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17. Thirty additional subjects, who did not meet the criteria for the controlled drinking treatment goal, were used in a parallel experiment that compared behavior therapy with conventional therapy when both had total abstinence as a goal. Since the Sobells reported no lasting differences between the two groups in the parallel experiment, we omitted it to simplify the exposition. We followed these patients also but do not report their results here.
18. "Screening factors included a reported minimal history of impulsiveness and past indications of exercise of self-control over other behaviors. . . . The staff also considered various other factors. . . . [such as] subjects having relatively few alcohol-related hospitalizations and arrests, being younger, reporting a shorter history of drinking problems, and having greater educational attainment" (*3*, p. 84).
19. This "myth" refers to the belief that alcoholics "will immediately or eventually proceed to drink to drunkenness should they ingest an initial drink" (*3*, p. 73).
20. For the second-year follow-up, drunk days were defined as "usually consumption of greater than 6 oz of 86-proof liquor or its equivalent in alcohol" (*7*, p. 196).
21. For the second-year follow-up, "usually consumption of 6 oz or less. . . ." (*7*, p. 196).
22. The Sobells also reported additional individual drinking data including the type of beverage, the length of longest binge, and the social environment and location in which drinking typically occurred (*7*, p. 203, table 4).
23. We also followed the 20 abstinence subjects, but less intensively. Eleven subjects and the widows of two others were interviewed. Another had already been reported to have died (*7*, p. 209), and six remain unaccounted for.
24. The alcohol-dependence syndrome is described by G. Edwards and M. Gross [*Br. Med. J.* **1**, 1058 (1976)]. To assess the severity of the syndrome, we have recently begun to use a questionnaire developed by T. R. Stockwell, R. J. Hodgson, G. Edwards, C. Taylor, and H. Rankin [*Br. J. Addict.* **74**, 79 (1979)]. However, since the lowest response category on the original questionnaire is "almost never," we needed the response "never" to discriminate subjects who did not have the syndrome at all from those with low levels of severity. Therefore, for each item, if a subject responded "almost never," we asked, "Which would be the most accurate response, "never" or "almost never"? Subject CD-E 18 obtained a score of zero, having responded "never" to all relevant questions. His responses were confirmed by his wife.
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30. Among the many who contributed to this research, we thank especially, R. C. Miller, M. Digan, N. H. Anderson, D. Dorinson, W. McQuillan, J. Wilkins, H. D. Steward and D. Steward, the Donwood Institute of Toronto, the San Diego Trial Lawyers Association, J. Fox, and the staff of Patton State Hospital.

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Hair-Cell Innervation by Spiral Ganglion Cells in Adult Cats

Abstract. *A horseradish peroxidase technique was used to trace the peripheral terminations of two types of ganglion cells in adult cats. It was found that large, usually bipolar ganglion cells end on inner hair cells and small, usually pseudomonopolar ganglion cells end on outer hair cells. Thus, a virtually complete segregation of afferent neural inputs from the two types of hair cells was directly confirmed.*

The cochlea receives sound stimulation and generates activity in fibers of the auditory nerve. Incoming mechanical signals are transduced by sensory cells (hair cells) that lie on the basilar membrane. The typical mammalian cochlea has one row of inner hair cells (IHC's) and three rows of outer hair cells

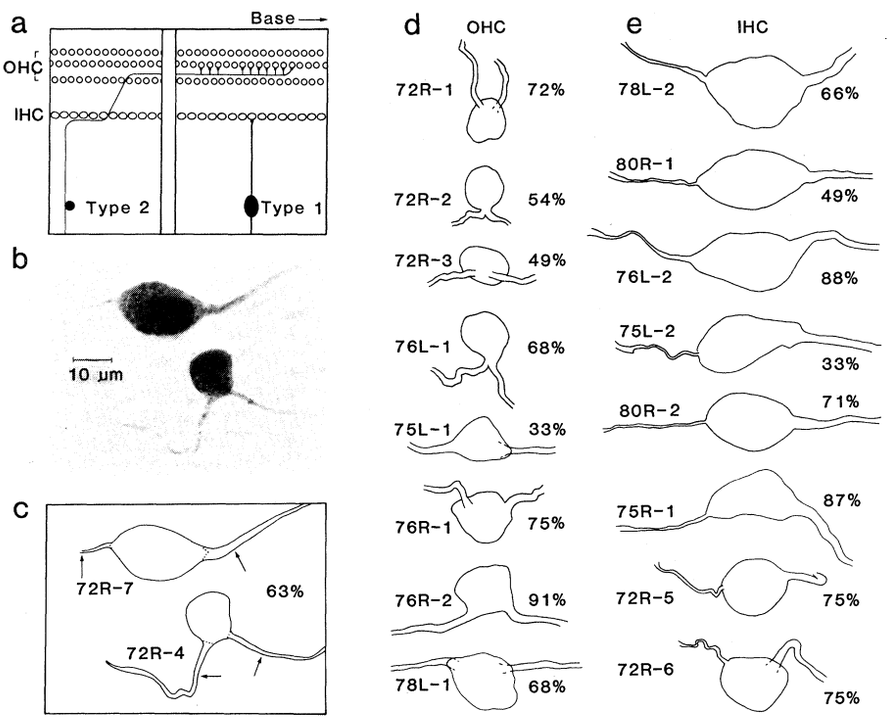

Fig. 1. Spiral ganglion cells in the cat cochlea. (a) Conceptualization of cochlear afferent innervation pattern. The type I (bipolar) neuron is shown projecting to IHC's and the type II (pseudomonopolar) neuron to OHC's. The diagram is not drawn to scale. (b) Photomicrograph of two neighboring labeled cells in the spiral ganglion of the middle turn. The peripheral processes are on the left and the central processes are on the right. The scale bar applies to (b) through (e). (c) Tracings of the photomicrograph in (b). The dotted lines show how cell body and processes are divided for the purpose of measuring cell area. The diameter of each process was measured at the narrowest points within 10 μm of the dotted lines. Cell area was determined by computerized planimetry. The arrows show where the diameters were measured for these two cells, which were located at a point 63 percent of the total distance from the base of the cochlea. (d) Camera lucida drawings of spiral ganglion cells with peripheral processes traced to OHC's. The drawings are arranged from top to bottom in order of increasing area of cell silhouette. Cell identification numbers are shown to the left and cell locations (percent distance from the base of the cochlea) are shown to the right. The dotted lines represent those portions of the processes partially obscured by the cell body but visible by focusing deeper into the section. (e) Camera lucida drawings of spiral ganglion cells with peripheral processes traced to IHC's. Drawings are labeled as in (d) and are arranged from top to bottom in order of decreasing area of cell silhouette.

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(OHC's), all extending the length of the cochlear spiral. At least two types of fibers form afferent synapses with the hair cells (*1–3*): outer spiral fibers (OSF's), which innervate OHC's, and radial fibers (RF's), which innervate IHC's (Fig. 1a). The RF's account for 90 to 95 percent of the total number of auditory nerve fibers, while OSF's account for only 5 to 10 percent (*4*).

Recent morphological studies have suggested that there are at least two types of spiral ganglion cells (*5–7*). According to Spoendlin (*8*), in the cat "about 95 [percent], the type I cells are large myelinated and bipolar with a round nucleus, a prominent nucleolus and many ribosomes in the cytoplasm. The remaining 5 [percent] are of the quite different type II about half the size usually unmyelinated and pseudo-monopolar with a lobulated nucleus, a small nucleolus and a filamentous cytoplasm." By comparing cell counts and fiber counts in normal and pathological cochleas, Spoendlin (*9*) concluded that type I cells give rise to RF's and type II cells to OSF's, but these correlations have never been directly demonstrated. Indeed, much of the Golgi research with neonatal animals, in which neurons are traced directly from hair cells to ganglion cells, contradicts this hypothesis (*1, 10, 11*), and there are difficulties in the interpretation of the pathological material.

In this study a horseradish peroxidase (HRP) technique was used to trace individual auditory nerve fibers from their peripheral endings on hair cells to their cell bodies in the spiral ganglion of adult cats. Iontophoretic injections of HRP (40 percent Sigma type VI in 0.1*M* tris, *p*H 7.3) were made through glass micropipettes (inner diameter, 30 to 35 μm) into the auditory nerve in the internal auditory meatus. This produced the diffuse (rather than granular) filling necessary for the continuous tracing of fibers. Currents up to 3 μA (10-second pulse, 50 percent duty cycle), applied for 400 seconds, yielded a low enough density of marked fibers to permit unambiguous reconstruction of single-fiber trajectories.

After 30 to 36 hours, the cochleas were fixed with glutaraldehyde and paraformaldehyde, thinned with stone burrs, decalcified (over a period of 6 to 8 days), and finally embedded in 20 percent gelatin. Serial 80-μm sections were incubated for 30 minutes in a solution of 0.05 percent diaminobenzidine, 1.0 percent dimethyl sulfoxide, 0.012 percent hydrogen peroxide, 0.013 percent cobalt chloride, and 0.01 percent nickel ammonium sulfate in 0.1*M* phosphate buffer (*p*H 7.3).

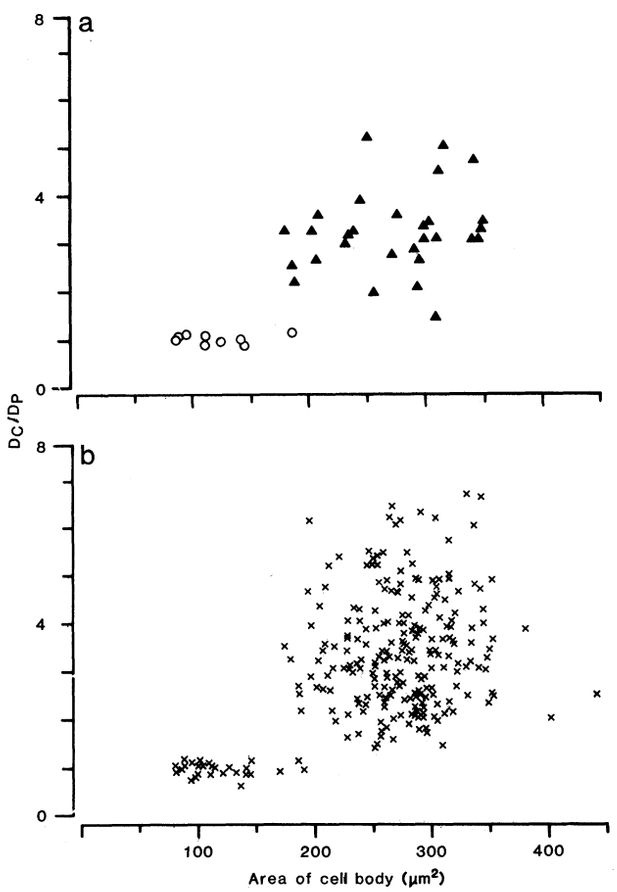

Fig. 2. (a) Ratio of central process diameter (D_c) to peripheral process diameter (D_p), plotted against cell area for a sample of neurons traced to OHC's (○) and a sample of neurons traced to IHC's (▲). Process sizes were measured as described in the legend to Fig. 1c. Mean central and peripheral process diameters were 1.25 and 1.23 mm, respectively, for cell bodies traced to OHC's and 1.73 and 0.56 mm, respectively, for cells traced to IHC's. (b) Ratio of D_c to D_p, plotted against cell area for 252 cells, both traced and untraced. The sample represents all parts of the cochlea except the most basal 2 mm and the most apical 1 mm.

A total of 61 fibers from eight cochleas were successfully traced with a camera lucida (total magnification, ×2550) from the peripheral endings to the cell bodies: 50 of these were RF's while 11 were OSF's. Fibers in the organ of Corti were carefully examined for possible branching. All RF's were unbranched, and OSF's were seen to branch only near the terminal region under the OHC's. No fiber was seen to innervate both kinds of hair cells.

The RF's and OSF's were easily distinguishable on the basis of fiber caliber. The central and peripheral projections of cells giving rise to OSF's were always less than 1 μm in diameter, while those of cells giving rise to RF's always had diameters greater than 2 μm (except in the region near the cell body). These size differences are consistent with the idea that RF's are myelinated while OSF's are not (*12*).

The OSF's were much more difficult to trace than the RF's because of their small caliber (often as small as 0.2 μm), especially in the region of the foramina nervosa. Consequently, many had to be dropped from the database. Three OSF's were continuously visible from ganglion cell to cochlear terminus. In eight other OSF's there were short (< 10 μm) stretches where the fibers were barely visible. Each could be traced with assurance, however, since no other fine fibers in the vicinity were labeled.

Morphological characteristics of the spiral ganglion cells giving rise to RF's and OSF's can be compared in Fig. 1. The upper cell in Fig. 1b innervated an IHC and fits Spoendlin's description of type I ganglion cells in being oval, bipolar, and large. The lower cell innervated OHC's and fits the description of type II ganglion cells in being round, pseudo-monopolar, and small. A larger sample of cells is shown in Fig. 1, d and e. Note that the absolute size of the cell body is imperfect in predicting the peripheral innervation pattern. For example, cell 78L-1 (Fig. 1d) innervates OHC's but is larger than cell 72R-6 (Fig. 1e), which innervates an IHC. The size overlap between the two ganglion cell populations may be an artifact of differential tissue shrinkage or some other systematic bias across animals. Such a bias is suggested by the fact that the smallest cells of each ganglion cell type were found in cat 72R while the largest cells of each type were found in cat 78L. In any one ear there was no overlap in sizes between the two types of ganglion cells.

The criterion of cell polarity is also

inadequate in predicting cell type. Although all frankly pseudomonopolar cells did innervate OHC's, the bodies of some of the cells giving rise to OSF's (such as 75L-1 and 78L-1) (Fig. 1d) were indistinguishable from those that gave rise to RF's.

The most reliable indicator of cell type across animals is the caliber of the processes, measured as they leave the cell body (Fig. 1c). As illustrated in Fig. 1e, the peripheral processes of cells giving rise to RF's were extremely thin in the vicinity of the cell body (*13*). Thus, the ratio of central process diameter to peripheral process diameter near the cell bodies was always greater for neurons innervating IHC's than for neurons innervating OHC's (Fig. 2a). The separation of cell types may be clearer with this measure than with absolute cell size because the ratio measure eliminates the problem of systematic size bias across animals.

Measurements of cell size and process ratios for a larger sample of spiral ganglion cells (including untraced cells) appear in Fig. 2b. These data suggest that in normal adult cats it should be possible to predict the peripheral termination of spiral ganglion cells with a high degree of certainty. These criteria should be of considerable practical importance in experimental work, since they can make tracing of peripheral processes unnecessary.

We conclude that at least two different types of spiral ganglion cells send projections deep into the internal auditory meatus. One group innervates OHC's by means of thin OSF's and the other innervates IHC's by means of thicker RF's. The two groups can be distinguished by certain morphological characteristics visible under the light microscope, and are generally consistent with Spoendlin's (*8*) descriptions of type I and type II ganglion cells.

To be entirely satisfied with such a view of the afferent innervation pattern, one needs to explain why these HRP data, which clearly support Spoendlin's conjectures, do not agree with the existing Golgi descriptions. Retzius (*1*), Lorente de Nó (*10*) (working with newborn mice), and Perkins and Morest (*11*) (working with newborn kittens) show drawings of large bipolar neurons traceable to OHC's. These neurons appear to be indistinguishable from those traceable to IHC's. Perkins and Morest also report the existence of single fibers sending branches to both IHC and OHC regions, a situation looked for but not found in our HRP data. Assuming that all the observations have been accurate, the simplest explanation of these discrepancies is that the morphology of spiral ganglion cells in the neonatal animal is significantly different from that in the adult. Specifically, during development the pseudomonopolar (type II) neurons of the spiral ganglion may pass through a transitional bipolar form similar to that described for the pseudomonopolar neurons of spinal ganglia (*14*).

The suggestion that the thin central axon of the OSF's corresponds to unmyelinated fiber components previously described for the auditory nerve (*15*) has two important implications. First, it means that we know nothing about the physiological response properties of these neurons, since small unmyelinated fibers are almost certainly not recordable with the techniques usually applied to the auditory nerve. Second, it suggests that little is known about the central terminations of these fibers, since relevant anatomical studies have concentrated on the larger fibers. Thus two avenues for future studies are clearly defined.

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13. The narrowing of the peripheral process of the type I neuron where it meets the cell body raises an interesting physiological issue. According to current ideas, this arrangement of a small fiber leading into a large cell body would reduce the safety factor for action potentials propagating through the cell body. Since the peripheral process is always large (diameter > 2 μm) until just before it reaches the cell body, one wonders why this arrangement occurs. A low safety factor would be useful if there were a need to inhibit impulses at this location. Perhaps the thin fiber region should be explored systematically for specialized structures, such as synaptic contacts.
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Flight Interneurons in the Locust and the Origin of Insect Wings

Abstract. *Interneurons involved in the generation of motor activity for flight in the locust were found in the first three abdominal ganglia as well as in thoracic ganglia. The evidence that sets of homologous flight interneurons occur in abdominal and thoracic ganglia supports theories that insect wings originated from movable appendages which were serially distributed along the thorax and abdomen and which were under central nervous control.*

Any overall theory of the evolution of insect flight must deal with the origin of the wings and their precursors, the "prowings" (*1*). The paranotal lobe theory (*2*) proposes that wings were derived from rigid expansions of the thoracic terga, which had a protective function. Through various possible functions such as epigamic display, thermoregulation, and parachuting (*3*), these paranotal lobes are believed to have become adapted first for gliding flight and then for flapping flight. The main alternative to this paranotal lobe theory is the pleural appendage theory (*4*) according to which wings were derived from movable and articulated pleural structures that were serially repeated along the thorax and abdomen. It has been proposed that the wings originated as protective covers for the spiracles (*5*) or the gills (*6*), or that they originated, from an uncertain beginning, as fins (*7*). After a stage in which they were used in ventilation and swimming movements, these precursors developed as flapping flight appendages—gliding flight being a subsequent sophistication to conserve energy (*4*). It is gener-

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