferent fibers respond to sound with latencies of from 5 to 50 msec (Robertson, '84; Liberman and Brown, '86). If type II neurons respond to sound, it is conceivable that information from type II and efferent fibers might arrive at CN regions simultaneously. Unfortunately, electrophysiological experiments using standard acoustic stimuli have not succeeded in demonstrating a class of responsive units that might correspond to type II neurons (Robertson, '84), possibly because type II neurons do not respond to ordinary sound stimuli. Sensory neurons with fine axons in the somatosensory system respond to noxious stimuli (Sugiura et al., '86). By analogy,

type II neurons might respond to high-intensity stimuli and produce the sensation of auditory pain (Licklider, '51), thus signalling the organism to escape from potentially damaging sounds. In this view, chemicals might be released from overstimulated outer hair cells, perhaps indicating cell injury. Future physiological experiments should determine whether such a functional role for type II ganglion cells has any validity.

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