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# Gap junctions between hair cells and supporting cells in the goldfish saccular macula. A freeze fracture study.

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## *Introduction*

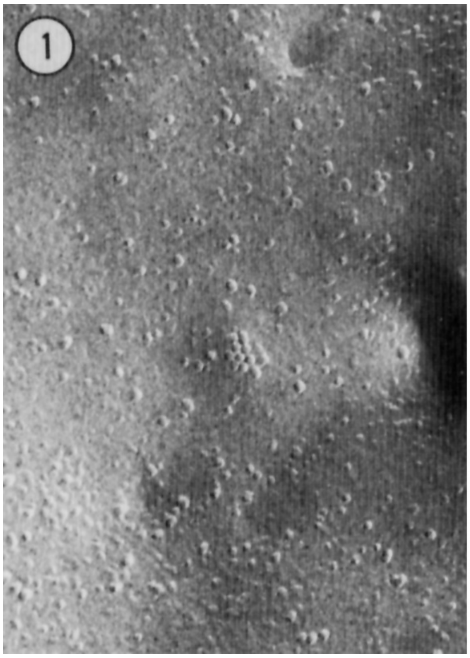
Gap junctions have been considered to be responsible for cell-cell communization and are found where electrical coupling or metabolic cooperation is present.[1,2] Many extensive gap junctions have been found between adjacent supporting cells in some acoustico-vestibular sensory receptors.[3] In the present study, gap junctions are found between hair cells and supporting cells in the goldfish saccular macula. The functional significance of these gap junctions is discussed.

## *Materials and methods*

Common goldfishes, about 10 cm in body length, were used. Saccular maculae were dissected out after decapitation without anesthesia. Specimens were fixed by immersion in 3 % glutaraldehyde and 0.1 M cacodylate buffer, pH 7.3, for one hour and cryoprotected in 25 % glycerol in the same buffer for two hours on ice. Specimens were frozen by immersion in Freon 12 at −155°C. Freeze fracture replicas were prepared using a Balzers freeze fracture device at −115°C.

## *Result*

Indentations formed by nerve terminals are found on the basal half of the receptor cell. Many small gap junctions are observed outside the indentation on the receptor cell membrane. They are probably formed between the receptor cell and supporting cell since the baso-lateral surface of the receptor cell is covered by supporting cells except for the region attached to the nerve terminal. They are usually small and may consist of only 11 particles (Fig. 1). However, rather large ones consisting of over hundred particles are occasionally encountered (Fig. 2). The diameter of constituent particles is 8.77 nm ± 0.50 nm which is smaller than that of supporting cell-supporting cell gap junctions, 9.50 nm ± 0.63 nm



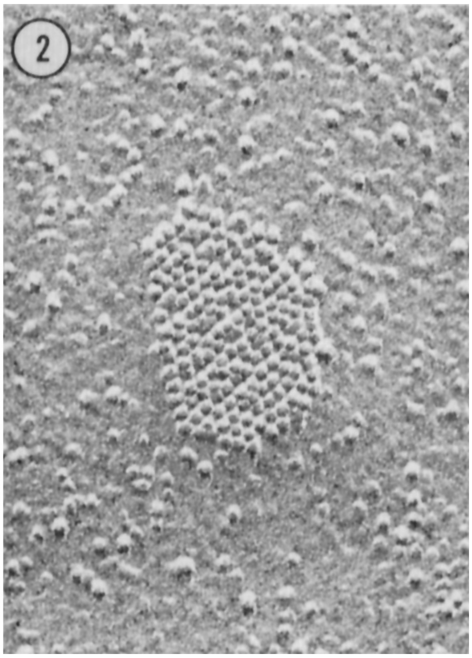




Fig. 1 Fig. 2

Fig. 1. A small gap junction between receptor cell and supporting cell consisting of only 11 particles. × 110,000

Fig. 2. Rather large gap junction between receptor cell and supporting cell. Particles are densely packed to form a regular hexagonal pattern. × 180,000

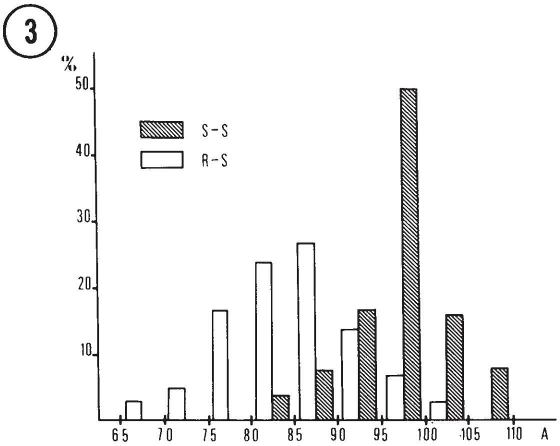



Fig. 3. Particle size distribution from supporting cells-supporting cells gap junctions (S-S) and receptor cells-supporting cells gap junctions (R-S).

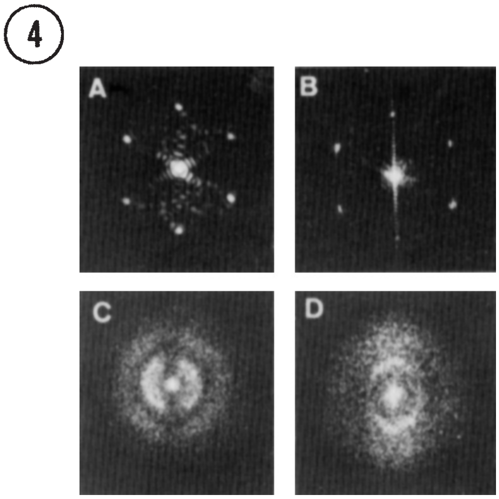



Fig. 4. Optical diffraction patterns.
A: from an aggregate of particles in a R-S gap junction.
B: from an aggregate of pits in a R-S gap junction.
C: from an aggregate of particles in a S-S gap junction.
D: from an aggregate of pits in a S-S gap junction.

(Fig. 3). They are packed into polygonal aggregate to form a regular hexagonal pattern. Optical diffraction patterns from these gap junctions indicate a regularity of particle and pit distribution. Whereas, those from supporting cell-supporting cell gap junctions do not (Fig. 4). They are frequently associated with desmosomes which is characteristic of the gap junction between receptor cells and supporting cells.

*Discussion*

Reports which demonstrate the electrotonic coupling between the hair cell and supporting cell in the vertebrate inner ears have appeared recently.[4,5,6] Although no electrical coupling has been directly observed between the hair cell and supporting cell in the saccular macula of goldfish, the small gap junctions described in the present study are probably related to electrotonic coupling between these cells. In the saccular macula of goldfish, since gap junctions were frequently found between hair cells and adjoining supporting cells and since adjacent supporting cells are extensively joined by large gap junctions[3,7] hair cells are conceivably connected by a low resistance pathway through surrounding supporting cells. Thus the electrical activity in one hair cell can influence the electrical activity in other hair cells through these connections. If so, the small gap junctions between hair cells and supporting cells might serve as a means for the processing of signals at the receptor cell level.

It has been postulated that, in freeze-fracture replicas of gap junctions, size and distribution of intramembrane particles depends largely on tissue preparation methods.[8,9] Peracchia[10] has reported a decrease in particle diameter and an increase in regularity of particle distribution after uncoupling procedures. In the present material, since receptor cell-supporting cell gap junctions and supporting cell-supporting cell gap junctions are situated side by side in the same sensory epithelium, it is unlikely that the effect of tissue preparation procedures differs substantially among these two types of gap junctions. Thus the present results may suggest either that receptor cell-supporting cell gap junctions are more easily uncoupled than supporting cell-supporting cell gap junctions by the same tissue preparation procedures, or these two types of gap junctions actually differ in structure reflecting the functional difference.

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## The acousticolateralis system and ionic environments

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It has been known that the endolymph of the mammalian inner ear contains high concentration of $K^+$ and that this ion is indispensable for the transduction process of the cochlea. However, little is known about the ionic mechanism of sound reception. The lateral-line organs of aquatic animals are homologous to mammalian inner ear. The organ has been shown to be chemoreceptors for ions and that the mechanical sensitivity was altered significantly by various ions.[1] Russell *et al.*[2] reported the presence of large amount of $K^+$ in the lateral-line cupulae, the overlying gelatinous structure on the top of the hair cells. Sand[3] described the effect of different ionic compositions of the external water medium of this organ on the mechano-sensitivity. He reported that the mechano-sensitivity was a function of the $Ca^{2+}$ concentration of the external medium. We confirmed the same $Ca^{2+}$ effects. Recently a model was proposed for the mechanism of neomycin ototoxicity.[4] In this model the basic groups of the antibiotic bind directly to polyphosphoinositides which are thought to be the sites of calcium binding in the membrane. To investigate the effect of neomycin on the mechano-sensitivity of the lateral-line organ, different concentra-