Journal of Neurocytology 8, 591–603 (1979)

Freeze-fracture studies on the synapse between the type I hair cell and the calyceal terminal in the guinea-pig vestibular system

R. L. GULLEY* and D. BAGGER-SJÖBÄCK†

Departments of Anatomy, Case Western Reserve University, Cleveland, Ohio and The University of Texas Health Science Center at San Antonio, San Antonio, Texas, U.S.A.

†*Department of Otolaryngology, Karolinska Hospital and King Gustaf V Research Institute, Stockholm, Sweden*

Received 15 September 1978; revised 4 January and 15 February 1979; accepted 27 February 1979

Summary

The apposition between type I hair cells and the calyceal terminals of vestibular ganglion cell peripheral processes was studied in the vestibular epithelium of the guinea-pig, using thin-sectioned and freeze-fractured specimens. Chemical synaptic junctions were exceedingly rare in thin-sectioned specimens, and were not seen in freeze-fracture replicas. Furthermore, no gap junctions were present between the hair cell and the calyx. There were, however, regions along the apposition where the membranes were closely apposed. At these regions, the hair cell was invaginated by cytoplasmic protrusions of the calyx and the plasmalemmata of the two cells were separated by only 6–7 nm. The number and conformation of the close appositions varied between different cells. In freeze-fracture replicas, the closely-apposed plasmalemmata of the hair cell and the calyx had no special distribution of intramembrane particles on either membrane leaflet. However, on the external membrane leaflet of the hair cell, a large patch of widely-spaced, large particles surrounded the regions of close apposition. The corresponding region of the plasmalemma of the calyx had no special distribution of particles on either membrane leaflet. The scarcity of chemical synaptic junctions, the absence of gap junctions between the cells and the unique arrangement of particles in the hair cell plasmalemma surrounding regions of close membrane apposition may indicate an unusual mode of synaptic transmission between the type I hair cell and the calyx.

Introduction

In the mammalian vestibular system, there are two types of receptor cells, the type I and type II hair cells. The type I hair cell is flask-shaped and is surrounded by a single calyceal terminal of the peripheral process of a vestibular ganglion cell

**Please address requests for reprints to Dr R. L. Gulley at his present address:* National Institute of General Medical Sciences, National Institutes of Health, Westwood Building, Room 920, Bethesda, Maryland 20205, U.S.A.

0300-4864/79/050591-13 $3.30/0

(Wersäll, 1956; Hamilton, 1968). The type II hair cell is cylindrical. Numerous peripheral processes of vestibular ganglion cells terminate along its base and sides. In addition to differences in the number, size and arrangement of afferent terminals on the two cells, the synaptic specializations between the two types of hair cell and the afferent terminals they contact are different. The type II hair cell has at least one chemical synaptic junction with each afferent terminal (Wersäll, 1956; Wersäll and Bagger-Sjöbäck, 1974); whereas, since few synaptic bodies are present in the type I hair cell, it has been suggested that transmission between the type I hair cell and the calyceal terminal might be electrical (Spoendlin, 1966; Hamilton, 1968). However, gap junctions, which typically mediate electrical synaptic transmission (Bennett *et al.*, 1967; Sotelo, 1975), have not been found in thin-sectioned material. At several regions along the apposition, the plasmalemmata of the two cells are closely apposed (Hamilton, 1968; Wersäll and Bagger-Sjöbäck, 1974), but the width of the extracellular space is greater than that of a gap junction. It has been suggested that synaptic communication may take place across these closely apposed membranes (Hamilton, 1968).

With the freeze-fracture technique, chemical synaptic junctions and gap junctions each have characteristic distributions of intermembrane particles (Goodenough and Revel, 1970; Pfenninger *et al.*, 1972; Heuser *et al.*, 1974; Gulley and Reese, 1977). Since this technique reveals large areas of plasmalemma, it is well suited for determining the presence and distribution of these junctions. This study uses the freeze-fracture technique to determine whether typical chemical synaptic junctions or gap junctions are present between the type I hair cell and calyx, and to determine whether the membranes at the close appositions have a distinctive arrangement of intramembrane particles which might suggest a synaptic function for these regions.

Methods and materials

Fourteen adult NIH strain guinea-pigs were sacrificed by intracardiac perfusion with a solution of 0.2 M sodium cacodylate, 20 mM $CaCl_2$ and 1% sodium nitrite followed by 3% glutaraldehyde, 2% paraformaldehyde, 0.1 M sodium cacodylate and 20 mM $CaCl_2$. Both solutions were at 37° C. Immediately following perfusion, the maculae of the saccule and utricle, and the cristae ampullaris of the semicircular canals were removed from both sides and placed in the aldehyde solution at room temperature.

The maculae and cristae from 12 animals were prepared for study with the freeze-fracture technique. After 2 h in the fixative, the tissue was washed briefly on 0.2 M sodium cacodylate with 20 mM $CaCl_2$ and placed in 20% glycerol in 0.1 M sodium cacodylate for 2 h. The tissue was frozen on gold discs in liquified monochlorodifluoromethane (Freon 22) and stored in liquid nitrogen. The tissue was fractured and replicated at −119° C on a Balzers 301 apparatus with two electron beam guns and a quartz crystal monitor for standardizing the thickness of the replica. The replicas, cleaned successively in cold methanol, Clorox and distilled H_2O were mounted on Formvar- and carbon-coated grids and examined in a Siemens Elmiskop 101 electron microscope.

Two animals were prepared for thin-section study. After 12 h in the fixative, the tissue was washed briefly in 0.2 M sodium cacodylate with 20 mM $CaCl_2$ and postfixed in 1.5% potassium ferrocyanide, 1% OsO_4 in 0.05 M sodium cacodylate at 4° C. The tissue was dehydrated in a graded series of methanol and embedded in Spurr's resin mixture. Thin sections were cut and examined in a Siemens Elmiskop 101 electron microscope.

Identification of the type 1 hair cell and the calyx in freeze-fracture replicas was accomplished by correlating the structures seen with thin-sectioned material. Cytological features including the shape and characteristic relationship of the calyx and hair cell made these elements easy to identify. Each of the specific features of the hair cell and calyceal plasmalemmata were identified in examples in which the identification of the cell to which the structure belonged was unequivocal. Each observation was confirmed in at least ten examples. Over 475 μm^2 of plasmalemma of the type I hair cell was examined in this study.

To assess the frequency of synaptic bodies in the type I hair cell, additional thin sections from four different cristae ampullares were cut perpendicular to the apical-basal axis of the epithelium and mounted on Formvar- and carbon-coated slot grids. Each hair cell in a single section was photographed at 3000x initial magnification. Adjacent sections were not photographed. When sections from the same specimen block were used, the sections were separated by at least 30 μm. The number of synaptic bodies and invaginations, the total perimeter of membrane of each cell and the perimeter of non-invaginated and invaginated membrane were analysed for each of 161 profiles of different hair cells. The measurement of perimeters was done on a Tektronix digitizer interfaced with a PDP 11–45 computer. The results from these sections were comparable to those observed in the other thin-sectioned material. The 161 type I hair cells examined in this part of the study had a total length of 4752 μm of membrane apposed to the calyx.

Results

In the maculae of the saccule and the utricle and in the cristae ampullares, the flask-shaped type I hair cell is enveloped by a single calyx. The shape of the portion of the hair cell surrounded by the calyx approximates a prolate spheroid with a maximum minor axis 9.2 μm in length and maximum major axis 17.4 μm in length. (The average minor axis was 6.8 μm in length; the average major axis was 15.1 μm in length.) The hair cell and calyx are separated by a regular, 30–35 nm wide extracellular space. The contour of the apposition between many hair cells and calyces is irregular (Fig. 1) due to invaginations of the hair cell by cytoplasmic protrusions from the calyx. The apposed plasmalemmata of the two cells forming these invaginations are separated by an extracellular space 6–7 nm wide (Fig. 3). The distribution of the invaginations on the hair cell is uniform, except that no invaginations are present at the neck of the hair cell at the level of the mouth of the calyx. Of the 161 profiles of different type I hair cells, 13% of the profiles, comprising 8% of the perimeter examined, have no invaginations (Fig. 2). About 26% of the profiles, comprising 27% of the perimeter examined, have no more than one invagination per 10 μm of plasmalemma (Fig. 2). About 22% of the profiles, comprising 22% of the perimeter examined, have at least one invagination per 5 μm of plasmalemma (Fig. 2). Hair cells with different numbers of invaginations appear randomly distributed in the epithelium, and adjacent hair cells with different numbers of invaginations are common.

The appositions between type I hair cells and calyces are notable for the paucity of synaptic junctions (Fig. 1). Of 161 thin-section profiles of different hair cells, only six profiles contain a synaptic body. Of these six synaptic bodies, two are in the cytoplasm and not directly adjacent to the plasmalemma in that section. The synaptic bodies are only found at sites where the plasmalemmata of the hair cell and calyx are separated by a distance of 30–35 nm. In other thin sections used in this

study, most cells have no synaptic body or other synaptic specialization such as an accumulation of vesicles near the membrane or around presynaptic densities. In the few profiles where synaptic bodies are present, no cell has more than one synaptic body.

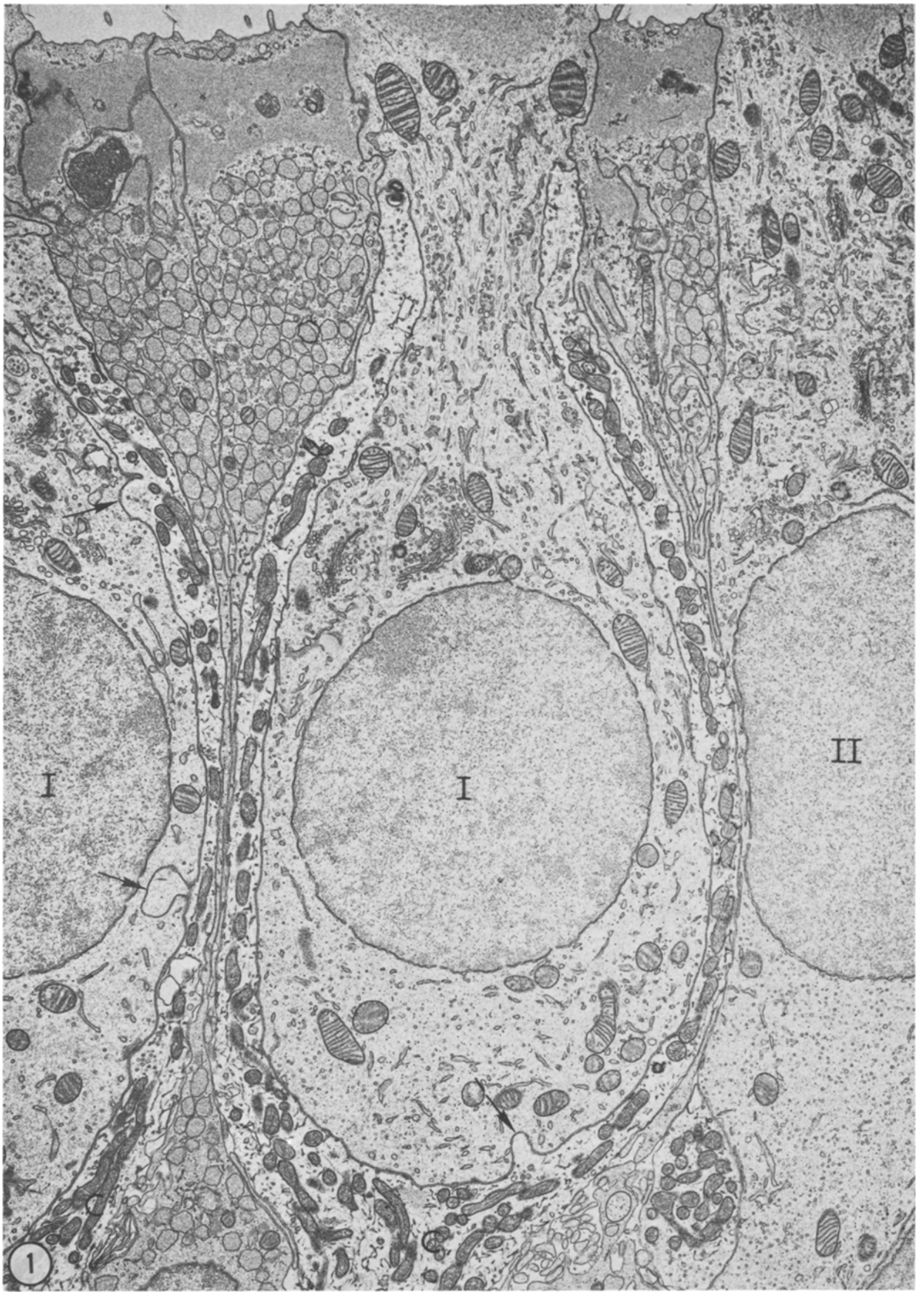
Some small clear vesicles are present in the cytoplasm of the hair cell (Fig. 1), including some near the plasmalemma. However, these vesicles do not cluster near the membrane and are not associated with any special region of the plasmalemma. No examples were seen of a smooth vesicle fused with the plasmalemma. Coated vesicles and coated invaginations of the plasmalemma are common. The coated invaginations are found exclusively on non-invaginated regions of the membrane. No puncta adherentia or other membrane specializations are seen between the hair cell and calyx.

With the freeze-fracture technique, the internal organization of large areas of hair cell and calyceal membrane are revealed. Regions of hair cell membrane which are not invaginated, and are separated from apposed membrane by 30–35 nm, can be distinguished from invaginated regions of membrane which are separated from apposed calceal membrane by 6–7 nm (Figs. 4 and 5). The cytoplasmic leaflet of the hair cell membrane is covered uniformly by small and medium-sized particles (Fig. 6). On this leaflet, there is no difference in the distribution of particles between invaginated and non-invaginated regions of the membrane. The hair cell membrane, including membrane at the base and within the invaginations (Figs. 4 and 5), generally has only scattered medium-sized particles on its external leaflet. However, on this leaflet, large irregularly-shaped patches of large intramembrane particles *are near or surround* the base of the invaginations (Figs. 4, 5 and 7). In replicas where the fracture exposes the external leaflet of the hair cell membrane and the apposed cytoplasmic leaflet of the calyx, the patches of large particles are on the region of the hair cell membrane which is separated by 30–35 nm from the calyx. These patches are not associated with a cytoplasmic organelle, such as a subsurface cisterna, since cross-fractures of the cytoplasm of the hair cell adjacent to the patches of particles reveal no underlying organelles (Figs. 4 and 5).

The typical arrangement of intramembrane particles associated with synaptic bodies (Raviola and Gilula, 1975; Gulley and Reese, 1977), was not seen on the plasmalemma of the type I hair cell. In fractures which exposed over 30 μm^2 of the plasmalemma of five different hair cells, no membrane specialization typical of a synaptic body or other synaptic active zone (Pfenninger *et al.*, 1972; Pfenninger and

---

Fig. 1. Thin section through a crista ampullaris. The section passes through two type I hair cells (I) and a type II hair cell (II). The body and neck of the type I hair cell are enveloped by a calyceal terminal (C). The contour of the apposition of the type I hair cell and the calyceal terminal is irregular. The type I hair cell is frequently invaginated by protrusions (arrows) from the enveloping calyx. The shape of the protrusions vary from shallow, dome-shaped protrusions to deep, finger-like or club-shaped protrusions. The details of the apposition of the type I hair cell and calyx are illustrated at higher magnification in Fig. 3. x 10 000.


I
I
II
1

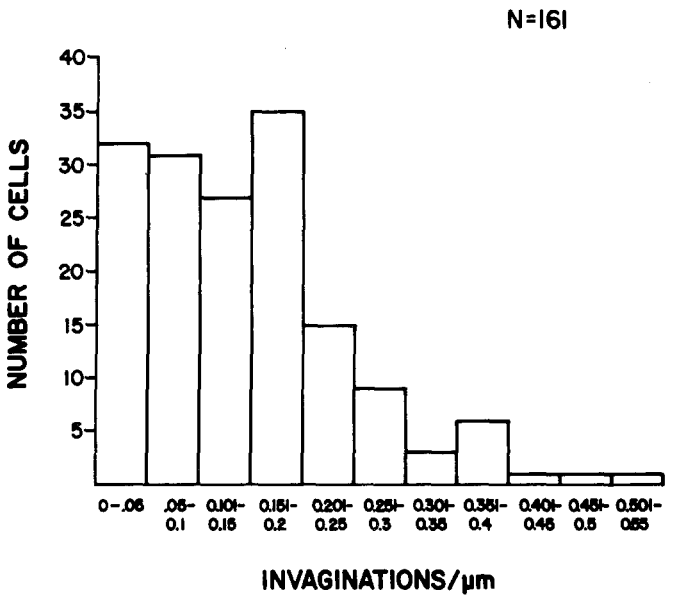


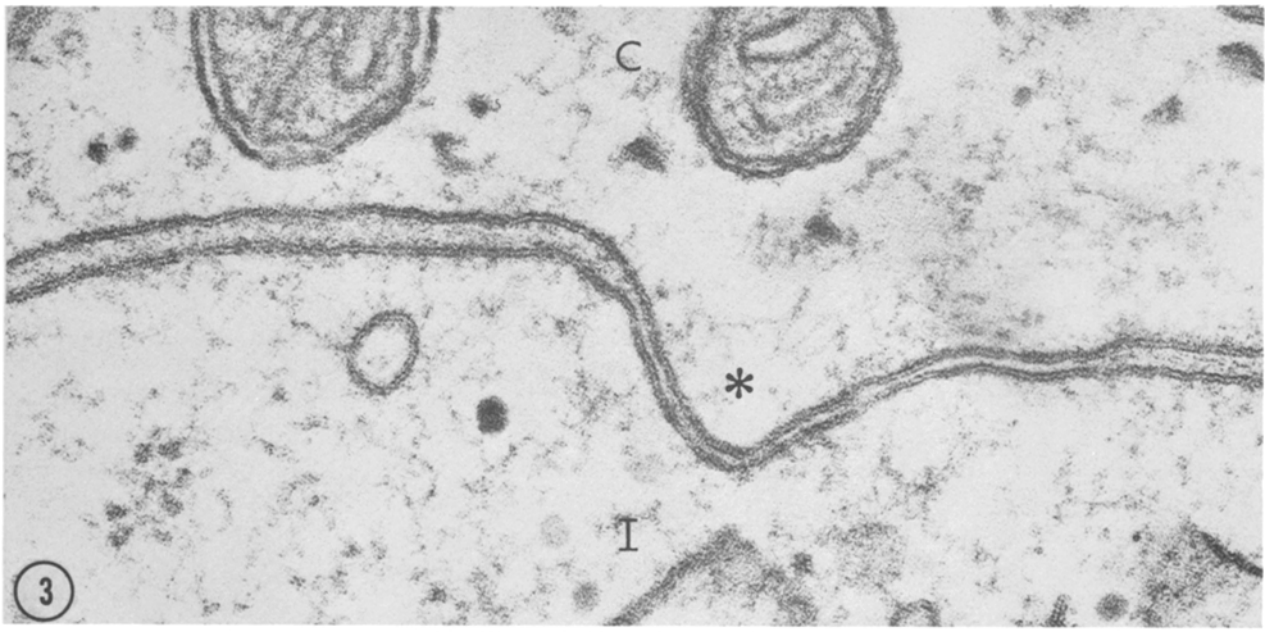
Fig. 2. Histogram showing the number of thin-section profiles of type I hair cells with a given frequency of invaginations along their perimeter. Profiles of 161 different type I hair cells were used in this analysis.

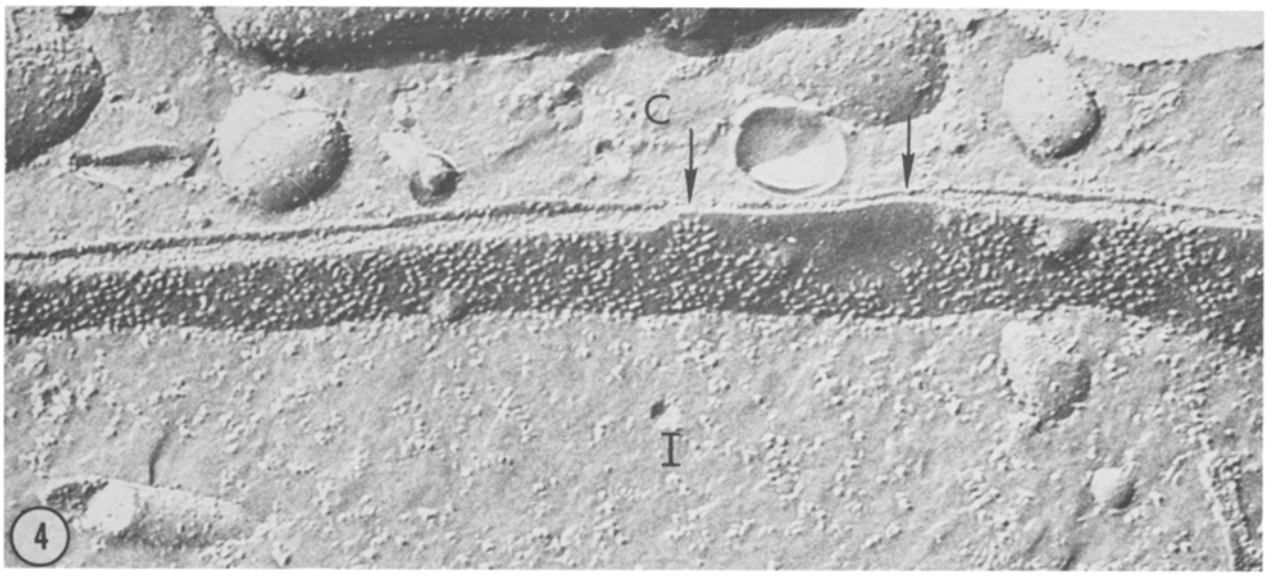
Fig. 3. Thin section through a portion of an apposition between a type I hair cell (I) and its calyx (C). A cytoplasmic protrusion (*) from the calyx invaginates the hair cell. The plasmalemmata are separated by 6–7 nm. x 140 000.

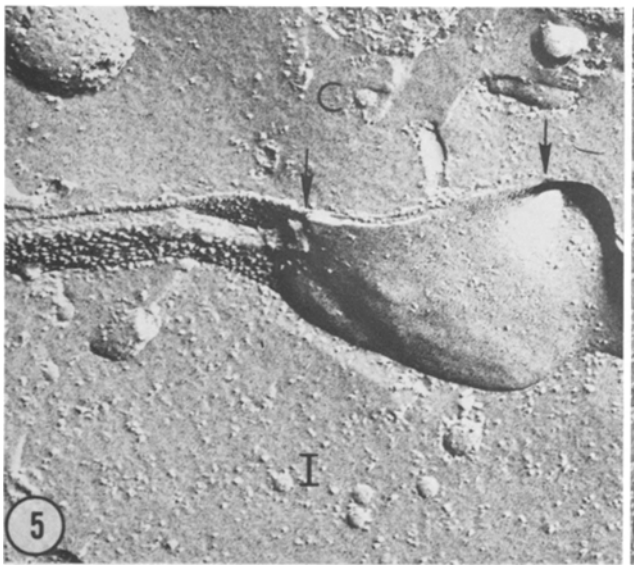
Fig. 4. A freeze-fracture replica of a crista ampullaris in which the fracture exposes a portion of the apposition of a type I hair cell (I) and its calyx (C) similar to that illustrated in Fig. 2. The fracture passes across the cytoplasm of the hair cell and exposes its external membrane leaflet. It then crosses the extracellular space between the cells and exposes the cytoplasm and a small part of the cytoplasmic membrane leaflet of the calyx. The plasmalemmata are separated by about 35 nm except where the calyx invaginates the hair cell (between arrows). There, the extracellular space between the cells is narrower. Surrounding the invagination, the plasmalemma of the hair cell has many large particles, 12–14 nm in diameter, on the external leaflet. The hair cell plasmalemma, within the invagination, has scattered medium-sized particles, on its external leaflet. x 40 000.

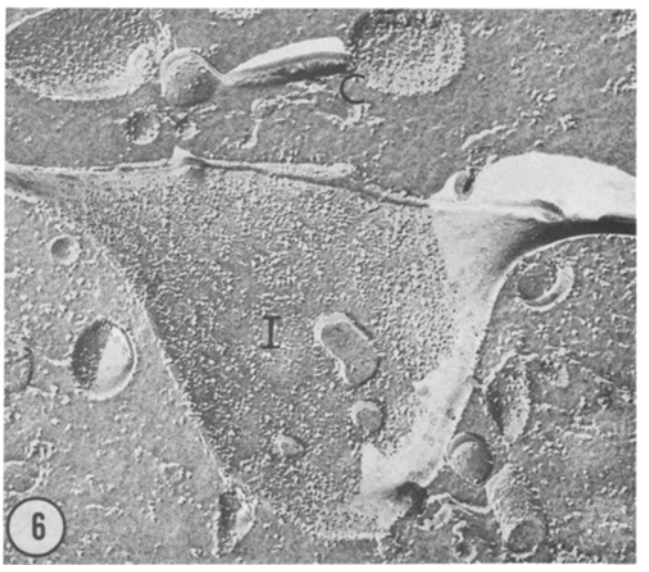
Fig. 5. A freeze-fracture replica of the apposition of a type I hair cell (I) and its calyx (C) in a macula utriculi. As in Fig. 3, the fracture crosses the hair cell cytoplasm and exposes its external membrane leaflet. The cytoplasm of the calyx and a small portion of its cytoplasmic leaflet are also exposed. Large, intramembrane particles cover the external leaflet of the hair cell membrane where it is separated from the calyx by an extracellular space about 35 nm in width. The plasmalemmata are more closely apposed at an irregularly-shaped invagination (between arrows). The external leaflet of the hair cell membrane has only scattered, medium-sized particles, in this region. x 57 000.

Fig. 6. In this freeze-fracture replica, the fracture exposes the cytoplasmic leaflet of a type I hair cell (I) within an invagination presumably caused by a protrusion from its calyx (C). This leaflet has numerous small to medium-sized intramembrane particles; however, on this leaflet the distribution of particles within the invagination is identical to that in other regions of the plasmalemma. Macula sacculi. x 70 000.


C
*
I
3


C
I
4


C
I
5


C
I
6

Rovainen, 1974; Gulley and Reese, 1977; Gulley *et al.*, 1978) was present. Large plasmalemmal deformations (Pfenninger and Rovainen, 1974; Heuser *et al.*, 1974; Gulley, 1978) are randomly distributed over the non-invaginated plasmalemma of the hair cell. These deformations are present within the aggregates of large particles (Fig. 4) and in unspecialized regions of non-invaginated membrane, but are not found on invaginated portions of the plasmalemma.

The plasmalemma of the calyx apposed to the hair cell has no aggregates or patches of intramembrane particles on either membrane leaflet. The cytoplasmic membrane leaflet of the calyx is uniformly covered by small to medium-sized particles both in evaginated and non-evaginated (Fig. 7) regions of the membrane. Similarly, no difference exists in the distribution of particles between evaginated and non-evaginated regions of the external membrane leaflet (Fig. 8). Hence, there is no specialization on either membrane leaflet of the calyx which is co-extensive with the patches of large particles in the hair cell plasmalemma. This asymmetrical distribution of particles suggests that the patches of particles on the hair cell membrane around the close apposition are not parts of puncta adherentia in which discrete plaques of particles are found on the external leaflet of *both* junctional membranes (Landis and Reese, 1974).

No tight junctions are present between the calyx and the neck of the hair cell.

Synapses of the efferent boutons on the calyceal terminal

One or more efferent boutons frequently contact the calyx near its base or are apposed to the unmyelinated portion of the parent nerve fibre. These boutons usually indent the surface of the calyx (Fig. 9). Small round synaptic vesicles cluster around small tufts of fuzz which randomly line the presynaptic membrane (Fig. 9). A thin postsynaptic density lines the apposed calyceal membrane. Subsynaptic cisternae, which are common at the efferent-type II hair cell synapse (Wersäll and Bagger-Sjöbäck, 1974), are not present at the efferent–calyx synapse.

In freeze-fracture replicas, the 'active zone' of the efferent bouton appears as a slight oval depression on the cytoplasmic membrane leaflet (Fig. 10). As at other chemical synapses, the number of large particles is greater and the number of small to medium-sized particles fewer within the active zone than in non-junctional regions of the membrane. Small plasmalemmal deformations, probably sites of synaptic vesicle exocytosis during transmitter release (Pfenninger and Rovainen, 1974; Heuser *et al.*, 1974; Gulley, 1978), are confined to the junctional region of the bouton (Fig. 10). Large plasmalemmal deformations, presumably associated with sites of membrane endocytosis in coated vesicles (Heuser *et al.*, 1974), are always found outside the junctional region. On the external membrane leaflet of the calyx opposite the active zone of the efferent bouton, there is an oval-shaped aggregate of large, loosely-packed particles (Fig. 10). In fractures such as that illustrated in Fig. 10, the size and shape of the aggregate seems to be similar to that of the presynaptic active zone. This impression is also supported by fractures of the calyceal membrane exposing entire aggregates of particles in which the size and packing den-

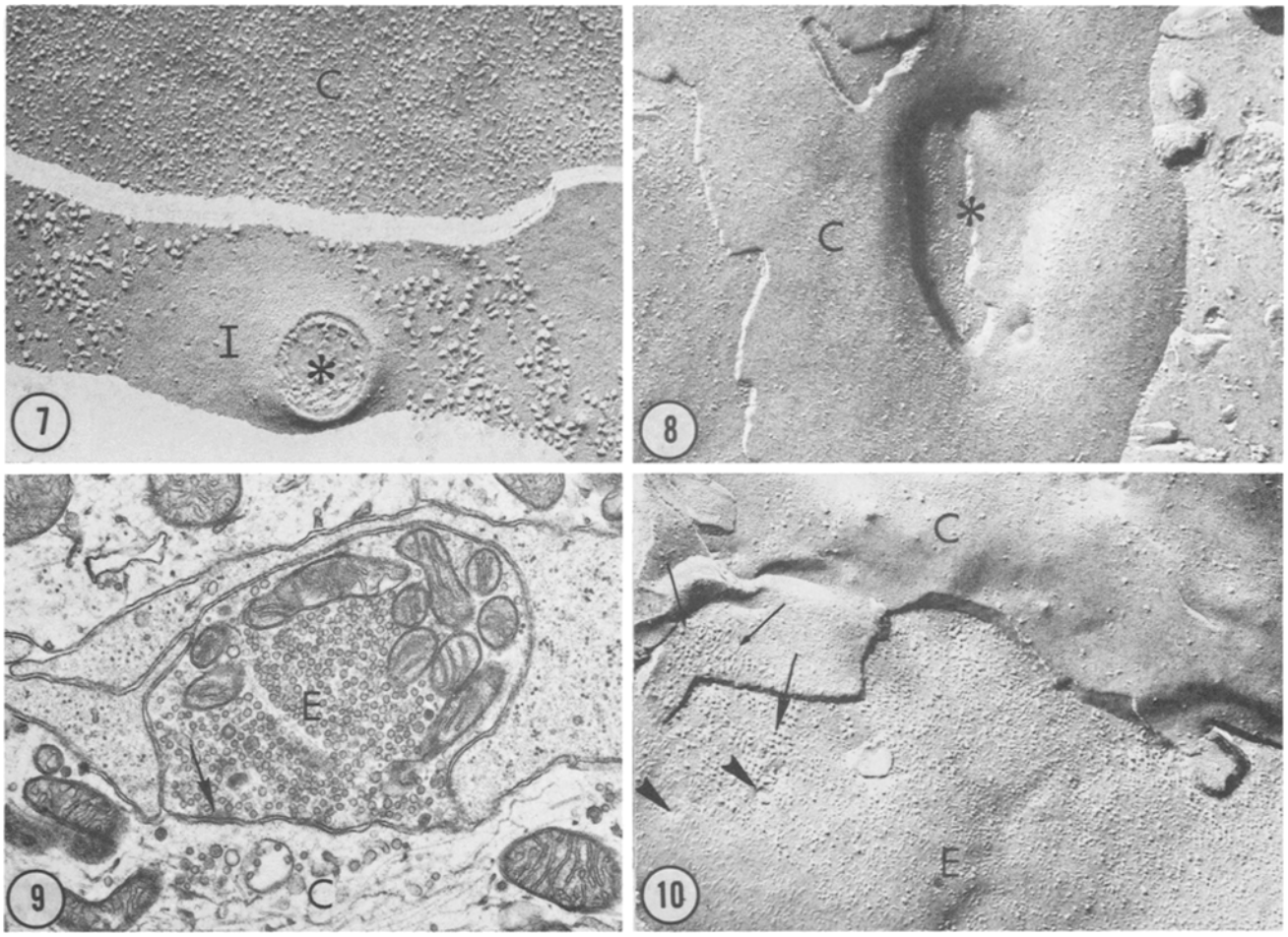


Fig. 7. In this freeze-fracture replica, the fracture exposes the external leaflet of a type I hair cell membrane (I) surrounding a finger-like protrusion from its calyx (*). The cytoplasmic membrane leaflet of the calyx (C) is also visible. On the external leaflet of the hair cell membrane, the invagination is encircled by large, intramembrane particles. There are a few particles on this leaflet at the base or along the sides of the invagination. Crista ampullaris. x 83 000.

Fig. 8. Freeze-fracture replica in which the fracture exposes the external membrane leaflet of a calyx (C). A protrusion (*) from the calyx invaginates the hair cell. A portion of the cytoplasmic leaflet can be seen through a hole in the calyceal membrane at the apex of the protrusion. Another portion of the hair cell cytoplasmic leaflet outside the invagination is seen at the left. The distribution of particles on the external leaflet of the calyx is identical in both evaginated and non-evaginated portions of the membrane. Macula utriculi. x 56 000.

Fig. 9. Thin section through a synaptic contact between an efferent bouton (E) and a calyx (C). The efferent bouton depresses the base of the calyx. The contour of the apposition is irregular. Small round synaptic vesicles cluster around small tufts (arrow) of fuzz which line the cytoplasmic surface of the presynaptic membrane. Crista ampullaris. x 60 000.

Fig. 10. Freeze-fracture replica in which the fracture exposes the cytoplasmic leaflet of an efferent bouton (E) and the external membrane leaflet of a calyx (C). Where the efferent bouton bulges into the calyx the cytoplasmic membrane leaflet of the bouton has more large intramembrane particles (arrows) than in non-junctional regions of the membrane. Small plasmalemmal deformations (arrowheads) also are found in this region. The region of the external leaflet of the calyceal plasmalemma over the presynaptic active zone has a cluster of large particles (small arrows) which appears to be co-extensive with the active zone. Macula utriculi. x 70 000.

sity of the particles are identical to that seen in the partial aggregates apposed to the presynaptic active zone. These complete aggregates are similar in size and shape to the presynaptic active zone.

Discussion

From the present study, three statements about the synapse between type I hair cells and calyceal terminals seem appropriate. First, using morphological criteria, typical, chemical synaptic contacts are exceedingly rare. Second, no gap junctions are present between the hair cell and the calyx. Third, the plasmalemmata of the hair cell and calyx are closely apposed at regions where the calyx invaginates the hair cell. In freeze-fractured specimens, no specialized patch of particles is present on either of the closely apposed plasmalemmata. However, on the external leaflet of the hair cell plasmalemma near the region of close apposition, patches of large particles are present.

The presynaptic plasmalemma of chemical synapses has a well-defined region, or regions, which is involved in the release of neurotransmitter by exocytosis. This region has been designated as the 'active zone' (Couteaux and Pecot-Dechavassine, 1970). In thin sections, this region is defined by tufts or a band of fuzz which lines the cytoplasmic aspect of the plasmalemma. Synaptic vesicles cluster around this active zone and are thought to release their contents by fusing with the membrane in this region. In many receptor cells, such as the rods and cones in the retina (Sjöstrand, 1958; Raviola and Gilula, 1975), the inner hair cell in the organ of Corti (Smith and Sjöstrand, 1961) and the type II hair cell in the vestibular epithelium (Wersäll and Bagger-Sjöbäck, 1974), the presynaptic density is modified to form a synaptic body or ribbon around which synaptic vesicles cluster. In freeze-fractured specimens, the cytoplasmic leaflet of the plasmalemma within the active zone has a greater number of large particles and fewer small particles than non-junctional regions of the plasmalemma (Heuser *et al.*, 1974; Gulley and Reese, 1977; Venzin *et al.*, 1977; Gulley *et al.*, 1978; Schwartz and Gulley, 1978). At the neuromuscular junction (Heuser *et al.*, 1974) and at ribbon synapses (Raviola and Gilula, 1975; Gulley and Reese, 1977), the large intramembrane particles of the active zone are arranged in rows which are co-extensive with the presynaptic density. Small plasmalemmal deformations, interpreted as sites of synaptic vesicle exocytosis during transmitter release (Streit *et al.*, 1972; Pfenninger and Rovainen, 1974; Heuser *et al.*, 1974; Gulley, 1978), are confined to the active zone region of the plasmalemma.

In the type I hair cell, few chemical synaptic contacts are seen in thin-sectioned material. No more than one contact is seen in a section through a cell and, in a given section, most cells have no synaptic contacts. In freeze-fracture replicas, no membrane specialization indicative of an active zone was found in the type I vestibular hair cell, even in replicas of cells where large areas of the plasmalemma were ex-

posed. This suggests that the number of chemical junctions at the hair cell-to-calyx apposition is, at best, infrequent. This is in contradistinction to the type II hair cell-to-afferent apposition where typical synaptic bodies are common in both thin-sectioned (Wersäll and Bagger-Sjöbäck, 1974) and freeze-fractured (Bagger-Sjöbäck and Gulley, unpublished data) material. From this, it is reasonable to suggest that the role of chemical transmission in the synapses of the two types of hair cell is different, and to question whether chemical transmission is the only type of synaptic interaction between the type I hair cell and calyceal terminal.

Regarding the latter suggestion, one should consider the possibility that the synapse between the type I hair cell and calyx is, at least in part, electrical. At another calyceal synapse in the avian ciliary ganglion, synaptic transmission is mediated both chemically and electrically (Martin and Pilar, 1963). There, both chemical synaptic junctions and gap junctions are present between the preganglionic calyx and ganglion cell (Brightman and Reese, 1969; Cantino and Mugnaini, 1975). If synaptic transmission between the type I vestibular hair cell and calyx is either electrical or a mixed chemical and electrical synapse, the absence of gap junctions, typically associated with electrical synaptic transmission (Bennett *et al.*, 1967; Sotelo, 1975), is noteworthy. At the gap junction, the plasmalemmata are closely apposed (2–3 nm), and each plasmalemma has an array of hexagonally-packed particles in register with particles on the apposing plasmalemma (Goodenough and Revel, 1970). The plasmalemmata of the hair cell and calyx at the protrusions of the calyx into the hair cell are closely apposed (6–7 nm). However, the distance between the plasmalemmata is greater than at a gap junction, and in freeze-fractured specimens, the closely apposed plasmalemmata have no special distribution of intramembrane particles on either membrane leaflet. Thus, the closely-apposed plasmalemmata do not have the structure of a typical electrical synapse and probably do not share a similar function.

While the closely-apposed plasmalemmata do not have a special arrangement of intramembrane particles, the hair cell plasmalemma does have a patch of large particles surrounding the membrane in the close apposition. The patches of large particles are only found on the hair cell membrane indicating that the membranes surrounding the close apposition are asymmetrical. There are no electrophysiological data about synaptic activity between the type I hair cell and the afferent terminal (see Goldberg and Fernandez, 1975); however, the proximity and asymmetry of the membranes might facilitate some other ephaptic (Arvanitaki, 1942; Grundfest, 1967; Rámon and Moore, 1978) mode of communication.

Acknowledgement

This work was supported by NIH Grant NS-R0113889 to RLG. We wish to acknowledge the valuable technical assistance of Ms Lauren Kimerer.

References

ARVANITAKI, A. (1942) Effects evoked in an axon by the activity of a contiguous one. *Journal of Neurophysiology* **5**, 89–108.

BENNETT, M. V. L., NAKAJIMA, Y. and PAPPAS, G. D. (1967) Physiology and ultrastructure of electronic junctions. I. Supramedullary neurons. *Journal of Neurophysiology* **30**, 161–79.

BRIGHTMAN, M. W. and REESE, T. S. (1969) Junctions between intimately apposed cell membranes in the vertebrate brain. *Journal of Cell Biology* **40**, 648–77.

CANTINO, D. and MUGNAINI, E. (1975) The structural basis for electrotonic coupling in the avian ciliary ganglion. A study with thin-sectioning and freeze-fracturing. *Journal of Neurocytology* **4**, 505–36.

COUTEAUX R. and PECOT-DECHAVASSINE, M. (1970) Vesicules synaptiques et poches au niveau des 'zones actives' de la jonction neuromusculaire. *Comptes Rendus de l' Academie des Sciences* **D271**, 2346–9.

GOLDBERG, J. M. and FERNANDEZ, C. (1979) Vestibular mechanisms. *Annual Review of Physiology* **37**, 129–62.

GOODENOUGH, D. A. and REVEL, J. P. (1970) A fine structural analysis of intercellular junctions in the mouse liver. *Journal of Cell Biology* **45**, 272–90.

GRUNDFEST, H. (1967) Synaptic and ephaptic transmission. In *The Neurosciences* (edited by QUARTON, G. C., MELNECHUK, T. and SCHMITT, F. O.) pp. 353–371. New York: Rockefeller University Press.

GULLEY, R. L. (1978) Changes in the presynaptic membrane of the synapses of the cochlear nucleus with different levels of acoustic stimulation. *Brain Research* **146**, 373–9.

GULLEY, R. L. and REESE, T. S. (1977) Freeze-fracture studies on the synapses in the organ of Corti. *Journal of Comparative Neurology* **171**, 517–44.

GULLEY, R. L., LANDIS, D. M. D. and REESE, T. S. (1978) Internal organization of membranes at end bulbs of Held in the rostral anteroventral cochlear nucleus. *Journal of Comparative Neurology* **180**, 707–42.

HAMILTON, D. W. (1968) The calyceal synapse of type I vestibular hair cells. *Journal of Ultrastructure Research* **23**, 98–114.

HEUSER, J. E., REESE, T. S. and LANDIS, D. M. D. (1974) Functional changes in frog neuromuscular junctions studied with freeze-fracture. *Journal of Neurocytology* **3**, 109–31.

LANDIS, D. M. D. and REESE, T. S. (1974) Differences in membrane structure between excitatory and inhibitory synapses in the cerebellar cortex. *Journal of Comparative Neurology* **155**, 93–126.

MARTIN, A. R. and PILAR, G. (1963) Dual mode of synaptic transmission in the avian ciliary ganglion. *Journal of Physiology* **168**, 443–63.

PFENNINGER, K., AKERT, K., MOOR, H. and SANDRI, C. (1972) The fine structure of freeze-fractured presynaptic membranes. *Journal of Neurocytology* **1**, 129–49.

PFENNINGER, K. and ROVAINEN, C. M (1974) Stimulation- and calcium-dependence of vesicle attachment sites in the presynaptic membrane; a freeze-cleave study on the lamprey spinal cord. *Brain Research* **72**, 1–23.

RAMÓN, F. and MOORE, J. W. (1978) Ephaptic transmission in squid giant axons. *American Journal of Physiology* **234**, C162–69.

RAVIOLA, E. and GILULA, N. B. (1975) Intramembrane organization of specialized contracts in the outer plexiform layer of retina. *Journal of Cell Biology* **65**, 192–222.

SCHWARTZ, A. M. and GULLEY, R. L. (1978) Non-primary afferents to the principal cell of the rostral anteroventral cochlear nucleus of the guinea pig. A correlative thin-section and freeze-fracture study. *American Journal of Anatomy* **153**, 489–508.

SJÖSTRAND, F. S. (1958) Ultrastructure of retinal rod synapses of the guinea pig eye as revealed by three-dimensional reconstructions from serial sections. *Journal of Ultrastructure Research* **2**, 122–70.

SMITH, C. A. and SJÖSTRAND, F. S. (1961) A synaptic structure in the hair cells of the guinea pig cochlea. *Journal of Ultrastructure Research* **5**, 184–92.

SOTELO, C. (1975) Morphological correlates of electronic coupling between neurons in mammalian nervous system. In *Golgi Centennial Symposium Proceedings* (edited by SANTINI, M.), pp. 344–65. New York: Raven Press.

SPOENDLIN, H. (1966) Some morphofunctional and pathological aspects of the vestibular sensory epithelia. In *Second Symposium on the Role of Vestibular Organs in the Exploration of Space*, pp. 99–116.

STREIT, P., AKERT, K., SANDRI, C., LIVINGSTON, R. B. and MOOR, H. (1972) Dynamic ultrastructure of presynaptic membranes at nerve terminals in the spinal cord of rats. Anesthetized and unanesthetized preparations compared. *Brain Research* **48**, 11–26.

VENZIN, M., SANDRI, C., AKERT, K. and WYSS, U. R. (1977) Membrane associated particles of the presynaptic active zone in rat spinal cord. A morphometric analysis. *Brain Research* **130**, 393–404.

WERSÄLL, J. (1956) Studies on the structure and innervation of the sensory epithelium of the cristae ampullaris in the guinea pig. A light and electron microscopic investigation. *Acta Oto Laryngologica* Suppl. **126**, 1–85.

WËRSALL, J. and BAGGER-SJÖBÄCK, D. (1974) Morphology of the vestibular sense organs. In *Handbook of Sensory Physiology*, Vol. VI/1 (edited by KORNHUBER, H. H.), pp. 123–71. New York: Springer-Verlag.