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A Study on the Fine Structure of the Saccular Macula of the Gold Fish*

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Summary. The fine structure of the saccular macula of the gold fish has been studied by means of the electron microscope.

The sensory epithelium of the macula consists of sensory cells and supporting cells. The surface of the sensory cell is studded with a group of sensory hairs consisting of one kinocilium and 50—60 stereocilia. In the dorsal half of the macula, the kino-cilium is located at the dorsal end of the sensory hair group. In the ventral half of the macula, the kino-cilium is located at the ventral end of the sensory hair group. In the intermediary portion of the macula, the sensory cells with opposite polarities are situated side-by-side. The relation between the microphonic potential and the position of the kino-cilium has been discussed.

Two types of nerve terminals are found situated on the basal surface of the receptor cells. The one contains no synaptic vesicle and the other contains a cluster of synaptic vesicles and a few cored vesicles. It is considered that the former corresponds to the afferent nerve terminal and the latter to the efferent one.

The families of the teleost fishes called ostariophysi are known to be more capable of hearing sound than the rest of fishes (v. FRISCH, 1936). The physiology of hearing in the ostariophysi has been much clarified recently (FURUKAWA and ISHII, 1967). The vibration of the swim bladder caused by the sound is transferred to the pressure changes of the endolymph of the sacculus mediated by the Weber's auditory ossicles and then the inward and outward flection of the membranous part of the saccular capsule is caused. Finally these changes lead to the vertical movement of the saccular otolith which is attached to the membranous part of the capsule. The upward movement of the otolith is resulted by the compression phase of the sound while the movement of the reverse direction is caused by the rarefaction of the sound. The tips of the sensory hairs of the saccular macula which is located in the saggital plane along with the body axis, touch to the lateral surface of the otolith (Fig. 1). Thus the vertical movement of the otolith stimulates the sensory cells of the macula. It is particularly interesting that the microphonic potential recorded from the dorsal half of the macula is different by 180° in the phase from that recorded from the ventral half of the macula. Whereas a combined microphonic potential of twice frequency is obtained when it is recorded from the intermediary portion of the macula (FURUKAWA and ISHII, 1967).

The fine structure of the saccular macula of a non-ostariophysi, a ray, has been described by LOWENSTEIN *et al.* (1964). They demonstrated that the hair cells are arranged in two separate rows which run along the longitudinal axis of the macula,

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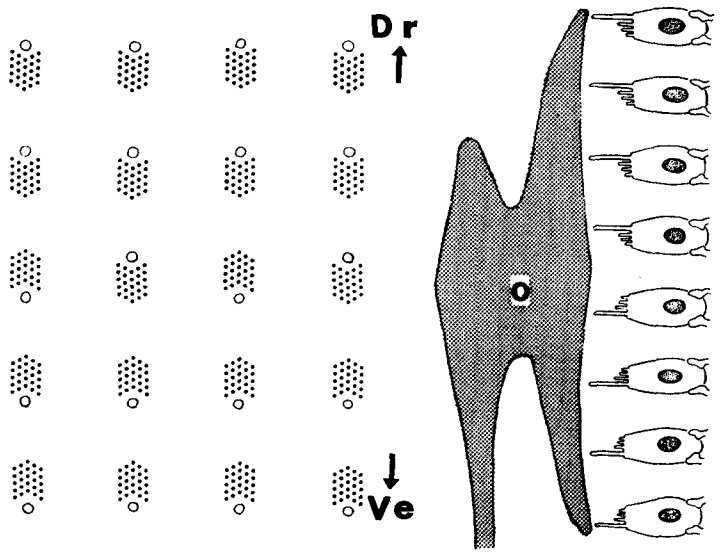

Fig. 1. The left half of the scheme shows the arrangement of sensory hairs in the saccular macula seen in the section tangential to the surface of the macula (saggital section) and the right half illustrates the relation between the otolith (*o*) and the sensory hair cells seen in the vertical section (frontal section). The arrows indicate the dorso (*Dr*)-ventral (*Ve*) direction. In the dorsal half of the macula, the kino-cilium is always located at the dorsal end of the each sensory hair group and in the ventral half of the macula, it points to the ventral direction. In the intermediary zone, hair cells with opposite polarities are mixed. An otolith is located over the sensory epithelium, and its lateral surface slightly touches to the tips of the sensory hairs

and that the hair cells in the dorsal and ventral rows are oriented in order that their kino-cilia point dorsally and ventrally respectively.

The fine structure of the saccular macula of the ostariophysi has never been described yet. As to nerve mechanisms, electrophysiological data clearly indicate that excitatory transmission from hair cells to afferent fibers in the present material is mediated chemically (FURUKAWA and ISHII, 1967). In addition, an inhibitory effect could be demonstrated after the excitation of the Mauthner cells in the mid-brain (FURUKAWA, 1966). Therefore, it is supposed that the both excitatory afferent and inhibitory efferent synapses are situated on the receptor cell.

The present paper deals specially with the fine structure and arrangement of the sensory hairs, the distribution of the sensory cells and the fine structure of the nerve terminals on the receptor cells in the saccular macula (macula neglecta) of the gold fish which is included in the ostariophysi.

Material and Method

The common Japanese gold fish was used as a specimen. The sacculus and lagena were dissected out from the anesthetized animal and the otoliths were removed before immersion into a fixative. Care was taken not to damage the sensory epithelium because the otolith is closely attached to the surface of the epithelium.

Two fixation procedures were employed: a) Fixed in 3% glutaraldehyde in 0.1 M phosphate buffer at pH 7.3 for two hours at room temperature and was rinsed in multiple

changes of the same buffer used in fixation with addition of 8% sucrose, then post-fixed in the cold 2% osmium tetroxide in the same buffer at pH 7.3 for one to two hours: b) Fixed in 2% osmium tetroxide in the same buffer at pH 7.3 with addition of 8% sucrose for two hours. The tissues were dehydrated through series of graded ethanol and embedded in Epon (LUFT, 1961). For the general observation, the frontal sections of the sacculus were prepared from rostal end of the macula to the caudal end. For the examination of the distribution of the receptor cells and the arrangement of the sensory hairs, the blocks were oriented so that the sections were always cut tangentially to the surface of the macula and from rostal to the caudal direction.

The specimens were double-stained after sectioning by 2% uranyl acetate and lead hydroxide or post-stained with lead hydroxide on the section after block staining by 2% uranyl acetate before dehydration, and studied with the Hitachi HU-11a electron microscope at 75 kv.

Results

The sensory epithelium of the saccular macula of the gold fish consists of two main types of cells, the sensory cells and the sustentacular or supporting cells. The sensory cell is a slender cylindrical cell with rounded base which never reaches to the bottom of the epithelium. As seen in the cross or oblique section of the epithelium, each sensory cell is surrounded by a layer of supporting cell cytoplasm (Fig. 2). A terminal bar is found surrounding the epithelial cells near the luminal surface. Many desmosomes are found on the contact surface between the adjacent cells (Fig. 3). The apposing plasma membranes of the basal part of the adjacent supporting cells apply very closely to each other for considerable extent from the basal surface to the deep into the epithelium (Fig. 4). At higher magnification, the outer leaflets of the apposing plasma membranes are seen to be separated by a narrow space of 10—15 Å in width (Fig. 5). Thus the intercellular space in the sensory epithelium is narrowed both at the luminal end and at the basal end.

The apical cytoplasm of the sensory cell is characterized by the existence of the dense material called cuticle in which the bases of the sensory hairs are embedded. In the tangential section through the apical part of the sensory cell, the cuticle is seen as an oval profile with one end lacking (Fig. 6). Many electron less dense holes are found in the cuticle arranged in rows. They are supposed to correspond to the bases of the stereo cilia.

Bundles of filaments are found randomly distributed throughout the cytoplasm of the receptor cell but predominatingly in the apical one (Figs. 3, 6). The cross section of the structure is seen as a tetragonal or hexagonal network of dense material instead of an aggregation of electron dense dots as in the case of the cross section of the bundle of filaments (Fig. 3, Insert). Consequently the whole structure is considered to either an aggregation of filaments with three edges protruded from the side with regular angle which are connected to the edges of the neighbouring three filaments, or an aggregation of tubules with tetragonal and or hexagonal profiles. The bundle of filaments or tubules mentioned above runs straight course and does not curve or bend for a considerable distance, which gives it a rigid appearance (Figs. 2, 3, and 6). In the apical cytoplasm, the bundles are accumulated around the cuticle beneath the cell surface at the level of the desmosomes and frequently one end of the bundle is seen continuing of the dense material associated with the desmosome (Fig. 6). The same kind of bundles are more abundantly found in the apical part of the supporting cell (Fig. 6) and so are

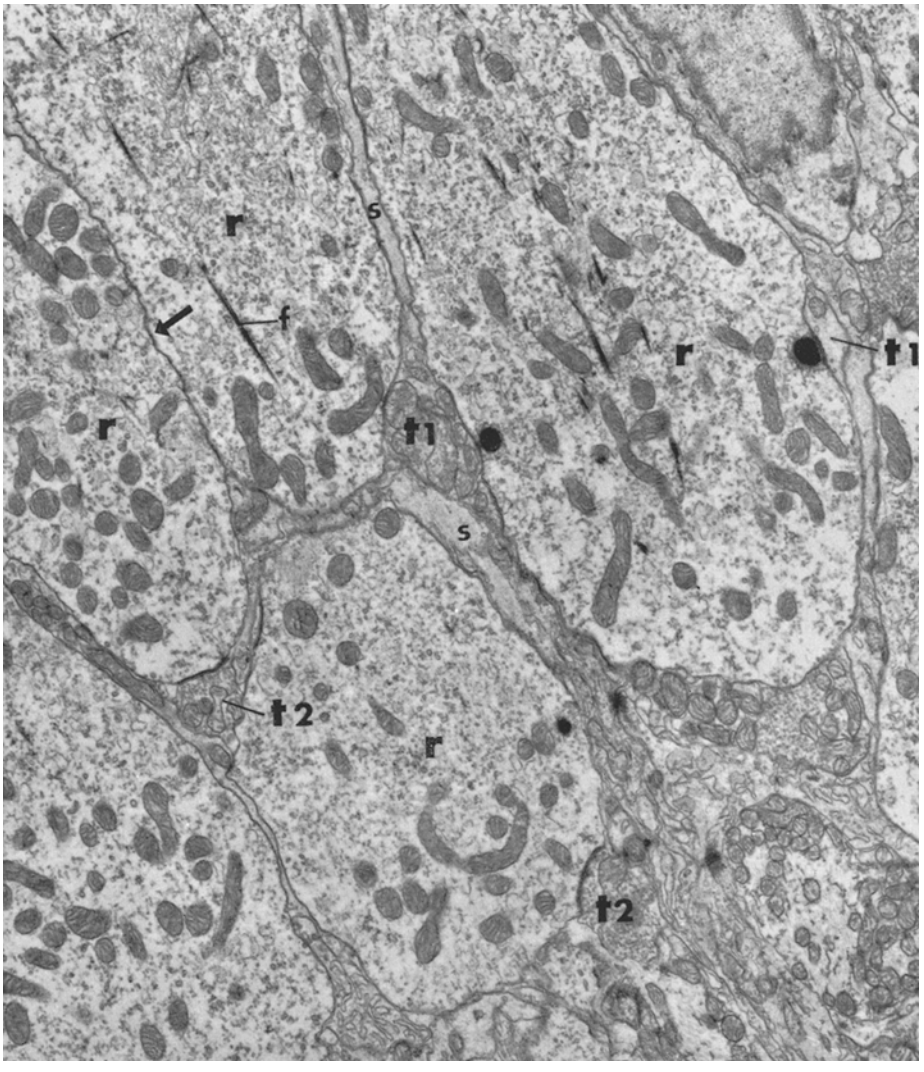

Fig. 2. A low power electron micrograph showing the basal part of the receptor cells in the saccular macula of the gold fish. Many small vesicles and dense filaments (*f*) are found in the receptor cell (*r*). Although the most of the receptor cells are separated to each other by a layer of supporting cell cytoplasm (*s*), sometimes the adjacent receptor cells are in close contact with a narrow intercellular space (arrow). Two types of nerve terminals (*t1* and *t2*) are found on the basal surface of the receptor cells. The *t1* is characterized by a associated dense body in the receptor cell and the *t2* is characterized by the presence of vesicles in the terminal. × 6,000

considered to be epithelial filaments modified. The basal cytoplasm of the receptor cell is occupied by vesicles of about 60—70 mμ in diameter. Sometimes a dense network of irregular tubules is seen to take the place of the vesicles mentioned above near the basal plasma membrane of the receptor cell (Figs. 7, 8). Many

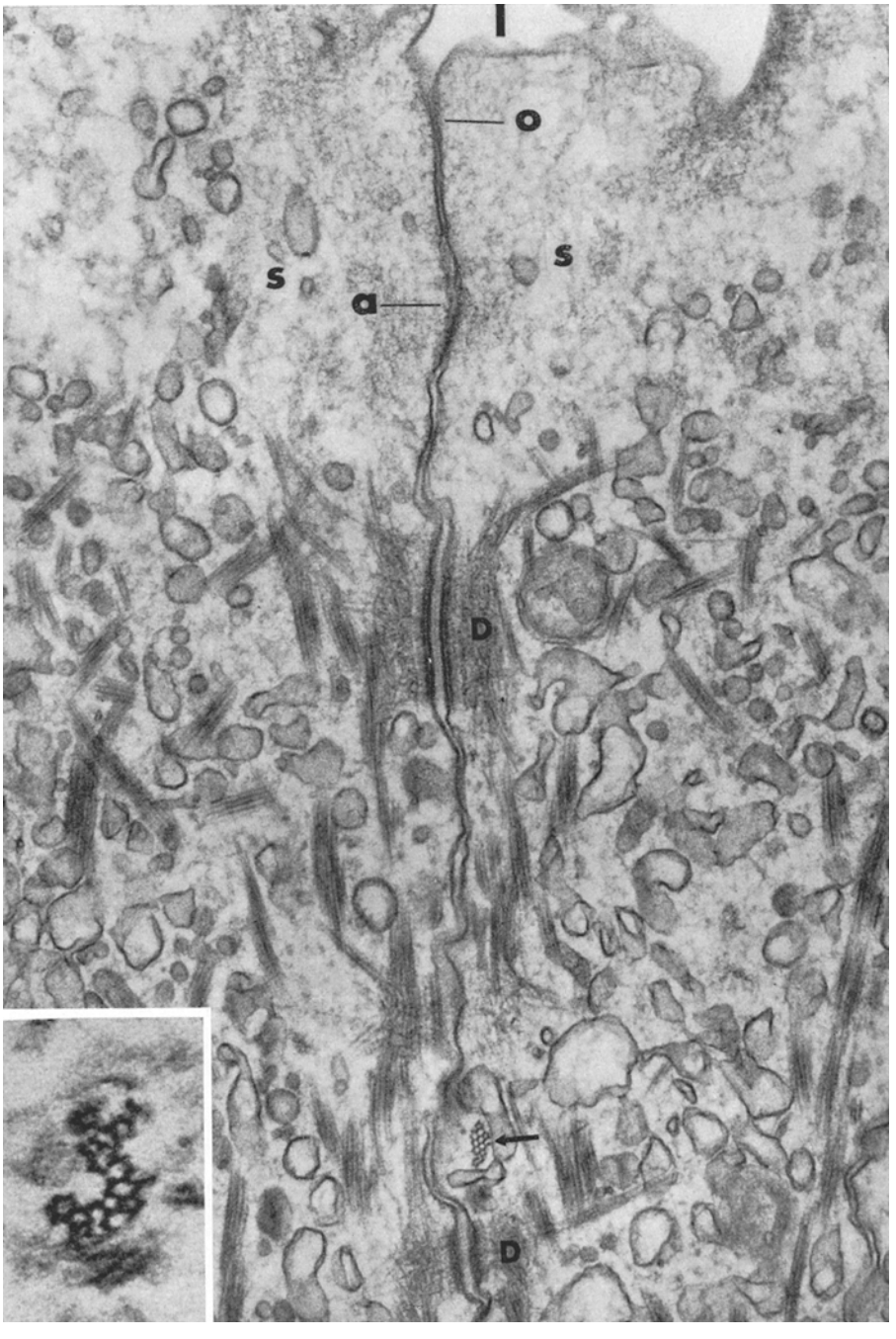

Fig. 3. A juxta-luminal junctional complex consisting of zonula occludens (*o*), zonula adherens (*a*) and macula adherens or desmosomes (*D*) is found on the contact surfaces between the adjacent supporting cells (*s*). The apical cytoplasm contains tubular or vesicular profiles of a granular reticulum and bundles of tubules or filaments. The tubules or filaments appear as hexagonal or tetragonal network as seen in the cross section (arrow and Insert). Some of them are seen to continue to the dense material associated with the desmosomes.
l: saccular lumen × 52,000, Insert × 132,000

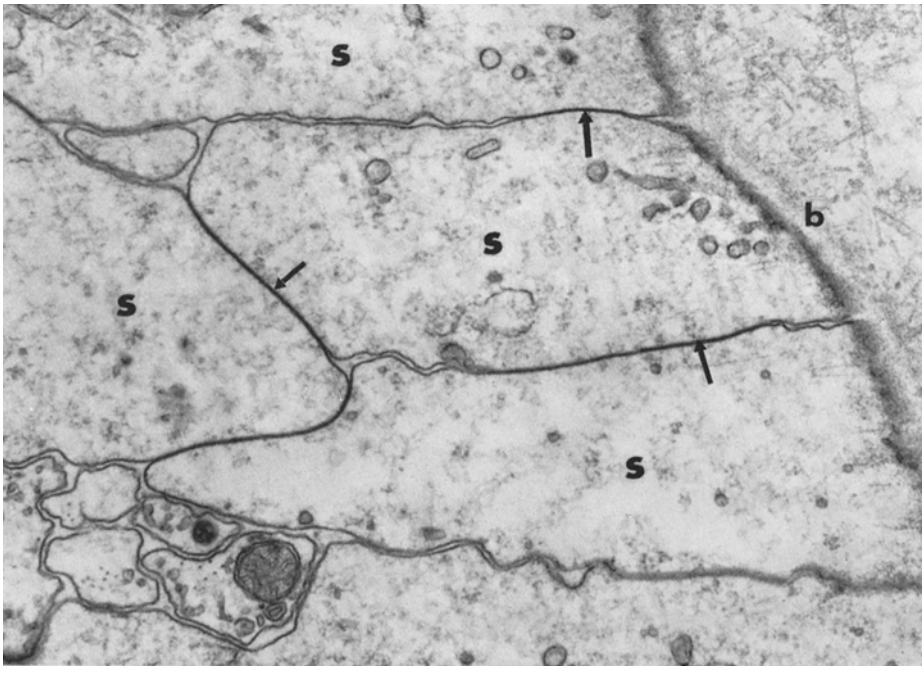

Fig. 4. An electron micrograph showing the basal part of the saccular sensory epithelium. The apposing plasma membranes of adjacent supporting cells (*s*) apply very closely to each other (arrows). *b* basement lamina. × 36,000

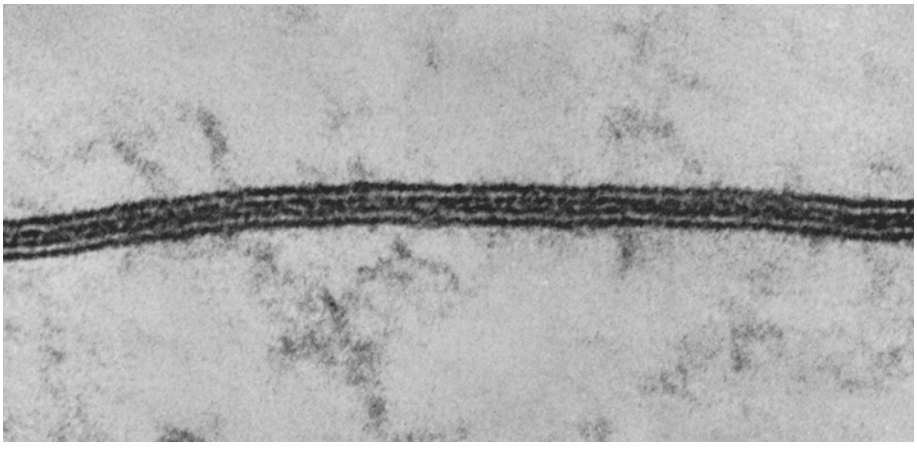

Fig. 5. A high power electron micrograph showing the detail of the close apposition of plasma membranes of neighboring supporting cells. The outer leaflet of the apposing unit membranes are separated by a space of about 10—15 Å. × 364,000

Fig. 6. A tangential section through the apical part of the sensory epithelium showing a oval profile of the receptor cell (*r*). In the receptor cell, a slightly electron dense mass, cuticle (*c*), which is roughly oval with one end lacking is found. In the cuticle, electron less dense round holes are seen. They are arranged in rows and are supposed to correspond to the bases of the

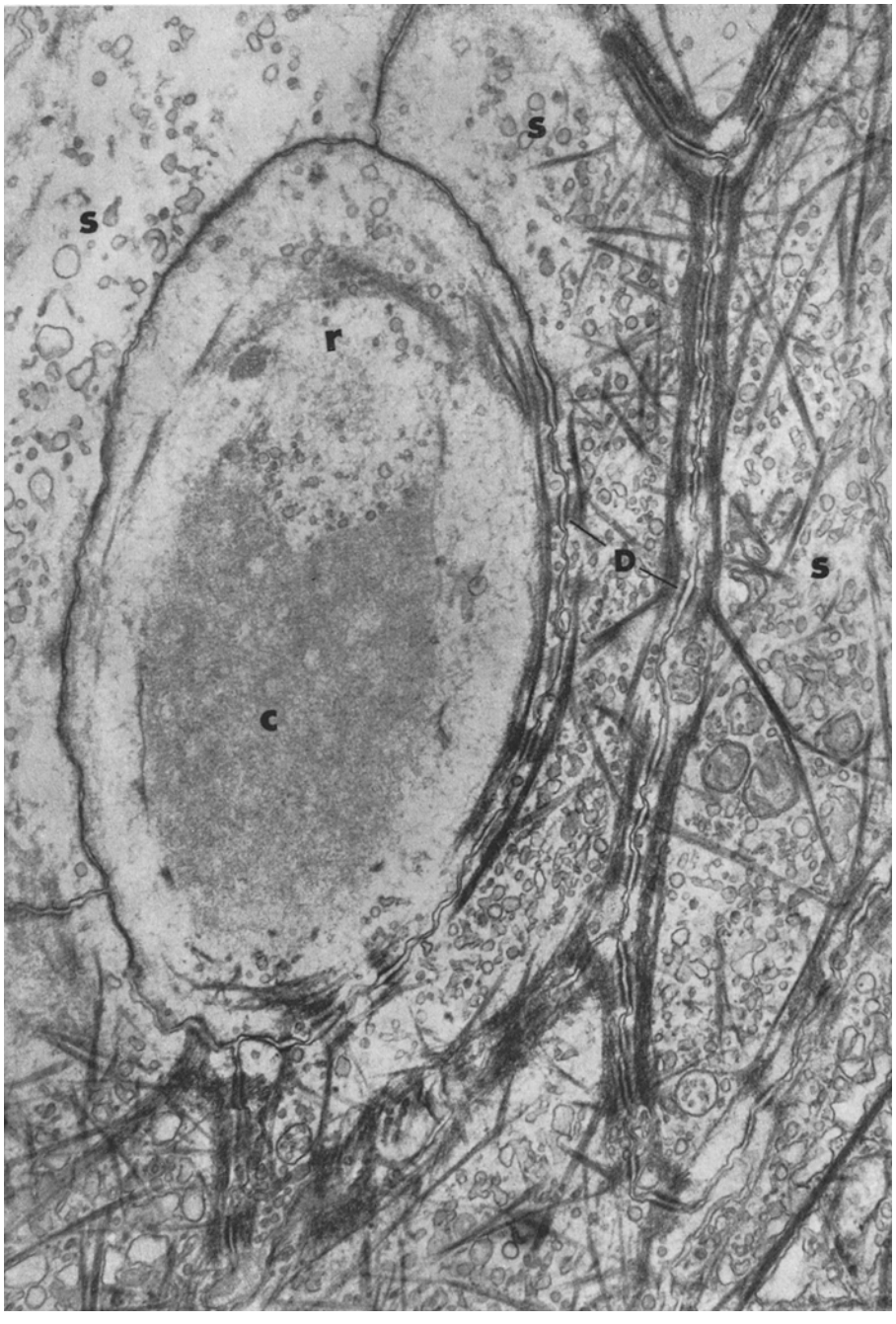

Fig. 6

stereo-cilia. On the contact surfaces between the receptor cell and the supporting cell (*s*) and between the adjacent supporting cells many desmosomes (*D*) are seen. At the area of contact a layer of dense materials is found in each cell beneath the desmosomes. Bundles of filaments are found both in the receptor cell and in the supporting cell but predominantly in the latter. In many places they continue to the dense material beneath the desmosomes. × 20,500

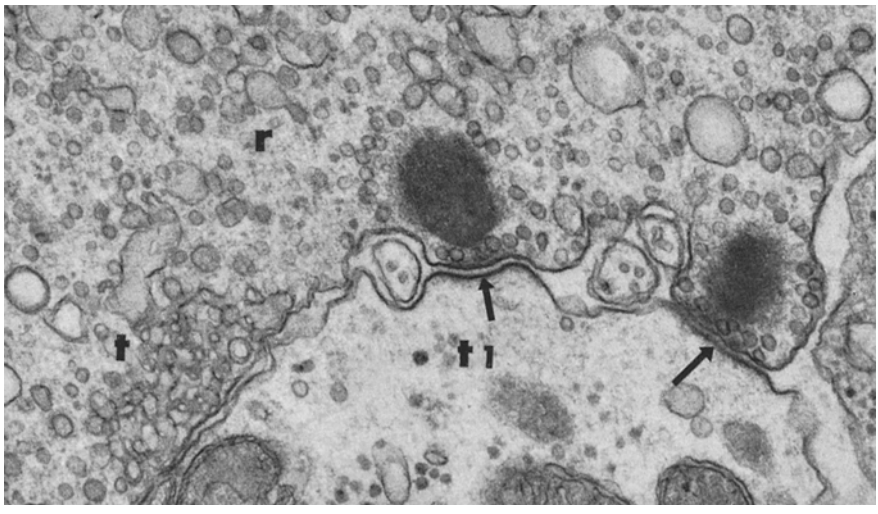

Fig. 7. An electron micrograph showing a type 1 terminal (*t1*) on the basal surface of the receptor cell (*r*). Mitochondria and glycogen granules are found in the nerve terminal but few vesicles. The electron dense bodies which are surrounded by vesicles are found in the receptor cell. They associate with the specialized areas of plasma membranes (arrow) where increase of electron density and accumulation of dense materials against them are found. A complicated tubular structure (*t*) of the agranular reticulum is found immediately beneath the plasma membrane of the receptor cell. × 46,000

coated vesicles are seen at the periphery of the network. Some of them are seen to be connected with the tubules (arrow in the Fig. 8). Sometimes even rather large vacuoles or cysterns are found being associated by an outer coating on the cytoplasmic surfaces. The relation between the tubular network and the synaptic vesicles is not clear.

Sensory hairs: The luminar surface of the receptor cell is studded with the sensory hairs consisting of one kino-cilium and 50—60 stereo-cilia. As seen in the section tangential to the surface of the epithelium, the stereo-cilia are arranged very regularly as in the case reported in the lateral line organs (HAMA, 1965; FLOCK and WERSÄLL, 1962), and the kino-cilium is always located at one pole of the group of the stereo-cilia (Fig. 9). Each axis of the group of the sensory hairs which passes through the position of the kino-cilium is parallel to each other and is directed vertically. The kino-cilium of the sensory cells in the dorsal half of the macula is always located at the upper pole of the group of the sensory hairs and on the other hand, that of the sensory cells in the ventral half of the macula always at the opposite pole. At the intermediate zone of the macula, the sensory cells with opposite polarity are intermingled side-by-side (Fig. 10). The arrangement of the sensory cells above mentioned agrees with that described by LOWENSTEIN *et al.* in the labyrinth of the ray (1964). The fine structure of the kino-cilium is similar to that of the common cilia or flagella. A basal body of the kino-cilium is found situated in the shallow depression of the cuticle.

Nerve terminals: Two types of nerve terminals are found on the basal surface of the receptor cell (Fig. 2). The one contains no vesicle (Figs. 2, 7, 11, 12, 13)

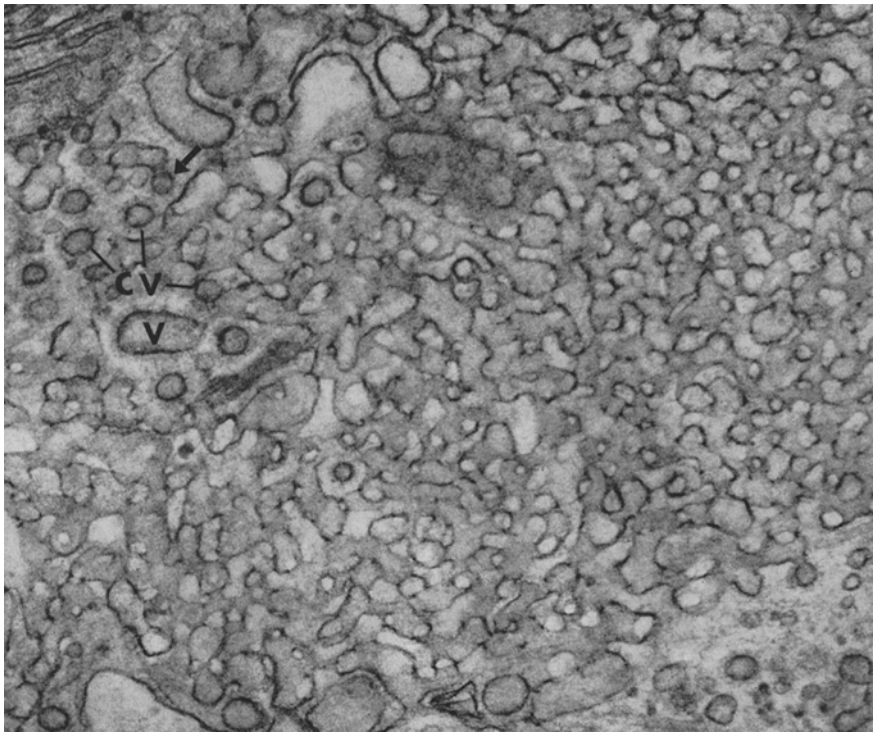

Fig. 8. Agranular reticulum found in the basal part of the receptor cell. Many coated vesicles (*cv*) are found at the periphery of the reticulum. Some of them are seen to continue to the latter (arrow). A vacuole with larger diameter (*v*) also has outer coating. × 67,000

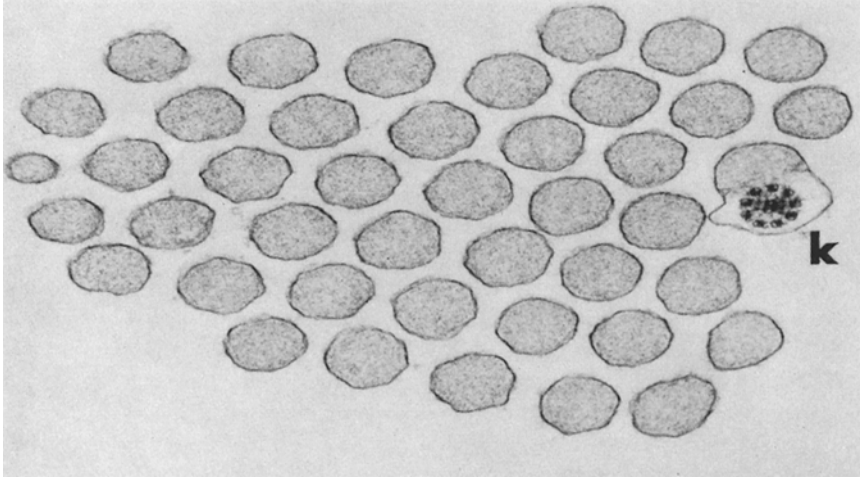

Fig. 9. A cross section of the sensory hairs. A kino-cilium (*k*) is always located at one end of the group of the stereocilia which are regularly arranged. × 42,000

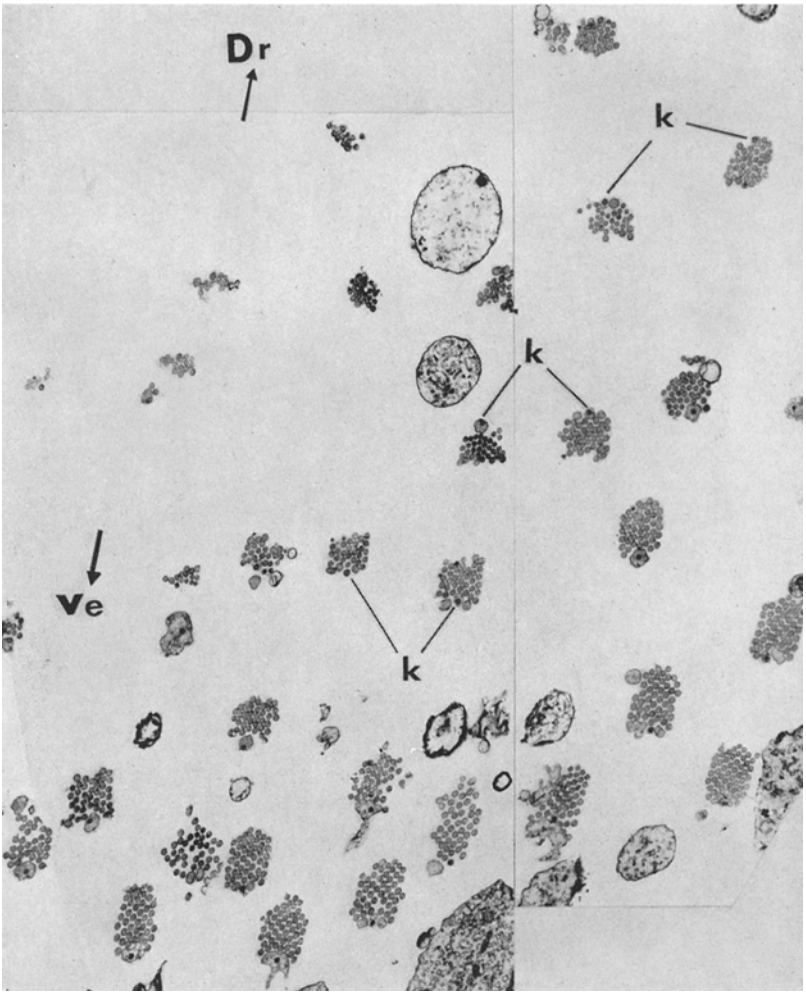

Fig. 10. This low power electron micrograph illustrates the arrangement of the sensory hair groups seen in the section parallel to the surface of the saccular macula. In the ventral half of the macula, the kino-cilium (*k*) is always located at the ventral end of the group of the stereocilia. On the other hand, in the dorsal half of the macula, it always points to the dorsal end of the sensory hair group. Arrows show dorso (*Dr*) ↔ ventral (*Ve*) directions. × 3,800

and the other contains a cluster of vesicles of 60 to 70 mμ in diameter and a few vesicles with an electron dense core (Fig. 14). The diameter of the cored vesicle is about 100 mμ. Hereafter, the former is referred as type I and the latter as type II.

The type I terminal: the apposing plasma membranes of the nerve terminal and the receptor cell are shown to have such specializations as increasing electron density and accumulating dense material in the cytoplasm backing the plasma membranes at the synaptic area. The accumulation of dense material is more evident in the nerve terminal side than the receptor cell side. The gap between the apposed plasma membranes is constant, about 30 mμ in width and is occupied

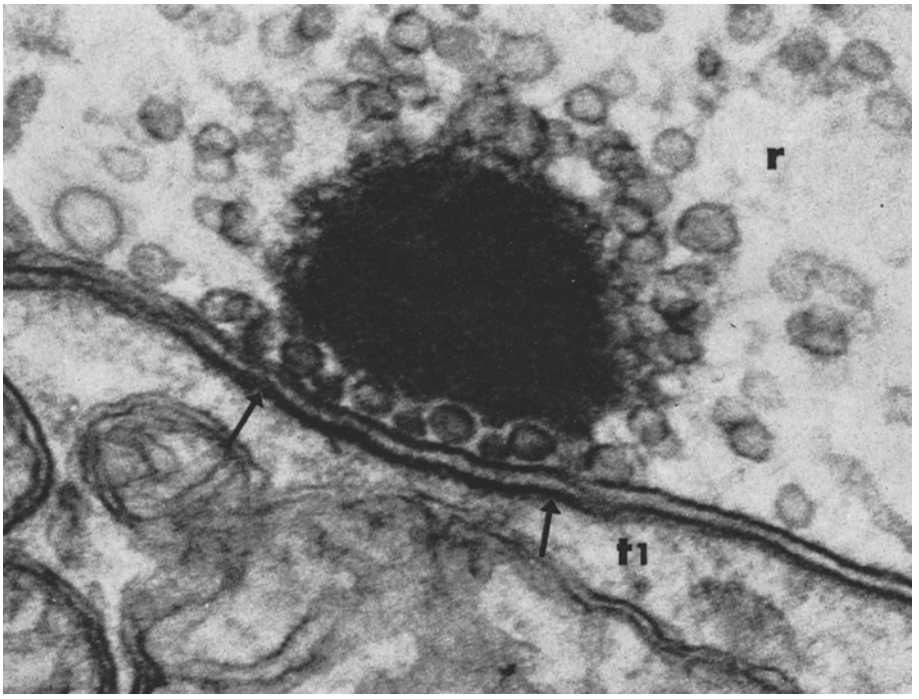

Fig. 11. An electron dense body which is surrounded by small vesicles is seen in the receptor cell (*r*) associated with the specialized area of synaptic membranes (between two arrows). Electron dense conical bodies of 40 mμ in diameter (presynaptic projections) are seen situated on the presynaptic membrane. No synaptic vesicles are found in the nerve terminal (*t1*). × 135,000

by slightly electron dense material. Sometimes, bridges of filamentous material crossing the gap at regular intervals are observed. In the receptor cell, a round profile of electron dense material, about 0.3 μ in diameter is found to be located near the synaptic membrane, being surrounded by a layer of vesicles (Fig. 11). The dense body is seemed to be analogous to "synaptic bar or rod" or "synaptic body" found in the vestibular organs of some vertebrates (WERSÄLL *et al.*, 1965) and in the lateral line organs of some fishes (FLOCK, 1963, 1965; HAMA, 1966). In the specimen which is double stained with uranyl acetate in alcohol and lead hydroxide, the conical dense bodies of about 40 mμ in the basal diameter are found situated on the synaptic membrane of the receptor cell with their base directed to the membrane. The center to center distance of these bodies is about 100 mμ. Protrusions of the dense material from the "synaptic body" mentioned before are in register with the tip of the conical dense material on the synaptic membrane, making compartments between the synaptic membrane and the "synaptic body". Each compartment contains a synaptic vesicle (Fig. 11). Sometimes two synaptic bodies are found in a synaptic region (Fig. 7). In one occasion, an oval profile surrounded by double membranes about 0.5 μ in its larger diameter was found situated in the basal cytoplasm of the receptor cell. The oval profile

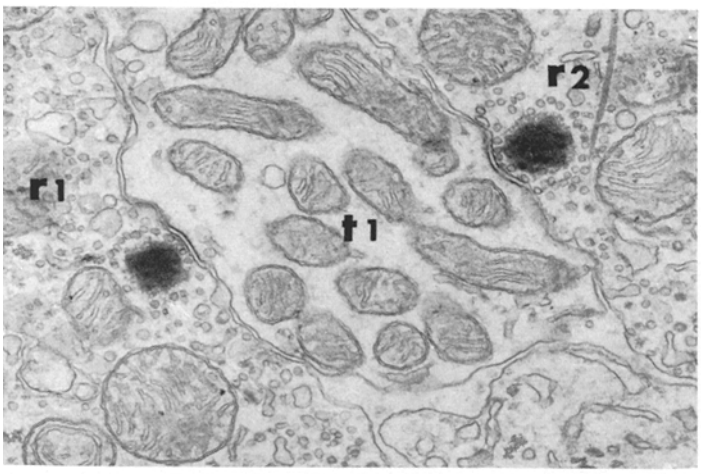

Fig. 12

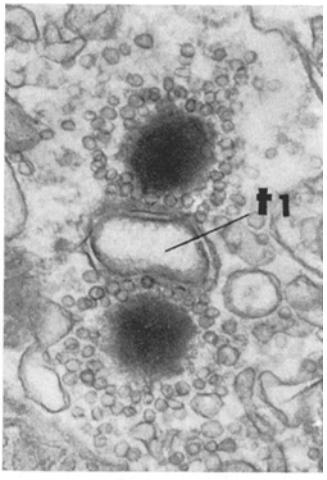

Fig. 13

Fig. 12. A nerve terminal (*t1*) is seen making synaptic contacts with two receptor cells (*r1* and *r2*). An electron dense body is seen in each receptor cell associated with specialized membrane areas. × 23,000

Fig. 13. Two electron dense bodies which are surrounded by vesicles are found on both sides of the oval profile of nerve terminal (*t1*). × 35,000

is found to have one synaptic body on its opposite sides (Fig. 12). The profile is probably the cross section of the process from the nerve terminal which invaginates into the receptor cell like the dendritic spine in the central nervous system. It has been frequently found that a nerve fiber contacts with neighbouring receptor cells to make type I synapses (Fig. 12). The type II terminal (Fig. 14): Along with the type I terminals, the type II terminals which contain a cluster of vesicles are frequently encountered on the basal surface of the receptor cell. The apposing plasma membranes of both the nerve terminal and the receptor cell are not seen to have such a specialization as other synapses have, but they merely apply to each other closely at the area of contact with extremely constant gap of about 20 mμ and run rather straight course and do not show irregularity such as jaggedness of the surface, which characterizes the junctional membranes. The gap substance does not show the specialization as to be found in other synapses. The nerve terminal is filled with vesicles of 60—70 mμ in diameter. Some of them are seen to open to the inter-cellular gap at the synaptic area (Fig. 14 arrow). Beside the vesicles mentioned above, a few vesicles with electron dense core are found somewhat separated from the contact surface. The diameter of the cored vesicles is about 100 mμ. Mitochondria and neurotubules are also provided in the nerve terminal. Immediately beneath the plasma membrane of the receptor cell at the area of contact, a flattened cistern is always located which is separated from the synaptic membrane by a constant narrow space of about 8 mμ in width. The both ends of the flattened cistern are commonly dilated and frequently connected with the tubular element of the agranular reticulum. A nerve fiber frequently forms type II terminals on the two receptor cells at the same time.

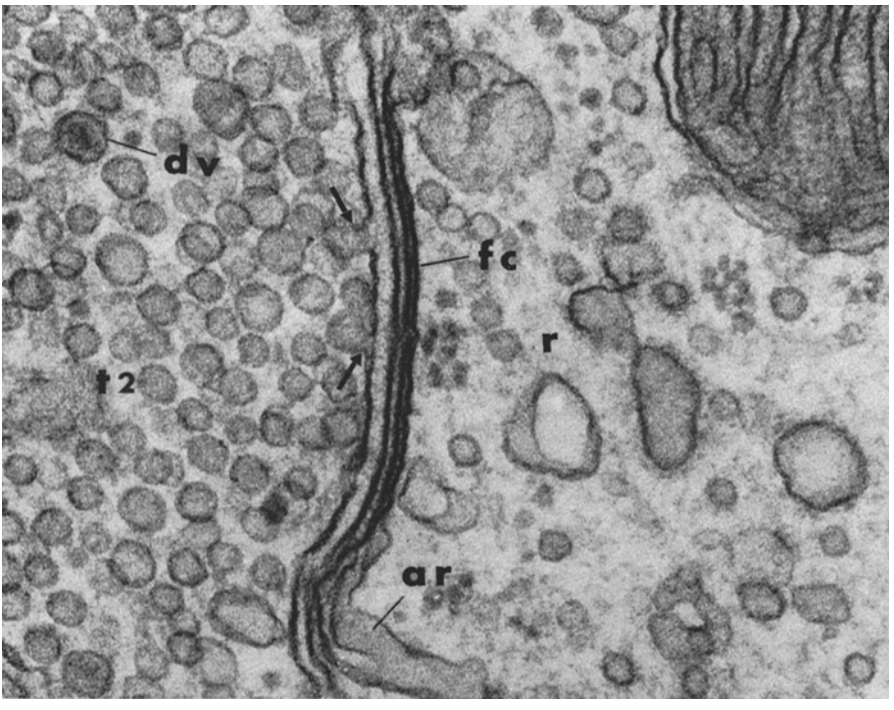

Fig. 14. A high power electron micrograph showing type 2 nerve terminal (*t2*) on the receptor cell (*r*) base. The nerve terminal is occupied by many vesicles of 45—100 mμ in diameter. Cored vesicles (*dv*) of 100 mμ in diameter are also found in the terminal. Some of the vesicular structures are seen to be continued to the surface plasma membrane (arrows). The space between the plasma membranes of the nerve terminal and receptor cell is constant, about 30 mμ in width. A flattened cystern (*fc*) is seen in the receptor cell situated beneath the synaptic membrane separated from it by a gap of about 8 mμ. The lateral ends of the flattened cystern continue to the agranular reticulum (*ar*) in the cytoplasm. × 110,000

Both type I and type II terminals are found situated side-by-side on the same receptor cell. However it has never been found that one nerve fiber forms type I and II terminals on the same or different receptor cells.

Nerve fibers: The saccular nerve has a ganglion just beneath the saccular macula. Most of the nerve cells in the ganglion have a myelin sheath around the perikaryon. The nerve fiber enters into the sensory epithelium with myelin sheath, loses it below the level of the receptor cell base, and makes synaptic contact with the receptor cells after running for some distance in the plane parallel to the surface of the epithelium.

Discussion

It has been clearly demonstrated in the gold fish sacculus that the microphonic potential (m. potential) responds in the dorsal half of the macula to the compression phase of the sound and in the ventral half of the macula to the rarefaction of the sound (Furukawa and Ishii, 1967). In other words, in the dorsal half of the macula, the m. potential responds to the upward movements of the otolith and is inhibited by its downward movements. To the contrary, in the ventral

half of the macula, it responds to the downward movements and is inhibited by the upward movements. When the m. potential is recorded from the middle portion of the macula, one sees that it is evoked at twice the frequency of the sound.

Regarding the fine structure of the saccular macula (Fig. 1) and the attitude of the m. potentials, it is clear that in the present material, the m. potential is evoked by the ciliary bending pointed to the kino-cilium and is inhibited by the reverse movement, which is in complete agreement with the previous descriptions on the vestibular organs (WERSÄLL, 1956, 1960; WERSÄLL *et al.*, 1965) and on the lateral line organs (FLOCK and WERSÄLL, 1962; FLOCK, 1965a, b). In their descriptions, it has been noticed that in the vestibular organs, the m. potential is evoked by the movement of the surrounding medium directed to the kino-cilium, and it is considered that in the lateral line organ, the twice frequency of the m. potential is resulted by the superimposition of two m. potentials with phase difference by 180° as the result of the coexistence of the sensory cells with opposite polarities.

The sensory hairs are anchored to the cuticle by a basal body and rootlets, and the firm intercellular attachment, which is formed by the terminal bar, together with the cuticle and surrounding epithelial filaments creates the rigidness of the surface of the sensory epithelium which may have a function transducing the mechanical impact on the sensory hairs to the electrical events.

According to the fine structure of the type I terminal — aggregation of the synaptic vesicles is found in the receptor cell and the accumulation of dense material to synaptic membrane is evident on the nerve terminal side — the excitation is supposed to traverse the synapse from the receptor cell to the nerve terminal, that is afferent in nature. The conical dense masses arranged on the synaptic membrane of the receptor cell resemble to those described by GRAY (1963) as dense projections at the synapse in the central nervous system. They may have a function of attaching or attracting the synaptic vesicles to the pre-synaptic membrane.

The electron dense body which is surrounded by a layer of vesicles has been found in the acustico-lateralis system and it, together with the analogous structure such as the pre-synaptic ribbon of the ampullar organ (SZABO, 1962) and of the retina (SJÖSTRAND, 1958), is seemed to be characteristic of the receptor afferent synapse, though the function of these structures is not clarified yet.

In the type II terminal, on the other hand, a cluster of vesicles is found accumulated in the nerve terminal, consequently the transmission across this type of synapse is considered to direct from the nerve to the receptor cell, that is efferent in nature. The same type of terminal was found on the base of the outer hair cell of the guinea pig cochlea and was proved to be efferent and inhibitory in nature (SMITH and SJÖSTRAND, 1961a, b; SMITH and RASMUSSEN, 1965; IURATO, 1962; KIMURA and WERSÄLL, 1962). The existence of the poly-synaptic inhibitory passway has been found in the gold fish auditory system (FURUKAWA and ISHII, 1967). Moreover, the same type terminal was found in the lateral line organ of the Japanese sea eel (HAMA, 1966) where the inhibitory innervation has recently been detected (KATSUKI *et al.* 1968) whereas the same type of terminal is very rare in the lateral line organ of the common eel (YAMADA and HAMA, unpublished data) where the efferent innervation was supposed to be absent

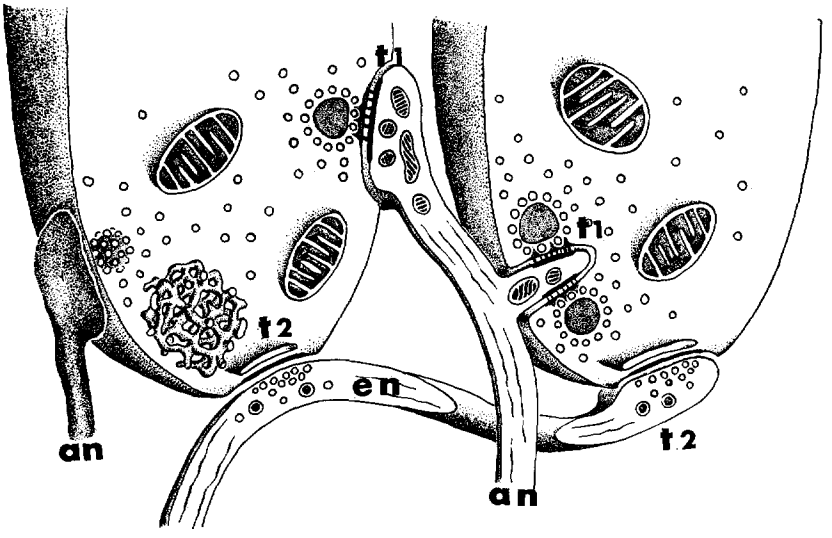

Fig. 15. This scheme shows the relation between the nerves with afferent and efferent functions and the types of nerve terminals. The afferent nerve (*an*) always forms type 1 terminals (*t1*) on the receptor cells. On the other hand, the efferent nerve (*en*) always forms type 2 terminals (*t2*) on the receptor cells. One nerve is frequently found to form terminals on the adjacent receptor cells

(KATSUKI *et al.*, 1951a, b). All these findings support the view that the type II terminal in the present material is efferent and inhibitory in nature. The flattened cistern associated with the post-synaptic membrane is always found in this type of synapse not only in the present material but also in other organs mentioned above and has been refered to as a subsynaptic sac. It is supposed that this structure may play a role in the inhibitory mechanism.

The cored vesicle was first detected in this type of synapse in the present study. The same type of vesicles have been found in the autonomic nerve terminals of various organs and it has been postulated that they might contain catecholamine. In the present case, they are few in number and are not found to be associated with the synaptic membrane, but they are always found to be located somewhat away from the synaptic membrane, so it is unlikely that they contain the transmitter substance. It is more conceivable that they might have a function of triggering the release of the transmitter substance which is contained in the synaptic vesicles responding to the signals from the center.

The trigger substance, if any, and the transmitter substance at the type II terminal in the present material have been not known, however, the existence of the cored vesicles only in the present material suggests that there should be any difference between the operation mechanisms of the type II synapse in the present material and of that in other organs mentioned before.

If one considers the fact that in the saccular macula the neighbouring sensory cells have the same polarity, it is interesting to note that one nerve fiber is easily found to make synaptic contact with more than one receptor cell, which has never been found in one section in the lateral line organs where the neighbouring

sensory cells have opposite polarities. It has not been possible because of the technical limitation in the present study to clarify whether one nerve fiber makes synaptic contact with two cells with definitely opposite polarities at the intermediary zone, while the presence of such a connection was suggested in an electrophysiological study (FURUKAWA and ISHII, 1967). The relation between the nerve fibers and the types of terminals is illustrated in the Fig. 15.

It is well understandable that the macular epithelium has a terminal bar near the luminal surface if one notices the fact that the K^+ content of the saccular fluide is much higher than that of the rest of the body fluide. However, the functional significance of the close apposition of the plasma membranes, which cover the adjacent supporting cells, existing to considerable extent near the bottom of the epithelium is not clear.

The same sorts of close apposition of plasma membranes with narrow space, 1.5—2 mμ in width, has been reported by several authors as to be characteristic for the electrotonical synapse (COGGESHALL, 1965; HAMA, 1965; REVEL and KARNOVSKY, 1967; ROSENBLUTH, 1965), and it is supposed that the close apposition of supporting cell membrane play a role in the ion mechanisms of the sensory epithelium.

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