

The Cochlea of the Dolphin, *Tursiops truncatus*: Hair Cells and Ganglion Cells* (ear/auditory papilla/basilar membrane/tonal differentiation)

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ABSTRACT A study of the cochlear hair cells in *Tursiops truncatus* showed 3451 inner and 13,933 outer hair cells, for a total of 17,384. This total is of the same order of magnitude as the value of 14,975 for the human ear. Determination of the ganglion cell population for the dolphin gave a total of 95,004 cells, which is about three times as many as in man.

The large number of hair cells in the dolphin ear suggests a high order of auditory proficiency in general, and especially a marked ability of tonal differentiation. The large ratio of ganglion cells to hair cells suggests unusual capabilities in the utilization of auditory information.

In two previous reports (1, 2), we described the general morphology of the dolphin cochlea and gave particular consideration to the form of the basilar membrane and its manner of suspension. This paper deals with the numbers of hair cells and ganglion cells and their distribution along the cochlea. These features are of interest because they relate to questions of the specificity with which the action of sounds on the ear can be represented at the level of hair-cell stimulation and in the initial involvement of the auditory nervous system.

THE HAIR CELLS

Early in the study of the anatomy of the vertebrate ear, it was observed that as we go from simpler to more advanced forms, we find a large increase in the size of the auditory papilla and in the numbers of its hair cells. Among the mammals, Retzius in 1884 found this increase in numbers in the series from the rabbit through the cat to man. It was readily inferred that this progression is related to the characteristics of these different ears in tonal range and frequency discrimination.

In determining the size of the hair-cell population, Retzius (3) used a method that had been developed earlier by Waldeyer (4) and Krause (5) in the study of inner ear anatomy, and had been applied by them in working out the numbers of hair cells in the human cochlea. The method was based upon a surgical approach consisting of a careful dissection away of the bony cochlear walls to expose progressively the delicate structures within, and allow a direct examination of these structures under suitable magnification. This method, revived of late under the name of the surface preparation, was the only method available for the study of deep-lying structures

before the development of serial sectioning. It requires much patience and skill, but provides an excellent orientation and appreciation of the forms of anatomical elements.

Waldeyer in 1872 examined the human cochlea by this procedure; his work was followed by that of Krause in 1876. These investigators exposed the organ of Corti in one region of the cochlea and counted the outer pillar cells over some convenient distance, from which they obtained the average spacing of these elements. They assumed that the hair cells (that were much more difficult to see under their conditions) had the same spacing as the outer pillar cells. Then, they measured the length of the auditory papilla (or the basilar membrane); by dividing this length by the spacing, they obtained an estimate of the number of elements in a row along the cochlea. Finally, by multiplying this figure by the number of rows, they found the total number of hair cells.

Retzius repeated these measurements with some refinements of technique and with the observation that in man there are only three rows of outer hair cells over most of the cochlea, whereas his predecessors had thought that there were four rows throughout. Retzius found four rows only in the apical region, where they appeared in a somewhat irregular manner. He evidently adhered to the strategem of counting outer pillar cells and the rather questionable assumption that the number of hair cells is the same.

For the rabbit, Retzius reported 1600 inner hair cells and 6100-6200 outer hair cells, for the cat 2600 inner hair cells and 9900 outer hair cells, and for man 3475 inner hair cells and 11,500 outer hair cells. His figures remain the best available for these three species.

For our estimation of the numbers of hair cells in the dolphin ear, we have used a more precise method.

Method

13 ears of *Tursiops truncatus* were available in serial sections, and seven of these, in which the preservation was particularly good, were selected for the present study. Detailed results are given on one of these, which is representative of the group.

A graphic reconstruction was made of the cochlear spiral by the method of Guild, as described in our earlier reports. This method provides a representation of the cochlea to scale, on which is indicated the locations of each of the serial sections in order, with all positions along the linear extent of the cochlea displayed from base to apex. (The outer spiral of Fig. 2, below, shows the form of this diagram.)

As the discussion of early efforts has brought out, three steps are required in the estimation of the numbers of hair cells: the determination of cell spacings along the cochlea,

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TABLE 1. Enumeration of hair cells in the dolphin cochlea

Cochlear segment	Location (mm)	Segment length (μm)	Inner hair cells		Outer hair cells			Inner + outer hair cells		
			Spacing (μm)	Segment total	Spacing (μm)	No. per row	Rows		Segment total	
Lower basal	0-16.5	16,500	13.30	1,244	9.17	1,800	3	5,400	6,644	
Upper basal	15.6-26	9,500	10.42	910	8.44	1,125	3	3,375	4,285	
Lower apical	26-33.5	7,500	9.42	797	8.08	930	3	2,790	3,587	
Upper apical	33.5-38.5	5,000	10.00	500	8.45	592	4	2,368	2,868	
				Totals:	3,451				13,933	17,384

measurement of the length of the sensory structure, and observation of the number of rows of hair cells.

(a) For sections cut in our usual plane, which is at right angles to the modiolar axis and roughly in the vertical plane of the head, the spacing of hair cells is best observed in sections tangential to the cochlear half-turns. There are four of these tangential positions in the 2-turn cochlea of *Tursiops*. In such a position, the hair cells are seen in extended arrays, which are portions of the spiral rows, and in a favorable cut there may be as many as 50-100. Over distances determined with a screw-micrometer ocular, these cells were counted to give a measure of the spacing. This determination was made in each of the tangential positions representing the beginning of lower basal, upper basal, lower apical, and upper apical regions, and were made separately for inner and outer hair cells.

(b) Distances along the basilar membrane are directly shown by the spiral diagram as described. The lengths of the different half-turns were determined separately, because the observations of hair-cell spacing revealed variations along the cochlea.

(c) The number of rows of hair cells is most easily determined in the transverse (radial) sections, those at the middle of a half-turn where the basilar membrane is sectioned perpendicular to its course. This determination can also be made in the oblique sections after experience with the material enables the observer to take account of the angle of cut. As reported earlier (1), there is a single row of inner hair cells throughout the cochlea of *Tursiops*, and three rows of outer hair cells from the basal end to about the middle of the lower apical half-turn. Here a fourth row of outer hair cells makes its appearance and continues in a somewhat irregular manner to the apical end. In the upper half of the lower apical half-turn, somewhat more than half of the sections show four rows of outer hair cells; the other sections show three rows. In the upper apical half-turn, most of the sections show four rows, but many have only three. These variations reflect the irregular row structure of the apical turn.

Results

Table 1 shows the results of the above observations. The second column gives the locations of the four cochlear segments, column 3 shows their lengths, and column 4 gives the mean of a number of measurements (5 per position) of hair-cell spacings for the inner hair cells. Similarly, column 6 shows the spacings for the outer hair cells. It will be noted that

these spacings are not uniform along the cochlea, but for both inner and outer hair cells are widest at the basal end, decrease in the next two half-turns, and then increase somewhat in the upper apical half-turn. For given cochlear regions, the inner hair cells have wider spacings, and these cells show greater regional variations.

The number of inner hair cells in their single row is obtained for each segment by dividing the length of the segment by the spacing. For the outer hair cells, the number per row is obtained in the same way, and then this figure is multiplied by the number of rows, as indicated. The number of rows was taken as 3 for the first three half-turns and as 4 for the last one. This overstatement of the rows in the last half-turn in some degree compensates for the irregular presence of a fourth row in portions of the lower apical half-turn.

The number of inner hair cells (column 5) is shown for each region, and the total is 3451. The number of outer hair cells (column 9) is likewise shown by region, and the total is 13,933. The totals for hair cells of both types are in column 10, and the grand total is 17,384.

THE GANGLION CELLS

Seen in a transverse section as in Fig. 1, the cell bodies of the cochlear ganglion appear in dense masses central to the organ of Corti, adjacent to the scala tympani, and usually partially enclosed by the bone of the modiolus. These cells are bipolar, sending their dendrites through channels in the modiolus to the hair cells and their axons centrally to form the cochlear branch of the eighth nerve.

The spiral course of the cochlear ganglion is indicated in Fig. 2. As shown, this ganglion runs inside the spiral representing the inner edge of the basilar membrane, and has the same form, except for a little shortening at the two ends. The form as shown in Fig. 2 was determined by measuring in a number of sections, as indicated by the open circles, the distances of the two edges of the ganglionic mass from the edge of the basilar membrane. As will be noted, the mass in cross section is a continuous ribbon, and not a nodular chain as in many other mammals, including man.

A method was worked out for determining to a reasonable degree of accuracy the size of this ganglion-cell population and the distribution of the cells along the cochlea.

Method

A sampling method was adopted, and a particular ear was selected for this study. The cells were counted in about 20%

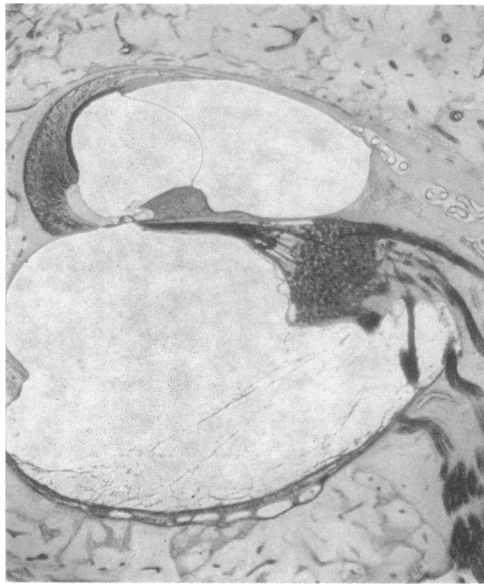


FIG. 1. Transverse section of the cochlea of *Tursiops truncatus*, at a position 10 mm from the basal end. Scale $\times 18$.

of the 525 serial sections that passed through the ganglion, and the numbers of cells in the remaining sections were found by graphic interpolation.

The cochlea was divided into seven parts that were dealt with separately. These were the four tangential regions and the midportions of the lower basal, upper basal, and lower apical half-turns. The short apical end-portion was included in the tangential region between the lower and upper half-turns.

A graph was made for each of these seven parts to facilitate the sampling procedure. As counts were made in selected sections, the results were marked on the graph; the process was continued until the form of the function was suitably represented. The choice of further sections for counting was guided by the graph; sections were taken at short intervals when the slope was large and changing, and at longer intervals in flatter and more constant regions. In some regions, every section was counted; this was always the case in tangential areas that were being entered or departed from by the series of sections.

In transverse sections like the one shown in Fig. 1, and other sections that were only moderately oblique, the counting was fairly easy, and the numbers per section were of the order of 100–200 cells. In the tangential regions, however, where the section passed through a large area of ganglion as in Fig. 3, the task becomes somewhat arduous, and the numbers in a single section rose to 1000 or more. The counting was aided by the use of a grid in the eyepiece of the microscope; an oil immersion objective was used, with a total magnification of $\times 1200$.

The ganglion cell bodies are oblate spheroids, with their long axes, those lying in the direction of the nerve processes, about 40% greater than their transverse axes. Measurements on a few cells in the upper part of the basal half-turn gave average values of $38.4 \mu\text{m}$ for the longer dimension and $25.2 \mu\text{m}$ for the shorter one. Other measurements made in the middle of the cochlea gave average values of 34.4 and $21.1 \mu\text{m}$ for the two dimensions.

These cells are not usually in contact, but are separated by distances that vary considerably. The average separation is around $2\text{--}3 \mu\text{m}$. Measurements between cell centers usually gave values between 24 and $41 \mu\text{m}$.

Actually it was the nucleoli that were counted. These bodies were prominently stained in our material and were easily identified by color and position in the nucleus. Their diameters ranged from 2.85 to $3.47 \mu\text{m}$, and averaged $3.06 \mu\text{m}$.

Results

After the sampling was completed, the numbers of ganglion cells were determined for successive regions along the cochlea; these numbers were referred to the radial position of the region with respect to the basilar membrane. A plot like that of Fig. 2 was used, and radial lines were drawn from the centers of curvature of the different half-turns to the millimeter points along the basilar membrane. The segments of the ganglion thus outlined were considered as containing the hair cells counted in a given section according to the degree to which its mass was included in the segment, which could be estimated from the graph. By this procedure, the data were expressed in terms of cell density, as ganglion cells per millimeter, and these data are presented in Fig. 4.

The curve shows a very rapid increase in ganglion-cell density at the basal end of the cochlea, rising to a peak value of nearly $3000/\text{mm}$, then shows a fall and a secondary rise to a maximum varying around $3200/\text{mm}$ in the middle of the basal turn. Beyond this maximum is an undulating decline to the apical end. The total number of ganglion cells, without correction for duplication in counting, was $104,400$. Because a given nucleolus may be cut through and appear in two adjacent sections, it is proper to reduce this number by a factor that takes account of the size of the nucleolus ($3 \mu\text{m}$) and the

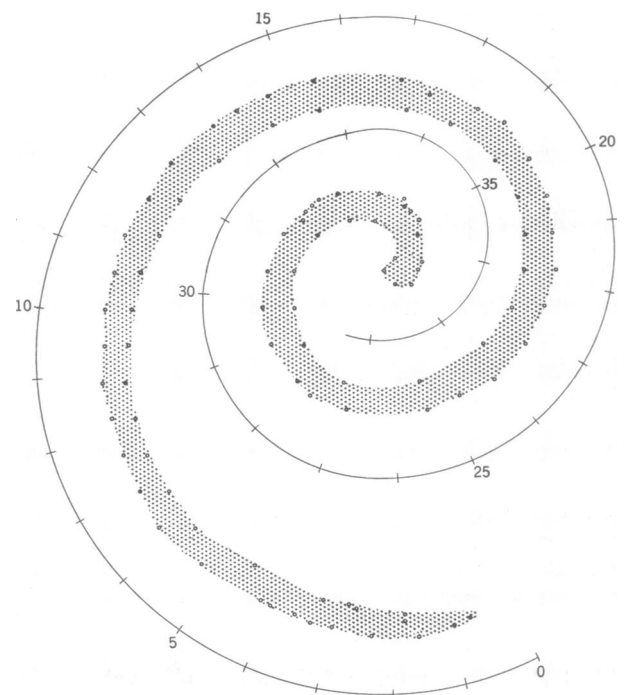


FIG. 2. The stippled spiral strip represents a cross section of the cochlear ganglion. Outside of this strip is the spiral line of the pillar cells, located near the inner edge of the basilar membrane. Scale $\times 15$.

section thickness (30 μm). This factor is 0.09, and the corrected number of ganglion cells becomes 95,004 (6), which should be taken as a minimum; the true value is probably greater both because empirical studies have shown that nucleoli are not split as often as theoretically would be expected, and because errors in the counting process are in the direction of overlooking units. Most likely, the true value is around 100,000 ganglion cells.

Discussion

It is of interest to compare the results obtained on the dolphin ear with those observed in man. Retzius reported for the human ear 3475 inner hair cells and 11,500 outer hair cells, for a total of 14,975 hair cells. The corresponding figures for *Tursiops*, as Table 1 has shown, are 3451 inner and 13,933 outer hair cells, a total of 17,384 hair cells.

The numbers for inner hair cells are similar for the two species; the slightly larger value for man can hardly be regarded as significantly different. The dolphin shows a preponderance of outer hair cells, however, and this advantage continues in the total. How seriously we should take this difference is a question, for both our results and those of Retzius are estimates. Also the Retzius data involve two assumptions that may well be questioned: that the hair cells are equal to the outer pillar cells in spacing, and that the spacing is uniform over the cochlea. Perhaps the only safe statement at present is that the hair-cell populations in man and dolphin are of comparable magnitude.

Data for numbers of ganglion cells in man and their variations in density along the cochlea were first reported by Guild, Crowe, Bunch, and Polvogt (7) in 1931. In 10 normal ears, they obtained an average total of 29,024 ganglion cells. Further counts made on 23 human ears by Dr. Mary Hardy and others were handled statistically and reported by Wever (8). These data showed an average total of 30,500 ganglion cells. The total of 95,004 reported here for *Tursiops* is thus about three times as great as that reported for the human ear.

The curves representing the varying density of ganglion cells along the cochlea as reported by Wever (8) for man and as

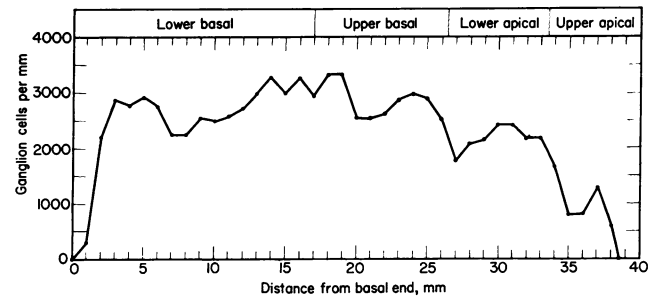


FIG. 4. The density of ganglion cells in a specimen of *Tursiops truncatus*, referred to position along the basilar membrane.

shown here for *Tursiops* are remarkably similar in form. For man as for the dolphin, there is an abrupt rise to a maximum density at the basal end, then a decline (though the decline is less marked than in the dolphin), and a further rise to a maximum in the middle of the cochlea. Finally, there is a rather rapid decline over the remaining region of the cochlea. The outstanding difference in the two functions is in the degree of density, which in man never exceeds about 1230 cells per mm, whereas in the dolphin it reaches 3400/mm. This difference in maximum density is in accord with the over-all difference in ganglion-cell numbers.

The long cochlea of the dolphin and the large population of hair cells suggest a high level of auditory capability in general, and especially a high degree of pitch discrimination. This inference depends, of course, on the condition that a considerable degree of specificity is maintained in the innervation of the hair cells. Our observations on the dolphin cochlea support this conception of specificity. As has been described in other mammals, the cochlear dendrites run out radially in nearly parallel bundles from the ganglion cells to the organ of Corti, and may be seen passing through the habenula perforata to the region below the inner hair cells. These fibers seem to form an unusually large number of endings on these hair cells as can be seen with the light microscope; they also form a very prominent inner spiral bundle in this vicinity. The further course of these radial fibers, and of other fibers passing out of the ganglion, could not be made out in our material, but we assume, as has been described for other mammals, that many of the fibers pass into the tunnel and outer spiral bundles, and with further branching and ramification serve the outer hair cells. It is clear that for the inner hair cells, at least, there is a high degree of specificity of innervation, and in general the ganglion cells at a given region of the cochlea serve the hair cells at the same level.

Of particular interest is a comparison in man and dolphin of the ratio between ganglion cells and hair cells. For the human ear, this ratio is almost exactly 2 to 1; for the dolphin it is a little over 5 to 1. This striking relation suggests two possible differences between man and dolphin in the central representation of cochlear processes: that the dolphin requires more neural pathways for the transmission of the high-frequency information that it deals with, or that this animal's system presents more details about cochlear events in general to the higher neural centers than man's does. Indeed, man and dolphin may differ in both these respects, and the representation of detailed information about high-frequency sounds, and especially their time and phase relations, may well be the basis for the dolphin's remarkable facility in echolocation.

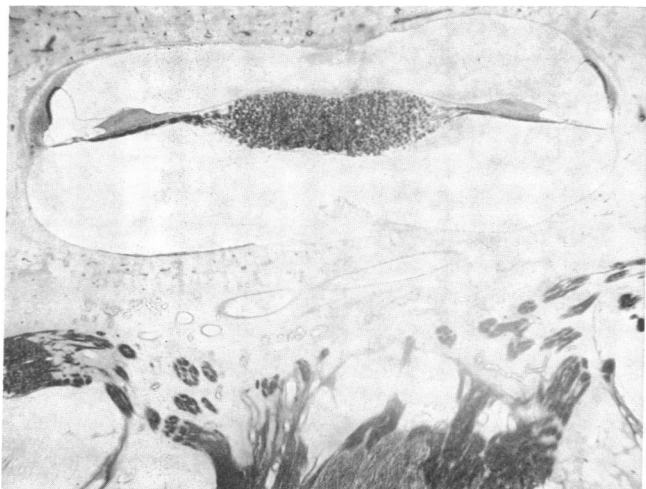


FIG. 3. A section showing the ganglionic mass in the tangential area between lower and upper apical halfturns, about 33.5 mm from the basal end of the cochlea. Below are shown numerous bundles of nerve fibers entering the main trunk of the cochlear nerve. Scale $\times 18$

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1. Wever, E. G., J. G. McCormick, J. Palin, and S. H. Ridgway, "The Cochlea of the Dolphin, *Tursiops truncatus*: General Morphology", *Proc. Nat. Acad. Sci. USA*, **68**, 2381-2385 (1971).
2. Wever, E. G., J. G. McCormick, J. Palin, and S. H. Ridgway, "The Cochlea of the Dolphin, *Tursiops truncatus*: The Basilar Membrane", *Proc. Nat. Acad. Sci. USA*, **68**, 2708-2711 (1971).
3. Retzius, G., *Das Gehörorgan der Wirbelthiere* (Stockholm, 1884), pp. 342-357.
4. Waldeyer, W., in "Hörnerv und Schnecke", *Handbuch der Lehre von den Geweben des Mensch und der Thiere*, ed. S. Stricker (Leipzig, 1872), Vol. II.
5. Krause, W., in "Allgemeine und Microscopische Anatomie", *Handbuch der menschlichen Anatomie*, ed. C. F. T. Krause (Hannover, 1876), 3rd Ed., Vol. I.
6. Abercrombie's correction formula was used. See Konigsmark, B. W., in "Methods for the Counting of Neurons", *Contemporary Research Methods in Neuroanatomy*, ed. W. J. H. Nauta and S. O. E. Ebbesson (Springer Verlag, New York, N.Y., 1970), pp. 315-340.
7. Guild, S. R., S. J. Crowe, C. C. Bunch, and L. M. Polvogt, in "Correlations of Differences in the Density of Innervation of the Organ of Corti with Differences in the Acuity of Hearing", *Acta Oto-Laryngol.*, **15**, 269-308 (1931).
8. Wever, E. G., *Theory of Hearing* (John Wiley and Sons, 1949), pp. 290-293, 309, 336.