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Morphometric and Ultrastructural Findings on Human Vestibular Ganglion Cells

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Abstract
Eight human temporal bones (4 patients) were fixed within 2 h after death by perilymphatic perfusion through the oval and round windows. After preparation, 30-μm-thick sections were cut for light microscopy and ultrathin sections for ultrastructural evaluation. Under the light microscope, the diameter, the circumference and the area of the vestibular ganglion cells were measured. The data were statistically analyzed. The histograms of cell measurements showed two maxima. When observing the diameter of cells, one was at 40 μm and the other at 28 μm. Under the light microscope, we could distinguish two cell types, which mainly differed in size and content of granules in the cytoplasm. Ultrastructurally we identified also two cell types: larger cells with many mitochondria, dark clusters of endoplasmic reticulum and a varying amount of dark-stained lysosomes in the cytoplasm and smaller cells with only few mitochondria, no lysosomes and an extended rough endoplasmic reticulum. None of the ultrastructurally analyzed ganglion cells were myelinated.

Introduction

The spiral and vestibular ganglia derive from a single statoacoustic ganglion in the early development of the inner ear. Two distinct types of ganglion cells were found in the spiral ganglion of mammals and humans by Spoendlin [1–5]: a majority of large bipolar cells and a minority of smaller cells with a number of distinct features. In a quantitative evaluation, Richter and Spoendlin [6] found two different cell types in the cat Scarpa's ganglion. Richter [7] found a reduction of vestibular hair cells and nerve cells in the vestibular ganglion in ears of aged humans.

The fine structure of the sheaths of vestibular ganglion cells was studied in rats, baboons and surgical specimens of humans by Ylikoski [8] and in bats by Ona [9].

Okami et al. [10] described four different cell types in the vestibular ganglion of the rat. Kitamura and Suzuki [11] could distinguish between myelinated and unmyelinated ganglion cells in the macaque monkey. Felix et al. [12] described the ultrastructure of human vestibular ganglion cells, found all cells unmyelinated and found also that the morphological features appear to be unique in human Scarpa's ganglion. The aim of this study was to evaluate the human vestibular ganglion at the light- and electron-microscopic level in order to determine possible morphological differences in the neuronal populations.

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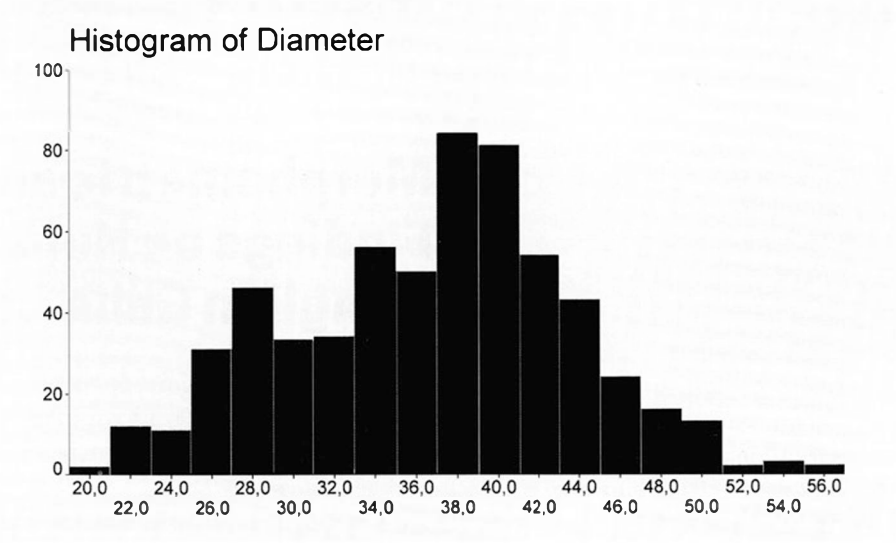

Fig. 1. Histogram of the diameter of the vestibular ganglion cells. x-axis: longest measured dimension of the cells in micrometers; y-axis: number of measured cells. Two clearly visible maxima, one between 38 and 40 µm, the other at 28 µm.

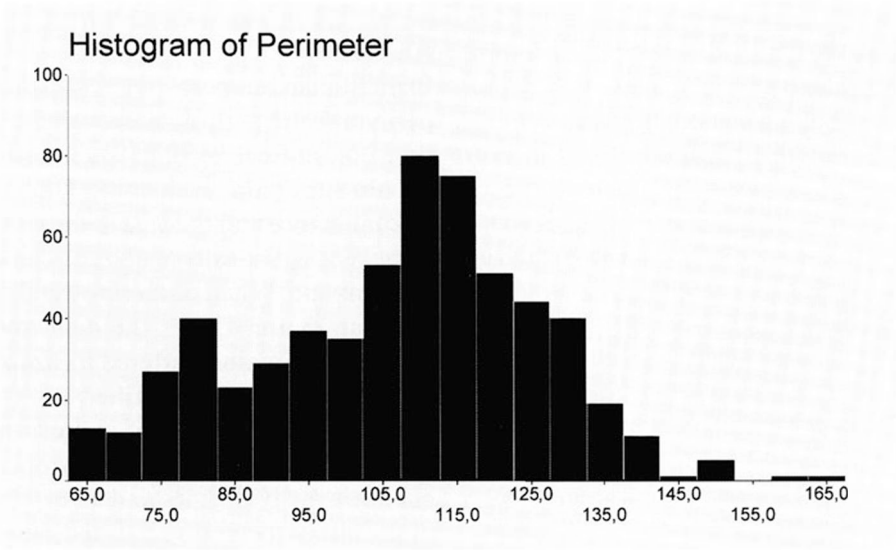

Fig. 2. Histogram of the perimeter of the vestibular ganglion cells. x-axis: measured perimeter of the cells in micrometers; y-axis: number of measured cells.

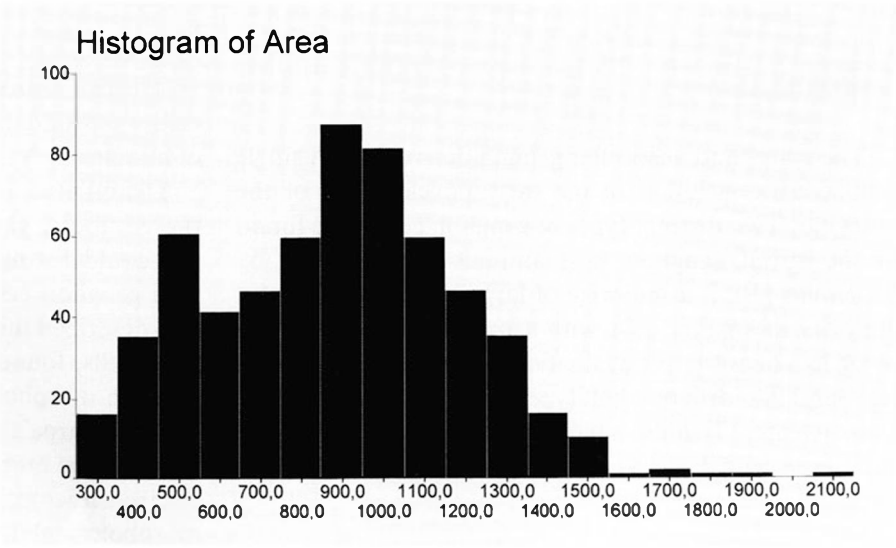

Fig. 3. Histogram of the area of the vestibular ganglion cells. x-axis: measured area of the ganglion cells in square micrometers; y-axis: number of measured cells.

Materials and Methods

In 4 patients, both ears were fixed within 2 h after death by perilymphatic perfusion through the oval and round windows using freshly prepared Karnovsky's fixative. Each temporal bone was then removed intact and further fixed by immersion in Karnovsky's solution for 48 h. None of these temporal bones were from subjects who had diseases or treatments that might have affected the vestibular organ. Both the facial and statoacoustic nerve complexes were carefully microdissected in phosphate buffer (pH 7.4) from the internal acoustic meatus making an effort to keep the entire nerve together with the pial sheaths and blood vessels [13].

Specimens were postfixed in 2% osmium tetroxide for 1 h followed by dehydration in graded alcohol and embedding in Spurr resin. 30-µm-thick sections were cut for light microscopy. The diameter, the perimeter and the area of 597 ganglion cells was determined by measurements with a computer-aided morphometric program (VidsIV; Bestebell Mobrey, Düsseldorf, Germany). Descriptive statistical analysis was performed with SPSS statistical software (SPSS for windows 6.0: SPSS Inc., Chicago, 1994). In order to prove the morphometric results, we performed an evaluation by light and electron microscopy.

Results

Morphometric Measurements

The diameter, the circumference and the area of the vestibular ganglion cells were measured. We only selected ganglion cells with a clearly visible nucleolus inside the nucleus. It was easy to measure the diameter of round cells. At ovoid-appearing cells, the measured diameter was the longest visible dimension of the ganglion cell. To measure the perimeter and the area of the ganglion cells, we rounded the projected depiction of the ganglion cells at a graphic tablet and analyzed the data with a statistical program. The exploratory analysis was done with 597 cell measurements in order to find latent structures in the data, concentrating on diameter, perimeter and the area of the ganglion cells. Graphical visualization of the data shows – according to size-related parameters – the possibility of two different types of cells among the sample. The histograms (fig. 1– 3) of the diameter, the perimeter and the area of the cells determine two concentrating maxima. In case of the diameter, one maximum lies at 40 µm, the other in the range of about 28 µm.

Light-Microsopic Evaluation

Serial 10-µm-thick sections were studied using Nomarsky interference contrast. We could distinguish between two cell types, a higher number of larger cells and lower number of smaller cells with clearly visible morphological differences (fig. 4a–d). In comparison to the smaller cells, a dark granulation with varying density appears in the cytoplasm of the larger cells. The larger cells seem to be darker than the smaller cells. Both types of cells contain a round nucleus with a clearly visible, mostly centrally located nucleolus.

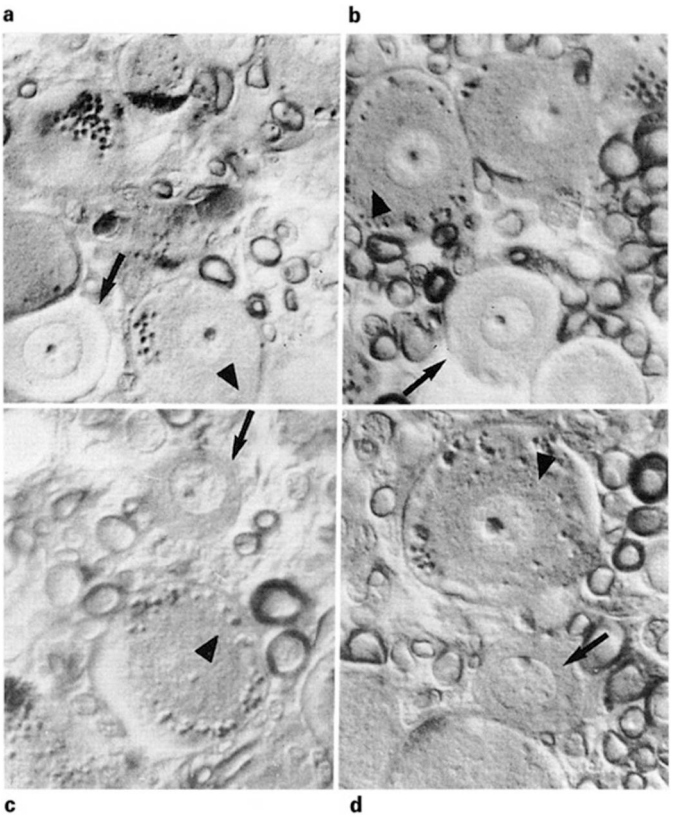

Fig. 4 a–d. Light-microscopic views of vestibular ganglion cells showing larger cells with granula (arrowheads) and smaller cells without granulation (arrows). × 2,200.

Electron-Microscopic Evaluation

The cells in the vestibular ganglion are round to oval in shape. In the more or less centrally located nucleus, 1 nucleolus is clearly visible. All cells are surrounded by 1–3 satellite cells. The cytoplasm of the stallite cells contains many mitochondria and coats the ganglion cell with a small layer. In none of our electron micrographs is the development of myelin sheaths identifiable.

Observing the cytoplasm and its organelles, a differentiation between two types is possible. We could find larger cells which contain many mitochondria and show dark clusters of rough endoplasmic reticulum with many surrounding ribosomes (fig. 5, 6). This may be the reason for the darker appearance in the light microscope. Scattered

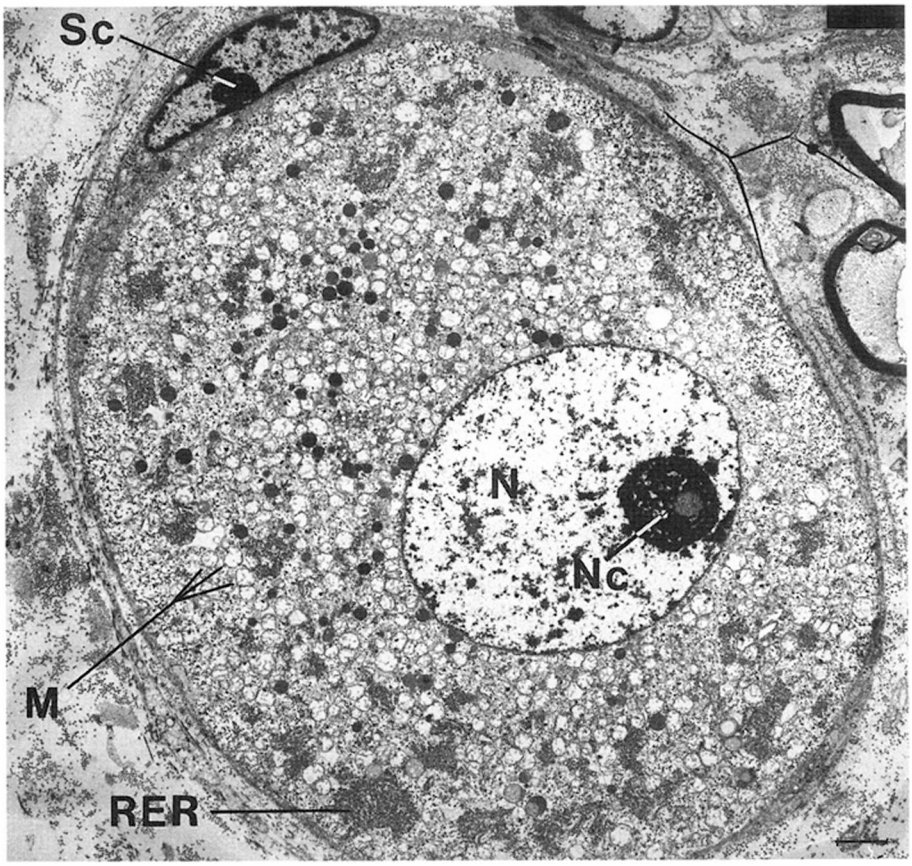

Fig. 5. Electron micrograph of a larger ganglion cell. N = Nucleus; Nc = nucleolus; M = mitochondria; RER = rough endoplasmic reticulum; Sc = satellite cell; scale bar = 5 μm.

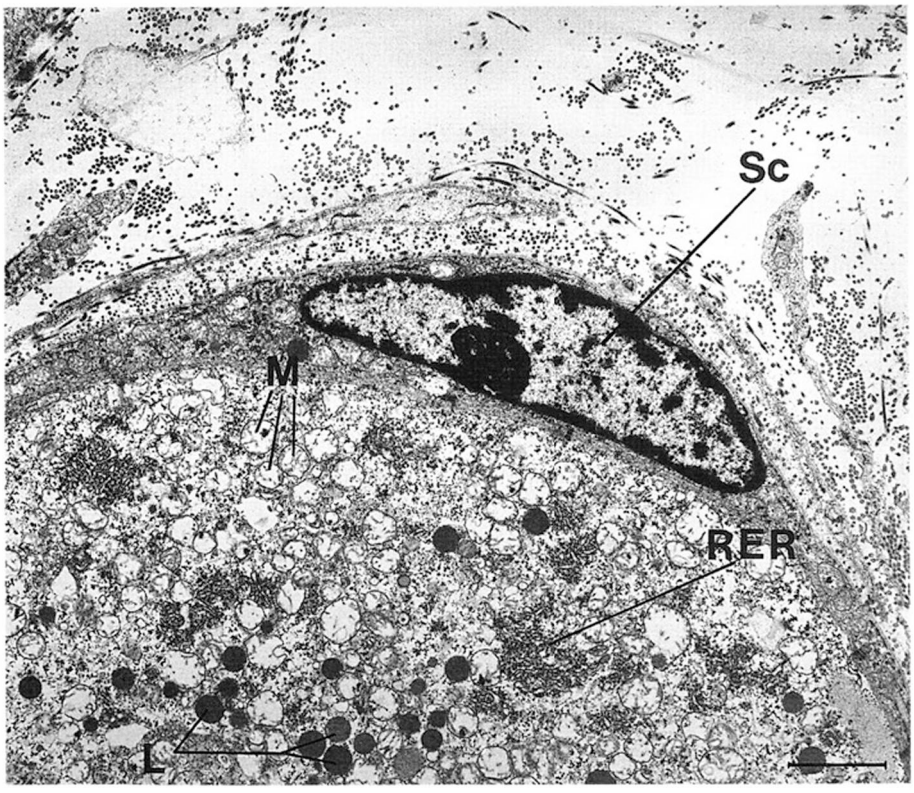

Fig. 6. Electron micrograph of a larger ganglion cell at a higher magnification. M = Mitochondria; RER = rough endoplasmic reticulum; Sc = satellite cell; L = lysosomes; scale bar = 5 μm.

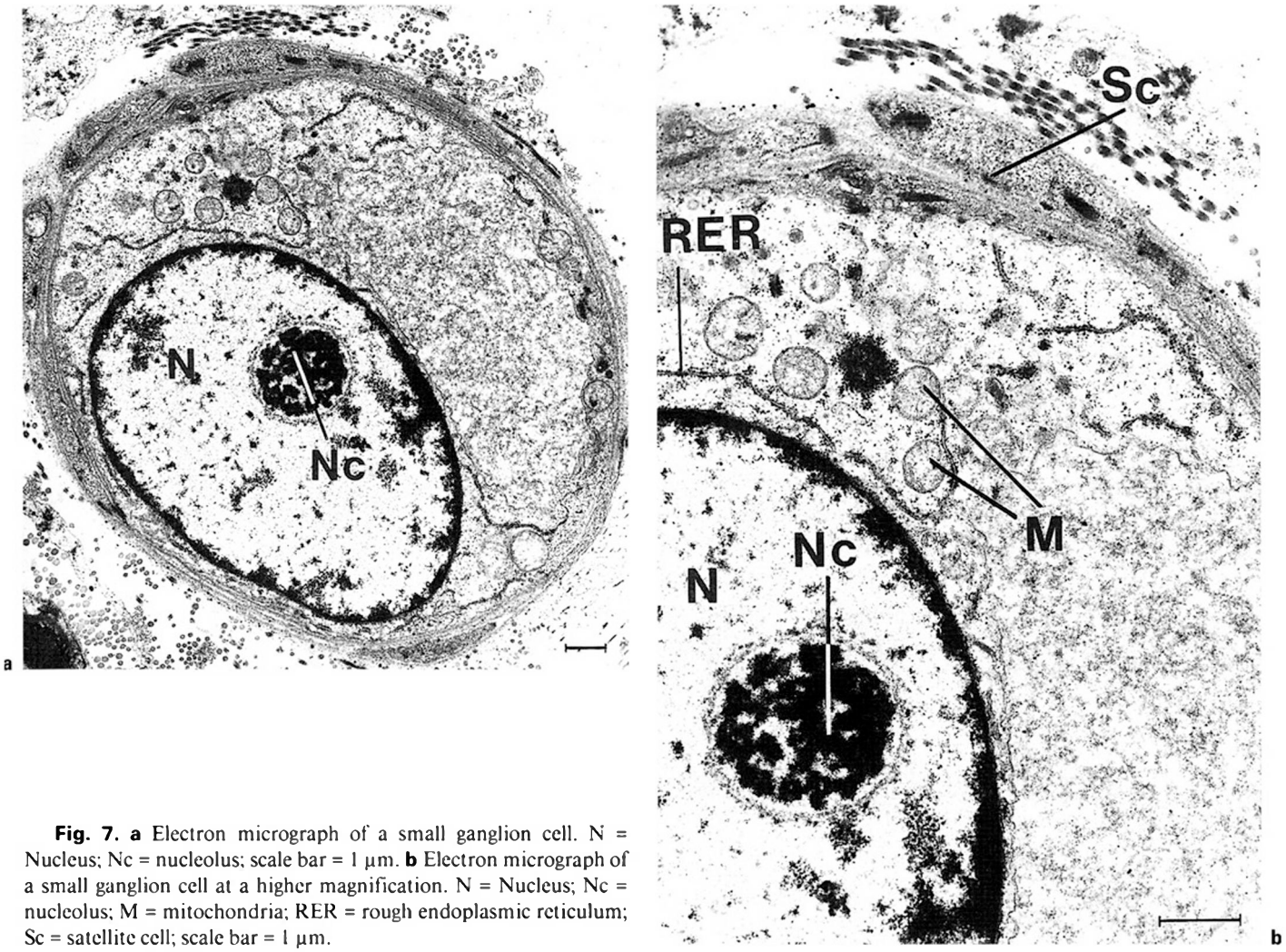

Fig. 7. a Electron micrograph of a small ganglion cell. N = Nucleus; Nc = nucleolus; scale bar = 1 µm. **b** Electron micrograph of a small ganglion cell at a higher magnification. N = Nucleus; Nc = nucleolus; M = mitochondria; RER = rough endoplasmic reticulum; Sc = satellite cell; scale bar = 1 µm.

in the cytoplasm are dark round lysosomes. These organelles appear in the light microscope as dark-stained granules which seemed in the ganglion cell to be located more peripherally.

The smaller cells have only few mitochondria, and the less developed rough endoplasmic reticulum is in contrast to the larger extended cells (fig. 7a, b). In these smaller cells, we could see no dark lysosomes.

Discussion

Ballantyne and Engström [14] studied vestibular ganglia in different species. They observed mainly large and myelinated perikarya. In the spiral ganglion of the cat and of humans, two distinct types of ganglion cells were described by Spoendlin [1–5]. By plotting the major axis of 340 neurons, Richter and Spoendlin [6] found two types of cells in Scarpa's ganglion of the cat: large neurons and small ones. The fine structure of the macaque monkey vestibular ganglion cells was studied by Kitamura and Suzuki [11]. They could distinguish between two types: myelinated and unmyelinated cells. However, they did not find histological differences in the cytoplasm of these two cell types. In the distribution curve of the cell size, only one single peak was visible and there was no significant difference between the size of myelinated and unmyelinated cells. Okami et al. [10] differentiated between four cell types in the vestibular ganglion cells of the rat. The main characteristic was the distribution of the rough endoplasmic reticulum and the Nissl granules. Ylikoski [8] studied the fine structure of the sheaths of vestibular

ganglion cells in rats, baboons and operative specimens of human vestibular nerves. He found the ganglion cells of the baboons encapsulated by a sheath of loose myelin and the neuronal cell bodies of the rats surrounded by a sheath of compact myelin. The human neuronal cell bodies were only ensheathed by a single layer of Schwann cell cytoplasm. In contrast to this finding, Ona [9] found myelin sheaths around the ganglion cells of humans by means of Levi's fixation using light microscopy. Regarding our electron-microscopic findings we agree with the observations of Ylikoski [8] that the human vestibular ganglion cells are unmyelinated. Also Felix et al. [12] described all human vestibular ganglion cells as being unmyelinated. One possible consequence of the lack of a myelin sheath might be to facilitate the exchange of intra- and extracellular fluid, allowing rapid diffusion of nutrients and metabolites across the perikaryal cell membrane. Under these conditions, the human vestibular cells might be more sensitive to environmental changes. The morphological features of the ganglion cells appeared to be unique in human Scarpa's ganglion.

In the present study, it was possible to distinguish two cell types in the vestibular ganglion. By light microscopy, we could find larger darker cells with dark granules and smaller lighter cells without granulation. At the electron-microscopic level, we were able to verify the light-microscopic observations.

The morphological difference does not always correspond to the functional one. Based on our electron-microscopic analysis, concerning the pronounced ultrastructural differences between the two cell types, it seems that the human vestibular ganglion contains cells with distinct responsibilities. From the present study, we cannot establish a relation of the two different types of ganglion cells to one of the two types of sensory cells in the vestibular organ. To extend our knowledge, immunohistochemical studies of the human vestibular ganglion cells are now in progress.

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