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Fine structure of the tuberous electroreceptor of the high-frequency electric fish, *Sternarchus albifrons* (gymnotiformes)

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Summary

Sternarchus emits low voltage biphasic pulses at about 700–900/s. These signals (and changes in them caused by external objects) are detected by the tuberous or phasic electroreceptors. We used electron microscopy to examine extracellular compartments in the current pathway to the receptor cells, which are delineated by cells joined by tight junctions. Highly specialized accessory cells were found to separate the receptor cells from the extracellular space continuous with the exterior. Except for synaptic specializations complements of intramembrane particles of cell membranes were unremarkable and did not correlate with presumed high and low resistivity. We propose an equivalent electrical circuit that is consistent with the morphological and physiological observations.

Introduction

Previous studies of electric fishes demonstrated that the electroreceptors responding to high frequency stimuli are tuberous organs whose basic structure is similar although there exists considerable variability in detail (Bennett, 1971b; Szabo & Fessard, 1974; Bullock & Heiligenberg, 1986).

The present study concerns tuberous receptors of a gymnotiform *Sternarchus albifrons* (Ellis, 1913, also known as *Apteronotus albifrons*). This species discharges at a high frequency, about 700–900/s (Bennett, 1971a). The receptor properties have been extensively studied both physiologically (Hagiwara *et al.*, 1965; Enger & Szabo, 1965; Scheich & Bullock, 1974; Bullock *et al.*, 1975; Hopkins, 1976; Viancour, 1979) and morphologically (Szabo, 1965; Lissmann & Mullinger, 1968; Srivastava, 1972, 1973; Srivastava & Szabo, 1974). The aim of this communication is to clarify the extracellular fluid compartments in the current pathway to the sensory epithelium and to characterize by freeze-etch the cell membranes and specialized cell contacts that affect the electrical properties. In addition, we describe the highly specialized covering cells, which are interposed between the receptor cells and plug cells.

Our findings allow formulation of an equivalent electric circuit that is consistent with both morphological and physiological observations.

Methods

Twelve *Sternarchus albifrons* were used. Fixation was by transcardial perfusion through the conus arteriosus under tricaine methane sulphate (Sandoz, Basel) anaesthesia (1:1000 w/v in the aquarium water). After brief perfusion with heparinized *Electrophorus* Ringer containing 167 mM NaCl, 3 mM $CaCl_2$, 1.5 mM $MgCl_2$ and 4 mM Tris at pH 7.4 (300 mOsm, Highstein & Bennett , 1975) to remove blood, the perfusate was changed to a mixture of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4, 560 mOsm). Small pieces (about 1 mm^2) of skin containing receptors were removed from the head and postfixed by immersion in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for several hours at 4° C. Small blocks of tissue were used for freeze-fracture procedures. After being kept overnight in 0.2 M phosphate buffer supplemented with 6.8% sucrose (pH 7.4, 520 mOsm), the specimens were immersed in 25% glycerol in Ringer solution at room temperature for 30 min,

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placed on gold specimen holders, rapidly frozen in liquid freon (−150° C) and kept in liquid nitrogen (−196° C). The frozen material was fractured at −110° C and etched for 5 min in a Balzers BAF 310 modified high vacuum freeze-etch unit, according to Moor and Muehlethaler (1963). Replicas were made by the evaporation of platinum and carbon, cleaned with methanol, hypochlorite bleach and 35% chromic acid, and then mounted on Formvar coated grids. Specimens for thin sectioning were post-fixed with 2% buffered OsO_4, dehydrated and embedded in Epon 812. Semi-thin sections were stained with Azur II-methylene blue solution or viewed by phase contrast. Ultrathin sections were stained with uranyl acetate and lead hydroxide.

Results

A diagram of the skin and a tuberous electroreceptor in a section perpendicular to the skin surface is shown in Fig. 1. The epidermis consists of four layers: (1) an innermost layer of large germinal and transition cells; (2) a layer of flattened cells, presumably responsible for the high electrical resistance; (3) an intermediate layer of homogeneous epidermal cells; (4) an outer heterogeneous layer including mucous secreting cells. The receptor consists of an approximately 0.15–0.4 mm deep tubular invagination of the epidermis. The layer of flattened cells (that are dense in phase contrast and darkly staining with Azur II and methylene blue) forms the wall of the invagination which runs down to the tuberous enlargement containing the actual receptor epithelium comprised of the receptor cells and supporting cells. The inner, germinal layer extends inward along the wall of the invagination and thins to a single sheet of large cells which ends at the start of the tuberous enlargement. A basal lamina or basement membrane underlies this cell layer in the epidermis and extends down around the entire invagination. The homogeneous layer extends down into the invagination where its cells become progressively more differentiated into a cellular plug. The extracellular spaces between the plug cells increase in width deeper in the invagination.

Eventually, these spaces merge into an extensive space we term the plug cell cavity. The floor of this cavity is formed by a single layer of large cells, the covering cells (Srivastava & Szabo, 1974). The covering cells form a cap that extends over the receptor cells and inward to contact supporting cells and cells of the wall near the bases of the receptor cells. The relatively small space between covering and receptor cell layers we term the receptor cavity. The receptor cells are attached at their bases to a thin unicellular layer of supporting cells, possibly one supporting cell per receptor cell. A basal lamina underlies the supporting cells. Myelinated branches of the single axon that innervates the entire organ pass through the supporting cell layer to contact each receptor cell. The branches of this nerve fibre comprise a distinct layer deep to the supporting cells and the basal lamina. A sheath of perineural endothelial cells covers the nerve fibre layer.

Blood vessels remain outside the wall of the invagination and form a plexus in the adjacent dermal tissue. At the base of the electroreceptor capillaries are found in the nerve fibre layer, where they provide a relatively direct blood supply to the receptor epithelium.

FINE STRUCTURE AND JUNCTIONAL RELATIONS

Outer epidermis

Extensive examination of thin sections and of freeze fracture replicas failed to reveal tight junctions in this region, and we conclude that the intercellular clefts are open to the exterior.

Plug cells

The plug cells form the core of the invagination (Fig. 1). The cells are vertically oriented and separated from each other by extracellular spaces that are progressively more enlarged deeper in the receptor (Figs 2, 3). These cells are linked by numerous spiny processes which form desmosomes. Peripherally there are extensive gap junctions between plug cells. There are occasional tight junctions.

Wall cells

The wall of the invagination consists primarily of densely packed flattened cells which are characterized in thin section by filament packed cytoplasm and numerous desmosomes (Fig. 3a). Freeze fracture reveals that the spaces between the desmosomes contain numerous tight junctional strands that are highly convoluted and infrequently branching (Fig. 3b). The desmosomes appear in P and E faces as concentrations of variably sized particles. Gap junctions are absent. The flattened cells of the epidermis also have extensive tight junctions, numerous desmosomes and no gap junctions. The presence of tight junctions and absence of gap junctions are consistent with the high electrical resistance of this layer. The wall cells extend down the invagination and progressively flatten to form a layer at the base of the receptor that is almost as compact as myelin (Fig. 4). Close to the supporting cells the lamellae end in cytoplasm filled loops with desmosomes. The most lateral wall cells contact the outer most supporting cells. Freeze etch shows numerous tight junctions in this region (see Fig. 12a).

Covering cells

The covering cells are approximately 30–40 μm in diameter and form a single layer overlying the receptor cells. The perikaryon of the covering cells is sharply divided into two regions (Figs 5, 6), one consisting of a conspicuous and highly ordered tubular system, the

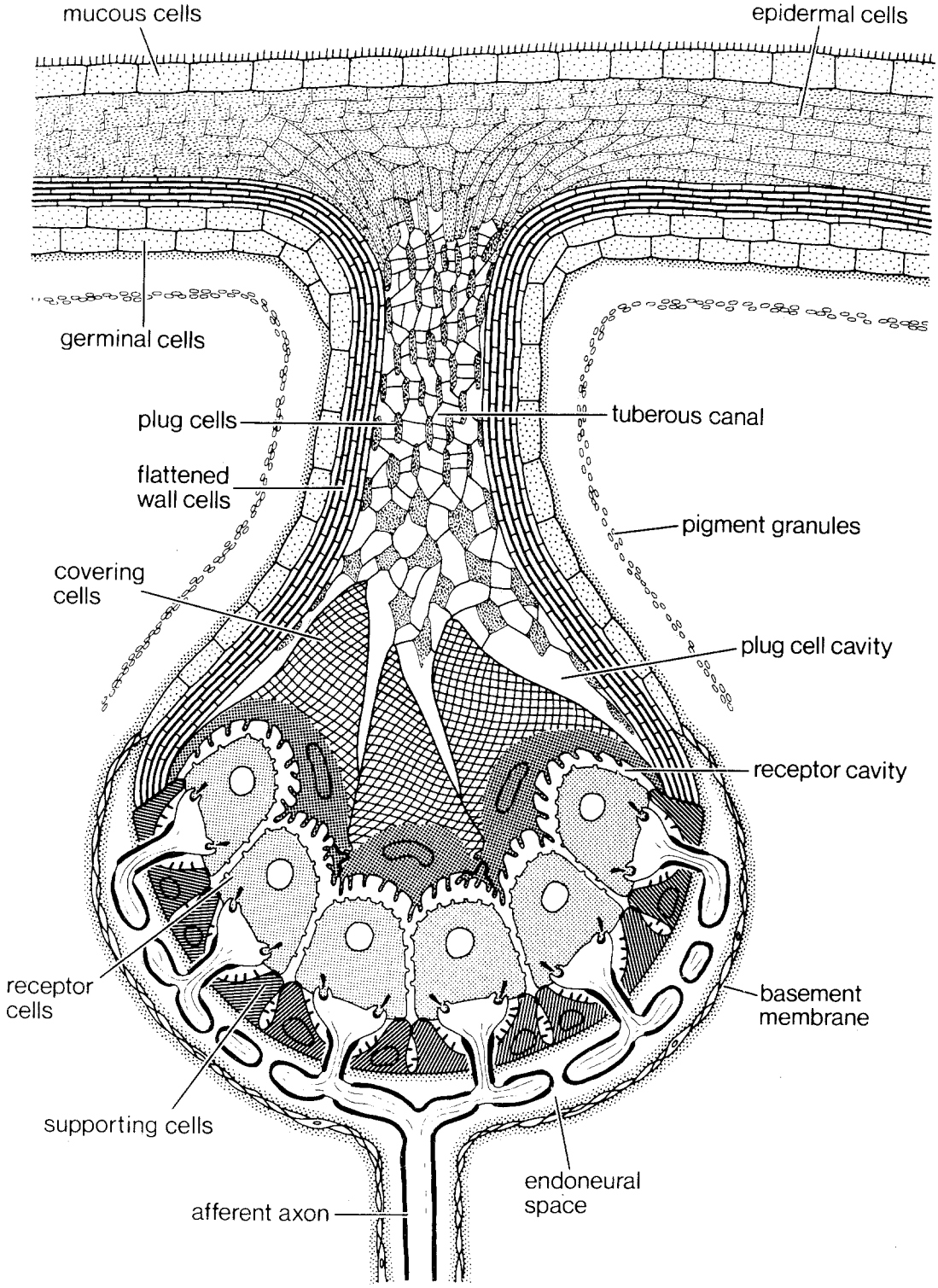

Fig. 1. Diagram of a longitudinal section of the tuberous electroreceptor.

other of a compact cytoplasm with Golgi apparatus and the usual organelles including a large number of closely packed and relatively small mitochondria. The tubular regions lie towards the body exterior while the more compact cytoplasm is adjacent to the receptor cells (Fig. 6a). The bases of the covering cells extend over the receptor cells to form a monolayer of interdigitating processes (see Fig. 8a). The clefts between covering cells are closed by multistranded tight junctions at their bases just above the receptor cells (Fig. 6b). Thus the covering cells provide a barrier between receptor cells and plug cells and divide the interior of the tuberosity into receptor and plug cell cavities. The covering cell monolayer abuts against the wall and

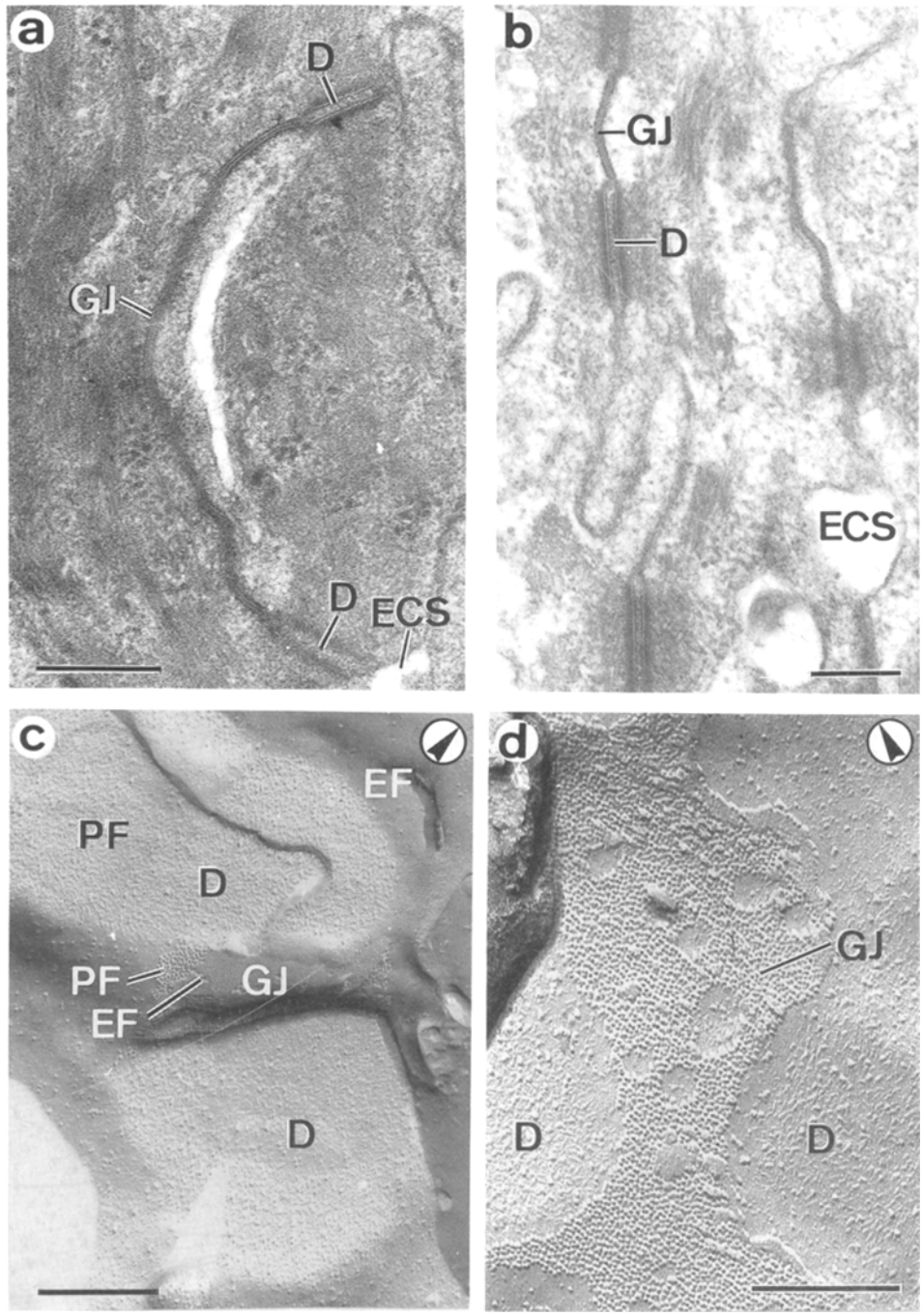

Fig. 2. Specialized cell contacts between outer plug cells. Junctional complexes consist of desmosomes (D) and gap junctions (GJ) as shown in thin sections (a and b) and in freeze-etch replicas (c and d). Desmosomes are characterized by moderately dense aggregations of intramembrane particles of varying size in both membrane faces (EF and PF). The extracellular spaces (ECS) are only moderately wide at this level of the plug. Calibration bar: 0.25 μm.

supporting cells at the periphery of the tuberosity and presumably forms tight junctions with them (Fig. 4b).

The tubular system consists of a three-dimensional labyrinth of evenly spaced channels forming a cubic lattice that can be almost crystalline in its regularity (Figs 5, 6a, 7). The channels open to the plug cell cavity and surface pores can be observed in a rectangular array corresponding to the anastomosing tubules (Fig. 7). The tubular system of the covering cells thus greatly increases their apical surface. Particle complements of P and E faces of apical and tubular membranes are unremarkable (Fig. 7b, d, e), similarly particle complements in the basal membrane do not appear unusual (not illustrated).

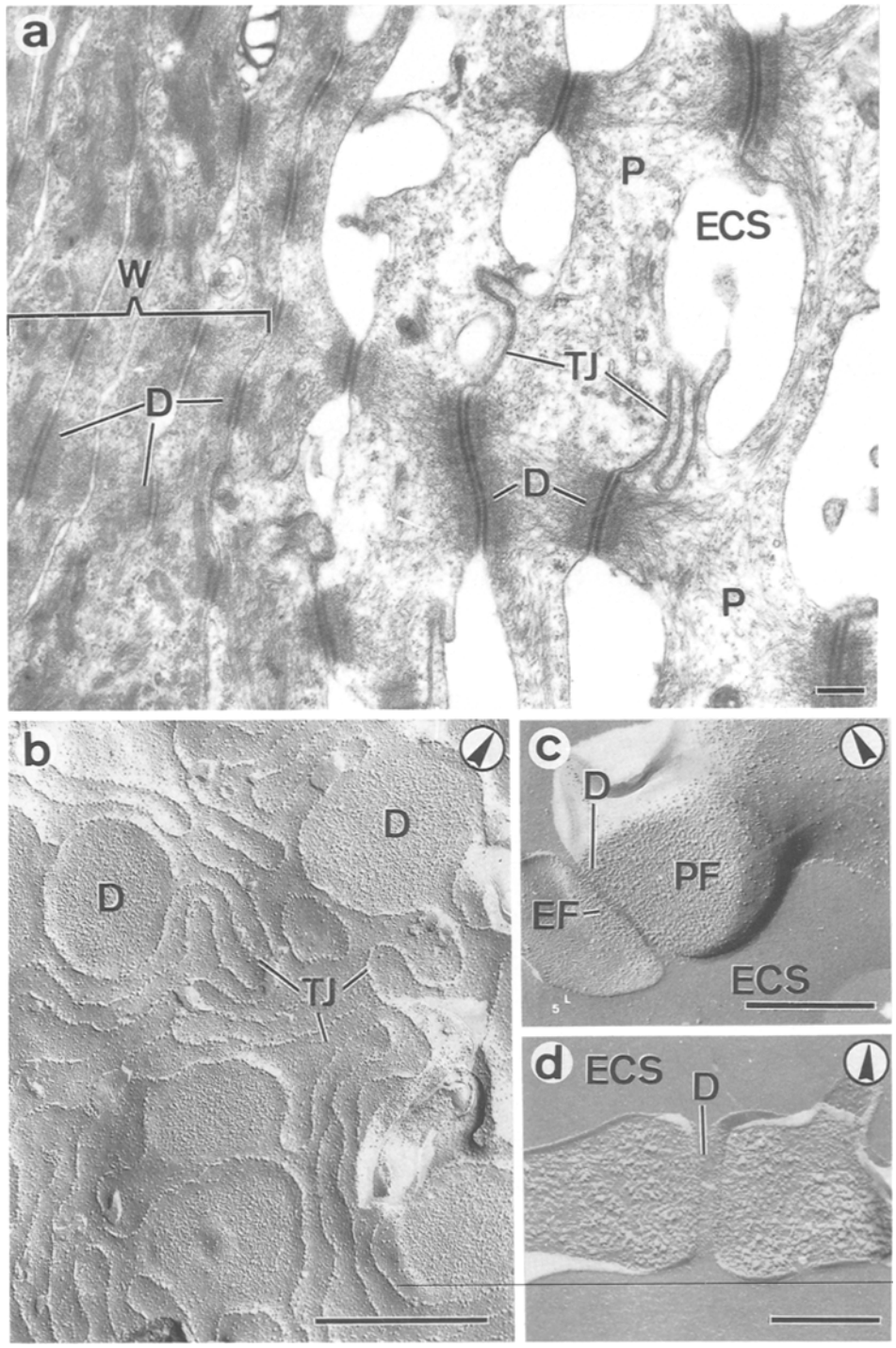

Fig. 3. Relations of plug and wall cells at a deeper level of the plug. (a) Longitudinal thin section showing plug cells (P) on the right and flattened wall cells (W) on the left. At this level the extracellular clefts (ECS) between plug cells are more widened. The plug cells are connected by desmosomes (D) and possibly by tight junctions (TJ). The wall cells are densely packed and connected by abundant desmosomes (D). Tight junctions are not obvious although in freeze-fractured material they are numerous. (b) Freeze-etch replica of flattened wall cells corresponding to those shown on the left in (a). In this largely P-face replica desmosomes (D) are characterized by moderately dense aggregations of intramembrane particles of variable size. The space between desmosomes contains many tight junctional strands (TJ) which branch infrequently. Gap junctions were not found. (c) Freeze-etch replica of a desmosome (D) connecting plug cells as shown on the right in (a). The fracture plane exposes the E-face on the left (EF) and the P-face on the right (PF). The extracellular space (ECS) is widened. (d) Cross-fractured desmosome (D) between spinous processes of two plug cells surrounded by enlarged extracellular space (ECS). The intracellular cleft contains fibrillar material. Calibration bar: 0.25 μm.

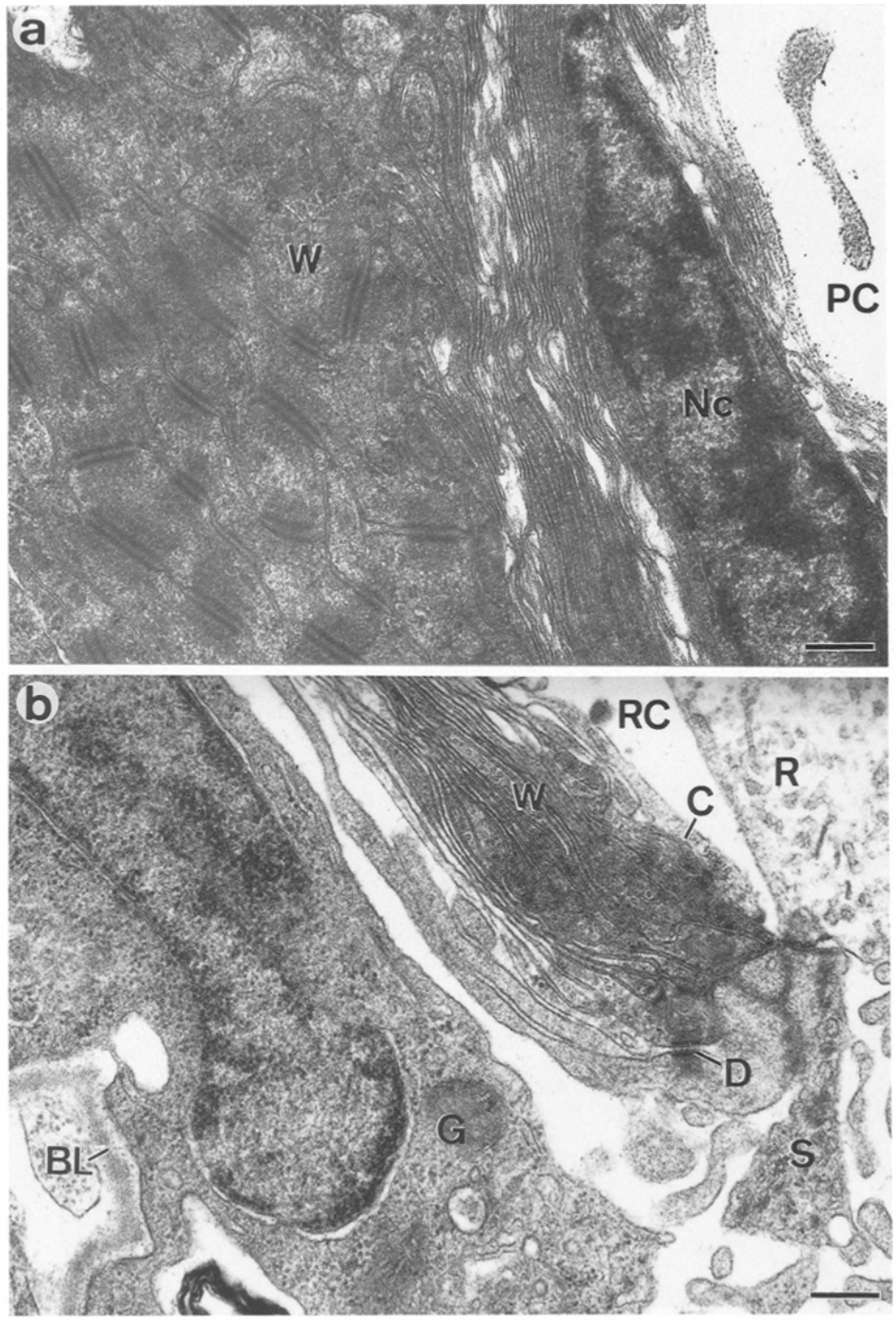

Fig. 4. (a) At the deepest level of the plug with extremely widened extracellular space (plug cell cavity = PC) the inner layer of the compact flattened wall cells (W) forms myelin-like membrane lamellae. Nc=nucleus. (b) At the base of the receptor cavity (RC) the myelin-like lamellae open up to paranodal-like loops which are connected by tight junctions (see Fig. 12a) and desmosomes (D). Some of these loops reach the supporting cells (S) and are connected with them by tight junctions. BL, basal lamina; C, covering cell; G, germinal cell; R, receptor cell. Calibration bar: 0.25 μm.

Receptor cells

These cells are cuboid, approximately 15–20 μm in diameter. The apical and lateral surfaces which comprise the outer face have a modest number of short microvilli (Figs 5, 6). Kinocilia are absent. The cytoplasm contains the usual organelles; it is particularly rich in small vesicles and tubules of smooth endoplasmic reticulum (Figs 8a, 9). Extended, flat cisterns of endoplasmic reticulum can be found subjacent to the apical surface. The lateral membranes just above the contact with supporting cells are separated in well-

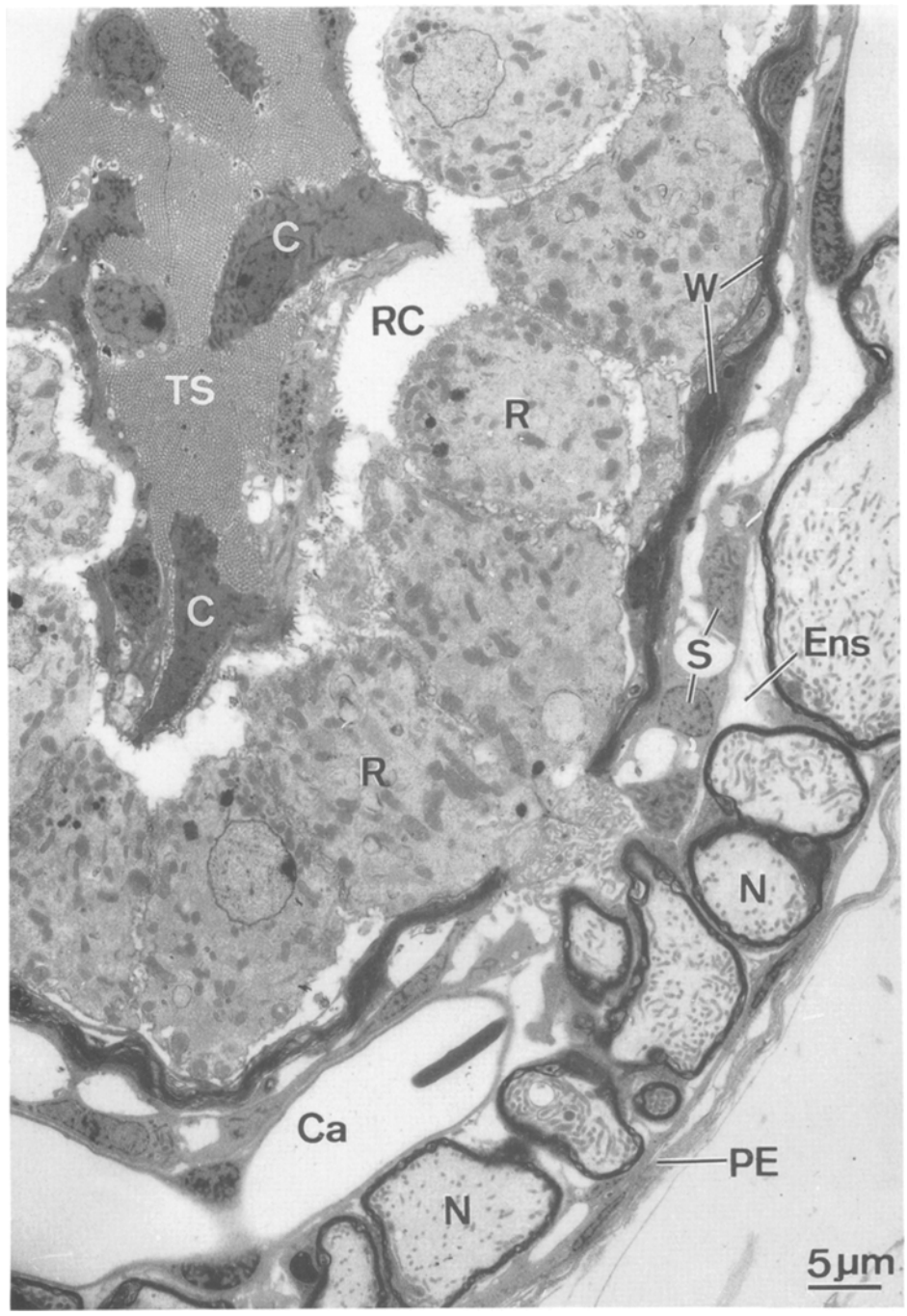

Fig. 5. Sensory region at the base of a tuberous organ taken at 1000 kV. Thickness of section 0.5 µm (by courtesy of Dr H. Ris, University of Wisconsin, Madison, USA). The nuclear and perinuclear regions of covering cells (C) are highly electron dense, and the tubular system (TS) exhibits crystal-like regularity. Ca, capillary; Ens, endoneural space; PE, perineural endothelium; N, nerve fibre; R, receptor cell; RC, receptor cavity; S, supporting cell; W, flattened wall cells (myelin-like).

fixed preparations. In a few cases where fixation was poor as judged by bulging of other cell profiles, the lateral membranes are much more closely apposed. These apparent junctions were probably artefactual. The particle complements of the apical membrane P and E faces are, like those of the covering cells, unremarkable (Fig. 8b).

Specialized contacts exist between the basal margin of receptor cells and the underlying supporting cells (Fig. 10b). Freeze fracture replicas show that these contacts consist of multistranded tight junctions (Figs 10c, 11b, 12). Each receptor cell is joined to its own underlying supporting cell or cells (Srivastava & Szabo, 1974) in a ring demarcating apical and basal

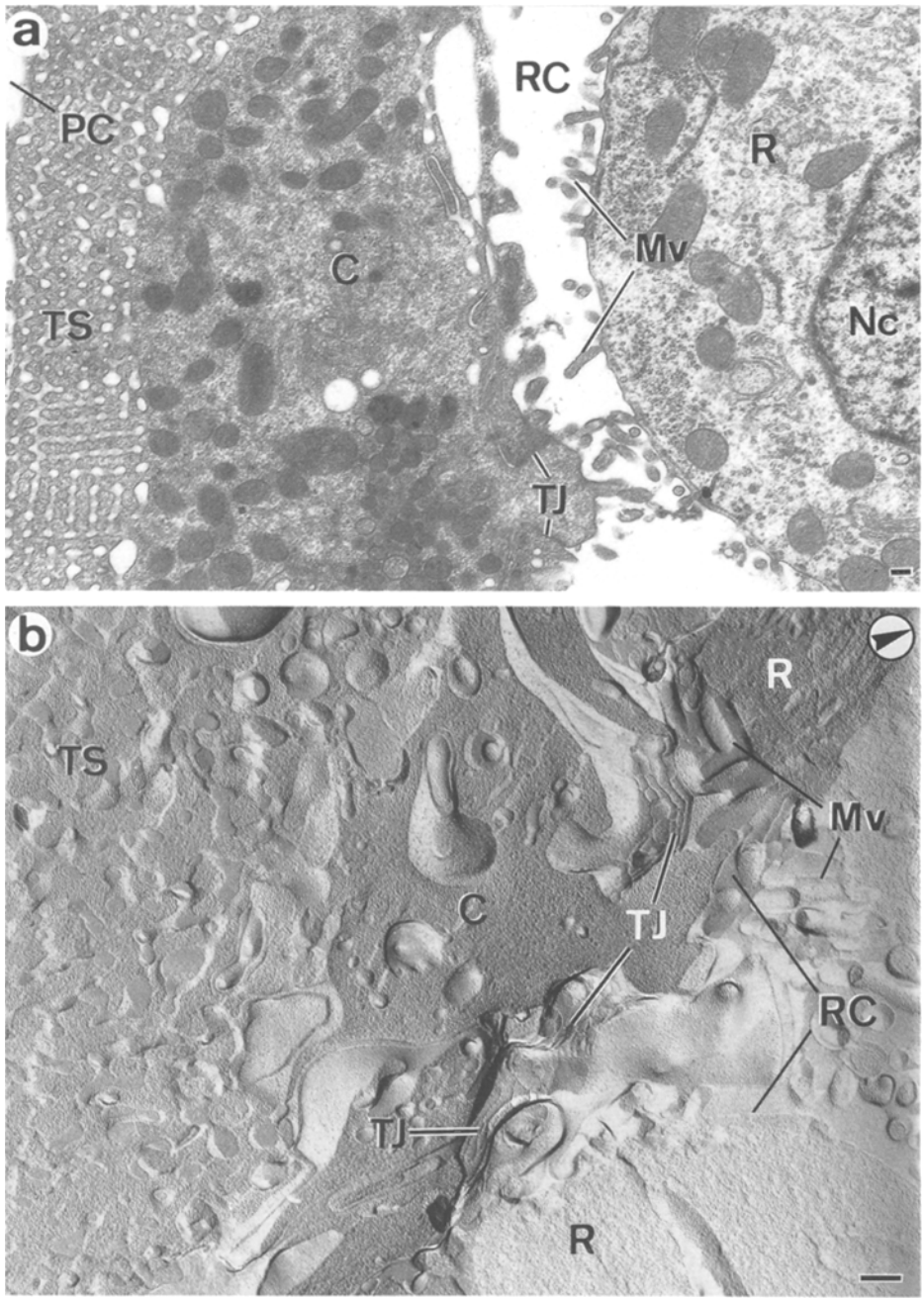

Fig. 6. Covering cells (C) in relation to receptor cells (R). (a) Thin section with the covering cell (C) on the left side showing the tubular system (TS) facing the plug cell cavity (PC) and the compact protoplasmic region with what are presumably tight junctional contacts with neighbouring covering cells (TJ) facing the receptor cavity (RC). The receptor cell contains mitochrondria and nucleus (Nc), as well as microvilli (Mv) on the surface. (b) Freeze-fracture of a nearly identical region. The tubular system (TS) on the left can just be recognized. The protoplasmic part facing the receptor cavity (RC) shows very clearly the tight junctions (TJ) with neighbouring covering cells. Two receptor cells (R) on the right show microvilli (Mv) on their surface. Calibration bar: 0.25 μm.

faces. Very close to the receptor cell contacts, adjacent supporting cells are joined by tight junctions, thus forming a junctional triplet (Fig. 10). Limited data suggest that the P face of the basal membrane is typically particle rich. The E face contains a moderate number of c.20 nm large particles that may be responsible for the cell's excitability (Fig. 12a).

Receptor synapses

A receptor cell synapses with the single afferent nerve

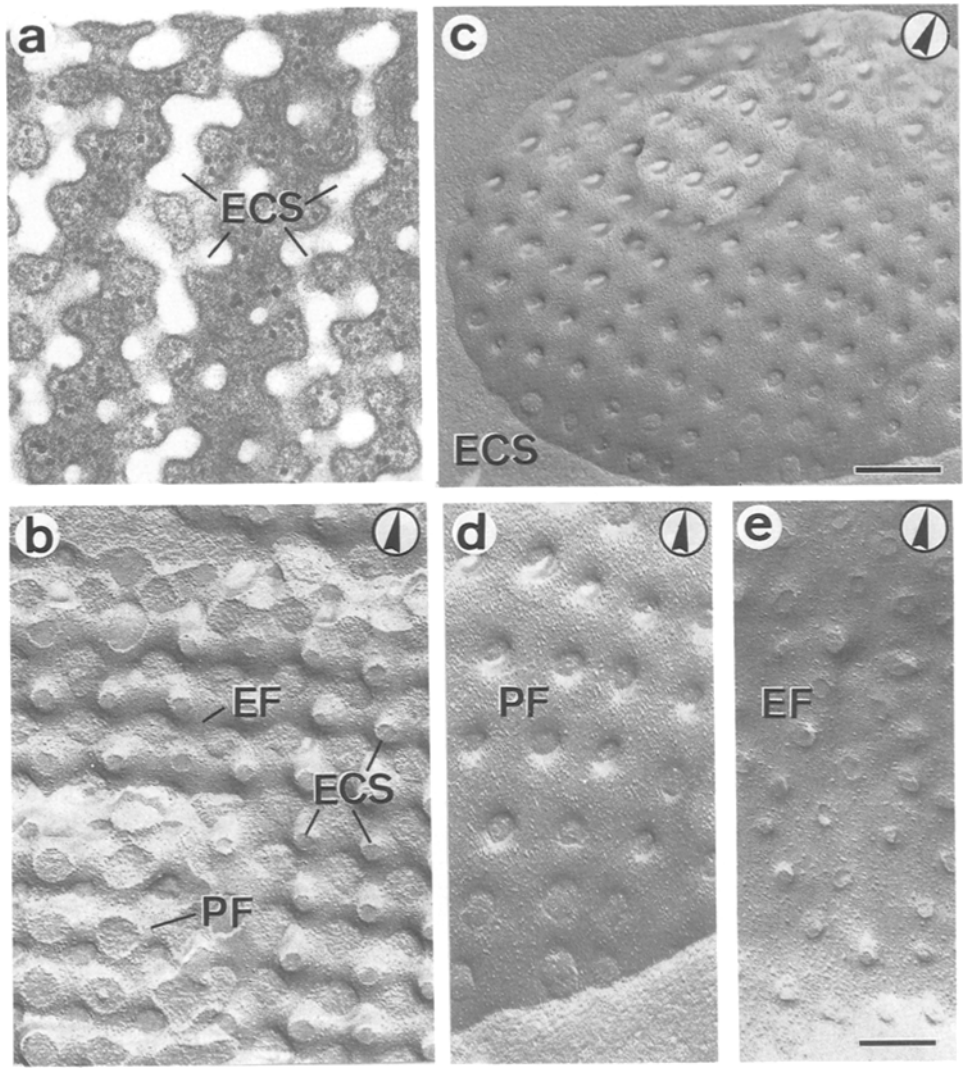

Fig. 7. Tubular system of covering cells. (a) A thin section showing that channels of the tubular system open to the plug cell cavity (top of figure) and are continuous with the extracellular spaces (ECS). (b) The tubular system in a freeze-fracture replica shows the rectangular alignment of channels. (c) A freeze-fracture replica of apical membrane of a covering cell showing the rectangular arrangement of openings of the tubular system in P-face (PF). (d) Higher magnification of (c) showing the particle rich P-face of the plasmalemma. (e) The apical E-face (EF) at the same magnification is also particle rich. Calibration bar: 0.25 μm.

fibre on a large terminal knob that covers most of its basal face (Figs 9, 11). Myelin extends close to the terminal, but occasionally the fibre branches to innervate several receptor cells after the last myelin segment. The afferent ending is filled with mitochondria and rich in microfilaments (Fig. 9a). Arranged in a circular fashion around the periphery of the synaptic apposition are the 'active zones', apparently as many as 16 in number (Fig. 11). Each active zone consists of a small protrusion of the receptor cell into a larger diameter process from the nerve terminal (Fig. 9). On the presynaptic side a dense body projects part way into the protrusion of the receptor cell. Cross-sections indicate that the dense body is rod-shaped (Fig. 11a, right). Many 50 nm clear vesicles are clustered against the dense body. Densities inside the presynaptic membrane at the end of the protrusion sometimes appear connected to the dense body and may anchor it in place (Figs 9b, c). Additional regions of cytoplasmic density are found along the basal surface close beside the synaptic sites. These densities may be aggregates of vesicles or a forming dense body (Fig. 9c). There are coated vesicles in the basal region of the receptor cells (Fig. 9b, arrow).

The synaptic cleft is cup-shaped with a membrane separation of about 30 nm. The postsynaptic membrane exhibits a conspicuous thickening and cytoplasmic density around the entire indentation by the

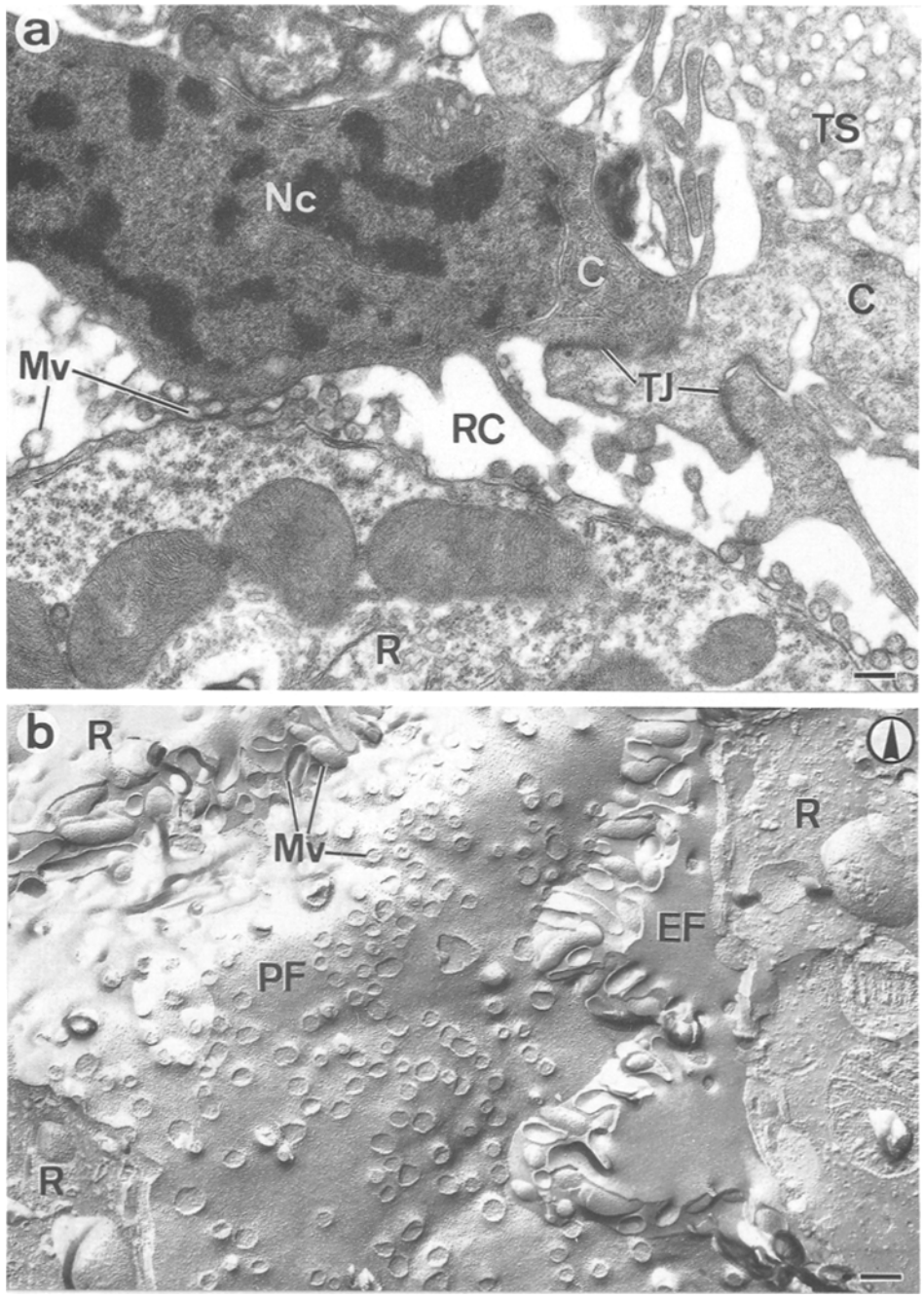

Fig. 8. Receptor cells. (a) A thin section of a receptor cell (R) with microvilli (Mv) bordering the receptor cavity (RC) and covered by covering cells (C) connected by tight junctions (TJ). The covering cell on the left shows the nucleus (Nc) apparently in mitotic prophase, the one on the right side shows the tubular system (TS). (b) A freeze-fracture replica showing three apposing receptor cells (R) with their P- and E-faces (PF; EF). Most of the microvilli (Mv) on the P-face are broken off. Calibration bar: 0.25 μm.

receptor process. Large 50–80 nm clear vesicles are clustered to the postsynaptic membrane at the base of the indentation (Fig. 9b–d).

In freeze fracture replicas the dense body of the presynaptic region consists of finely granular material (Fig. 9d, e), while in the synaptic indentation the postsynaptic EF membrane contains a dense aggregate of highly ordered particles (Fig. 9e). We were unable to identify gap junctions between receptor cell and axon terminal in our material, which included relatively large areas of membrane in the central region of the synaptic apposition (Figs 9a, 11b, 12a).

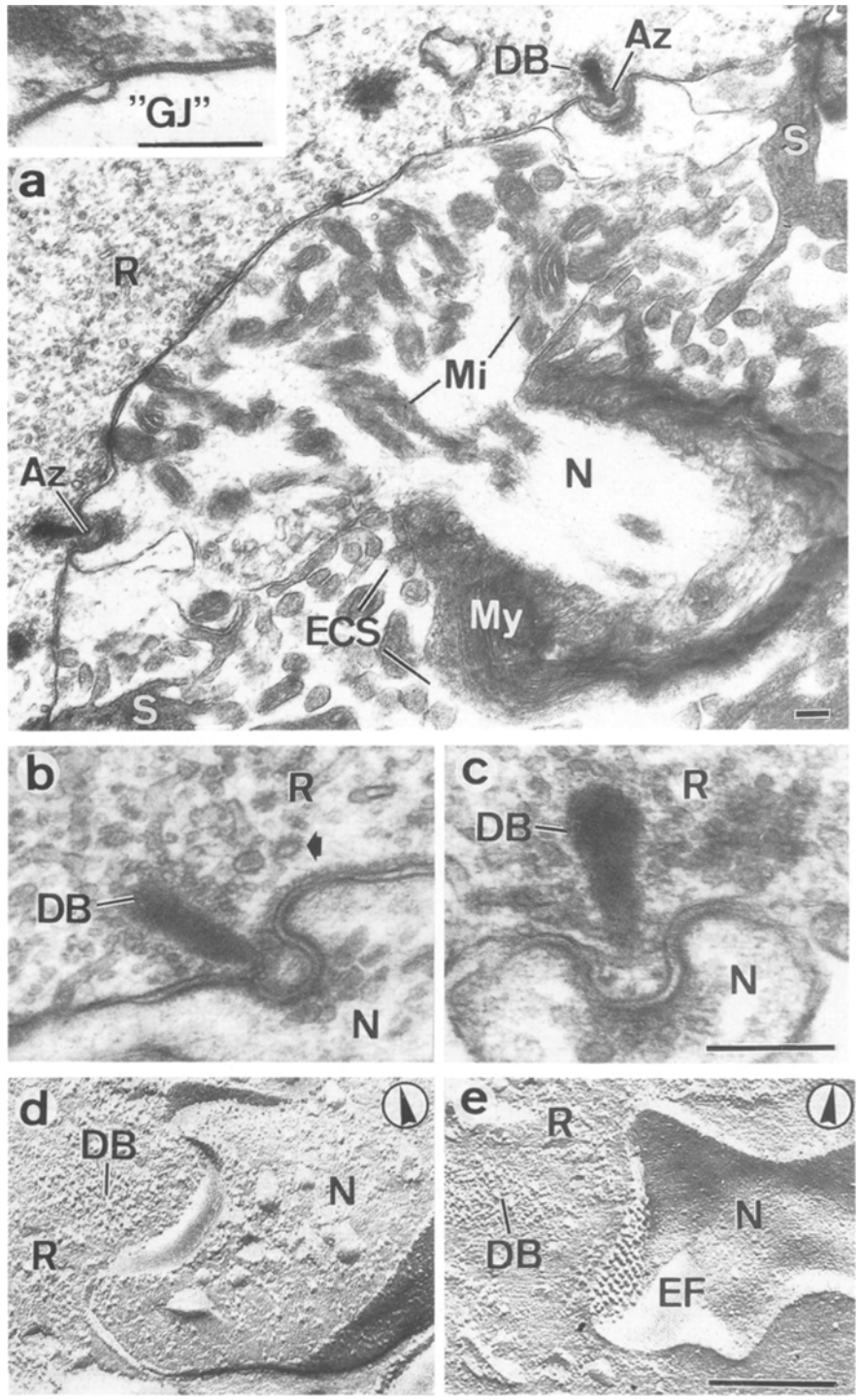

Fig. 9. Receptor synapse morphology. (a) A large axon terminal (N) contacts the receptor cell (R). Individual active zones (Az) are characterized by prominent presynaptic dense bodies (DB) and protrusions of the receptor cell into the axon. The receptor cell contains large numbers of tubules and clear vesicles. The nerve terminal contains numerous mitochrondria (Mi). The myelin sheath ends close to the terminal. The villous processes surrounding the axon are from supporting cells (S). *Inset* in the upper left shows at higher magnification a close membrane apposition in a badly fixed preparation. Although suggestive of a gap junction ("GJ") this apposition is probably artefactual. (b–c) Enlargements of active zones. Clear synaptic vesicles are clustered against the presynaptic dense body. The synaptic cleft may contain dense material. The postsynaptic membrane and subjacent cytoplasm exhibit increased density, and there is a conspicuous aggregation of vesicles at the end of the protrusion. These vesicles are somewhat larger than the presynaptic vesicles. (d–e) Freeze-fracture replicas of active zones. The presynaptic dense body (DB) appears as a fine granularity. (d) The postsynaptic vesicles are clearly visible. (e) There is a regular array of large particles on the postsynaptic E-face of the nerve terminal. Calibration bar: 0.25 μm.

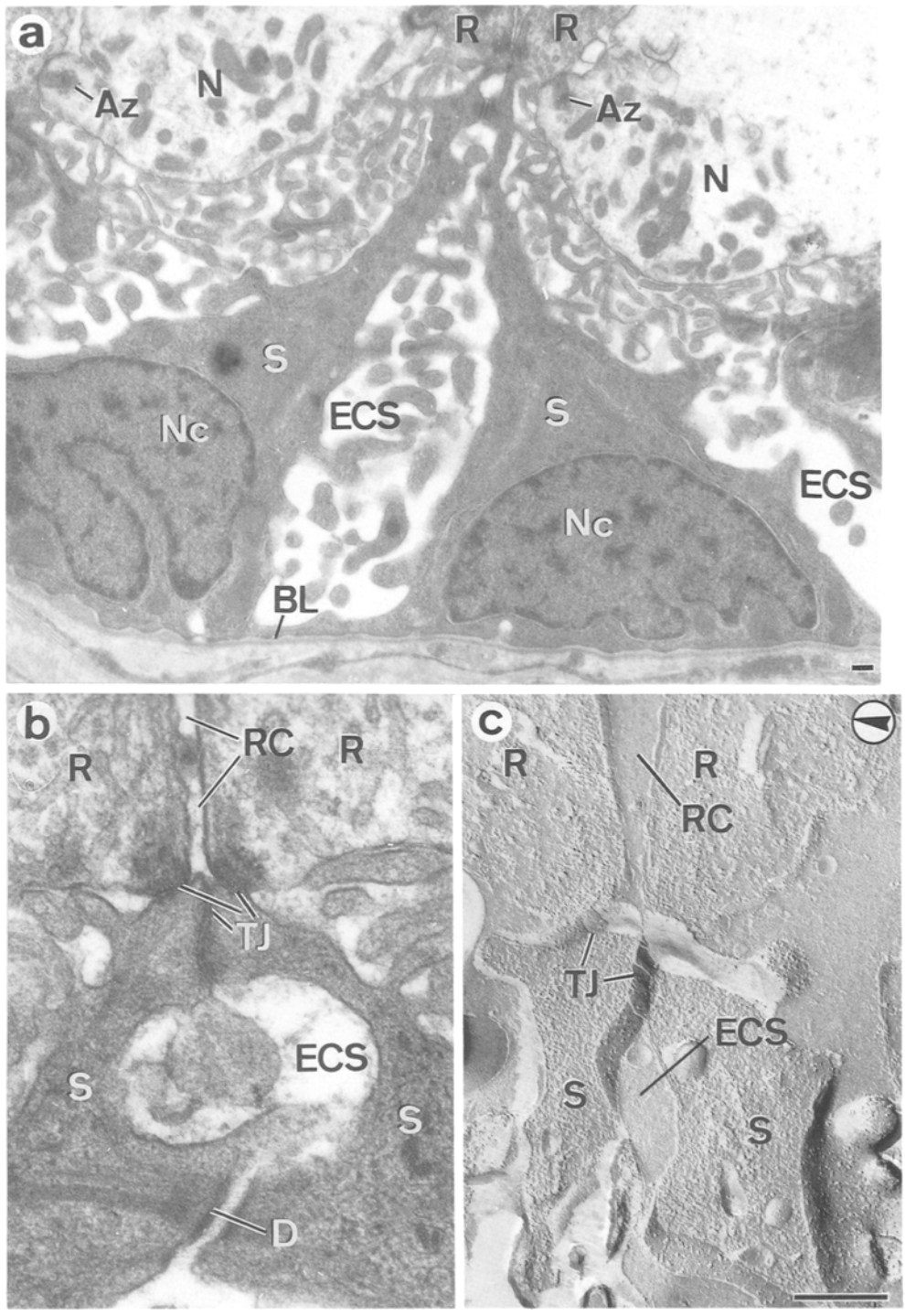

Fig. 10. Relation between receptor and supporting cells. (a) Two supporting cells (S) extend from receptor cells (R) to basal lamina (BL). The supporting cells form tight junctions with two receptor cells and with each other. AZ, active zone. Villous extensions of the supporting cells extend into the space between them and also surround the nerve terminal. N, nerve terminal; Nc, nucleus; ECS, extracellular space. (b) Higher magnification of the contact of two receptor cells (R) with two supporting cells (S). D, desmosome; ECS, extracellular space; RC, receptor cavity; TJ, probable tight junction. (c) Freeze-etch replica showing two tight junctional (TJ) strands in E-face between the left receptor (R) and supporting cell (S) and in P-face between supporting cells. RC, receptor cavity; ECS, extracellular space. Calibration bar: 0.25 μm.

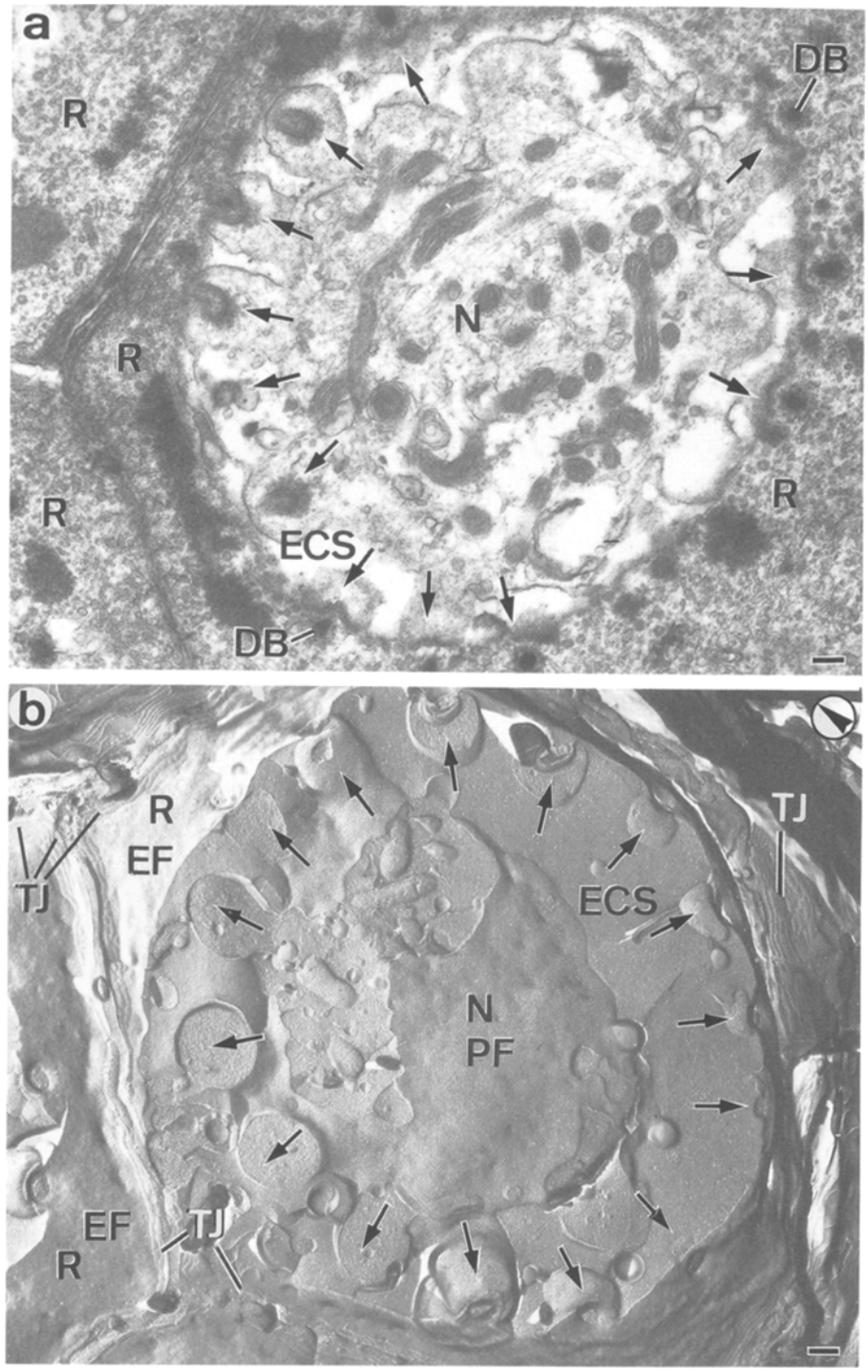

Fig. 11. Receptor synapse viewed tangentially. There are about 16 active zones arranged in a circle along the circumference of a receptor cell (arrows). (a) In thin section the nerve terminal (N) appears as a circular electron lucent area surrounded by synaptic sites. The receptor cell cytoplasm is darker. Presynaptic dense bodies (DB) are round in cross section (right). Two neighbouring receptor cells (R) are seen on the left. (b) Freeze-etch appearance of a cross-fractured synaptic region. The profile corresponds closely to that seen in (a). The E-faces (EF) of two receptor cells (R) on the left are attached to the border of underlying supporting cells by multistranded tight junctions (TJ, see Fig. 10). The plane of fracture passes through the postsynaptic processes deep to the active zones on the left and passes above the active zones on the right with intermediate levels above and below. In the centre the P-face (PF) of the nerve terminal (N) can be seen. Mitochrondria are numerous in the cytoplasm of the axon terminal. ECS, extracellular space. Calibration bar: 0.25 μm.

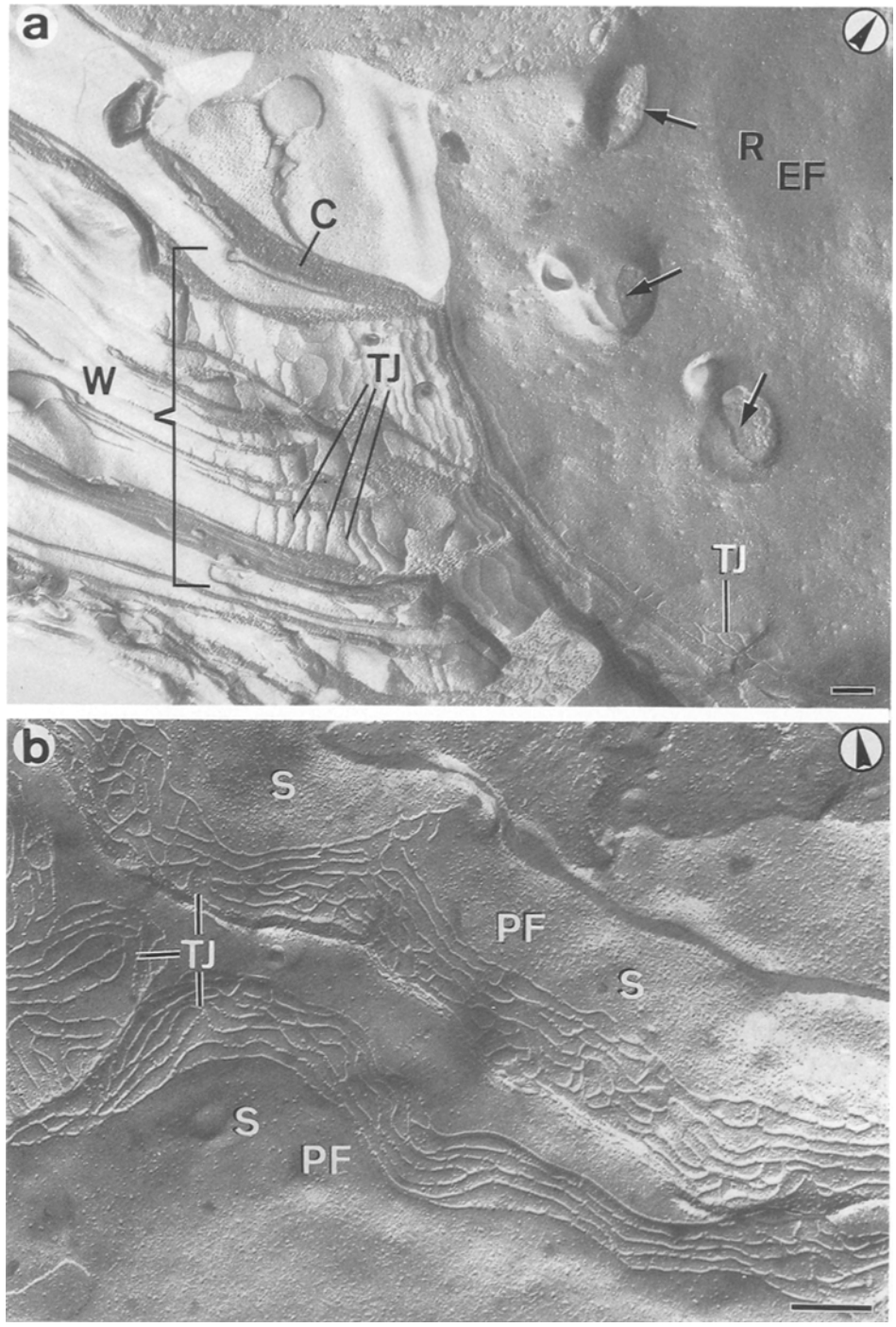

Fig. 12. Relation between receptor cells, supporting cells and flattened wall cells. (a) A freeze-fracture replica shows the basal E-face (EF) of a receptor cell (R) with three regions near active zones (arrows). The tight junctions (TJ) on the border of this cell belong to an underlying supporting cell (see Fig. 4b). On the left are paranodal like loops of flattened wall cells interconnected by multistranded tight junctions. The uppermost loop belongs probably to a covering cell (C) (see Fig. 4b for a similar situation in thin section). (b) Supporting cells (S) showing in P-face (PF) the multistranded tight junctions (TJ) facing receptor cells. Calibration bar: 0.25 μm.

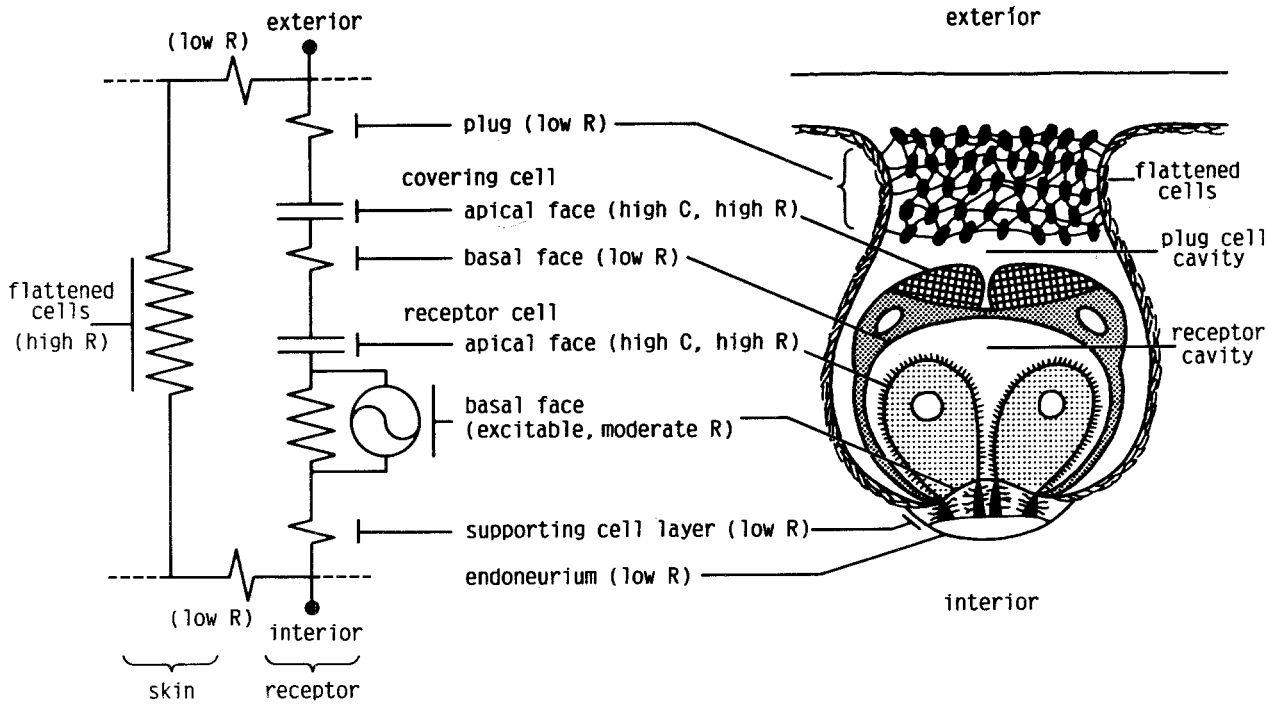

Fig. 13. Diagram of the receptor and its probable electrical equivalent circuit.

We did sometimes see close appositions in this region in thin sections, but only in poorly fixed material (Fig. 9a, inset). They were probably a fixation artefact.

Supporting cells

These cells extend from the basal surfaces of the receptor cells to the inner aspect of the sensory epithelium marked by a basal lamina (Fig. 10a). A supporting cell contacts a single receptor cell and the relation may be one to one (Srivastava & Szabo, 1974). As noted above, where receptor cells are closely abutting, their supporting cells are joined by tight junctions to the receptor cells and to each other (Figs 10b, c, 12). Also at the edge of the receptor epithelium the supporting cells are joined by tight junctions to the outer most wall cells and possibly the layer of covering cells (Figs 4b, 12).

The nuclei of the supporting cells are located basally. The cytoplasm is dense. There is considerable extracellular space in the supporting cell layer into which the cells give off numerous pleomorphic processes that are connected occasionally by desmosomes (Fig. 10). Branches of the afferent nerve cross the supporting cell layer to contact each receptor cell. The fibres are myelinated close to the synapses and Schwann cell nuclei can be seen in the nerve fibre layer. Spaces occur between the bases of the supporting cells and around the penetrating nerve branches. The extent to which a single supporting cell forms a complete cuff around each afferent branch is unclear.

Discussion

With the aid of the freeze fracture technique it was possible to identify gap (communicating) and tight (occluding) junctions. We can infer therefrom the current pathways between the exterior and the interior of the body across the receptor epithelium and the compartmentation of the intervening extracellular spaces (Fig. 13).

Intercellular clefts present between superficial cells of the epidermis should allow ready passage of current across this layer. The absence of tight junctions between cells of this layer was validated by extensive examination of freeze fracture replicas. As one moves inward the cells become specialized into plug cells, and there is a progressive widening of the extracellular spaces. The plug cells are attached to each other by extensive desmosomes, which peripherally are often combined with gap junctions. These gap junctions and those of the overlying unmodified epidermal cells would lower the resistance between cell cytoplasms and conceivably provide an additional current pathway that is intracellular (as suggested by Srivastava & Szabo, 1974). The few tight junctions between the plug cell processes would have little effect on extracellular current flow.

The layer of flattened cells of the epidermis invaginate to form the wall surrounding the plug. These cells are interconnected by multistranded tight junctions in much of the appositional region between

them. Numerous desmosomes are also present in the appositions. The flattened cells are presumably responsible for the high resistance of the skin (Bennett, 1967), which is consistent with the occurrence of tight junctions and the absence of gap junctions. The presence of many cell layers in series would also reduce capacitive currents, which would be important because of the high frequency of the electric organ discharge. (The absence of gap junctions and occlusion of intercellular clefts raise an interesting question as to how essential metabolites are provided to the more superficial cells.)

Although the extracellular spaces of the epidermis and plug connect the external environment (freshwater) and the receptor organ interior, there is a barrier interposed between the plug and receptor cells which consist of the covering cells. These elements have an extraordinarily large apical surface provided by the tubular system. Extensions from their basal regions are joined by tight junctions and form a continuous layer over the receptor cells. The appearance is of two cell types, the large covering cells and a flattened layer (Lissmann & Mullinger, 1968), but our observations indicate that there is only a single cell type which is differentiated at outer and inner regions (see also Srivastava & Szabo, 1974).

The next barrier is found at the level of the bases of the receptor cells. Here, tight junctions are formed between receptor and supporting cells and between adjacent supporting cells to form three closely neighbouring junctions. Supporting cells are also connected by tight junctions to the outer wall cells. The junctions of receptor cells with supporting cells separate the apical and lateral (outer) membrane of the receptor cells from the basal (inner) membrane and isolate the receptor cavity from the extracellular space of the synaptic region. They also force transepithelial currents to flow through the cell interiors rather than the intercellular clefts. Beneath the supporting cell layer lies the nerve fibre layer; the extracellular spaces in these regions are continuous with each other. The nerve fibre layer contains capillaries. The impedance between the endoneurial space of the nerve fibre layer and the general body interior is unknown, but presumably is low compared with the impedance across the receptor cells.

The morphological features have a number of implications for receptor function. Physiological studies indicate the presence of a blocking capacity in series with the transducing membrane (Bennett, 1971b). This inference is based on the fact that the receptors are insensitive to DC signals. Thus responses at the onset of long pulses are identical to responses at termination of equal and opposite pulses; the receptors respond to changes in potential and not to its absolute level. It has also been shown with sinusoidal stimuli that sensitivity of afferent discharge is greatly reduced at low frequency (Hopkins, 1976).

A further property indicating the presence of a blocking capacity is the absence of a DC component in externally recorded responses of the receptor cells (Bennett, 1971b). These responses, whether oscillatory or impulse-like, have positive and negative phases with equal integrals over time. Their polarity is appropriate for regenerative depolarizing responses of the basal membranes, and it was postulated that these are the active membranes.

The earlier morphological descriptions of gymnotid tuberous receptors were consistent with the outer membrane of the receptor cells acting as the series capacity. It was known that the intercellular clefts between receptor, supporting and wall cells were closed by tight junctions which would require current to pass through the receptor cells (Lissman & Mullinger, 1968; Szamier & Wachtel, 1970). The receptor cells protruded into the receptor cavity and their surface was further increased by microvilli. If this membrane were of high resistance and normal capacitance, it could act as the blocking capacity. It was thought that the cells overlying the receptor cells and the supporting cells did not provide much series resistance, because the receptor is very sensitive to potentials between external medium and the body interior just beneath the skin. In addition, the many extracellular spaces between plug cells and exterior had been described (Wachtel & Szamier, 1966).

In *Sternarchus* (and in *Eigenmannia*, another gymnotid) an additional element, the covering cells, must be considered. These cells may provide a tight epithelium covering the receptor cells and blocking the pathway to the exterior. Because their apical surfaces are highly proliferated by a tubular system, their outer faces could conceivably provide the blocking capacity. If so, the inner faces would have to be of low resistance, and ion flow through intercellular clefts would have to be restricted, consistent with the presence of tight junctions. The proliferated membranes of both covering and receptor cells may constitute a blocking capacity. An alternative, nonelectric function of the covering cells may be in maintaining osmotic or ionic concentrations as considered more fully below.

Another element in series with the transducing membrane is the perineural sheath, which may seal off the endoneurial space from the general body interior. Although these cells may be joined by tight junctions (cf. Akert *et al.*, 1976), there is no proliferated membrane that would be a candidate for the blocking capacity. Also, the nerve fibre layer contains capillaries, and the presence of a complete barrier is uncertain. For these reasons we believe it to be of low resistance.

Based upon the foregoing considerations a simpli-

fied morphological picture and revised equivalent circuit of the receptor are given in Fig. 13. Provisionally we indicate that outer faces of both covering and receptor cells are of high resistance. In considering the resistances of all the elements in series, receptor sensitivity is a relevant parameter. We expect that much of the voltage across the skin is developed across the basal membranes of the receptor cells, because these receptors are known to be sensitive to submillivolt stimuli (Bennett, 1971b). If the transducing membrane were more sensitive, more of an applied voltage could be developed across series elements. The actual trans-skin potentials produced by the electric organ in this species have not been determined. For adequate sensitivity it is only important that the basal membranes of the receptor cells have a relatively high impedance at the electric organ frequency compared with that of other parts of the current pathway. Since biological membranes can have widely varying specific resistivities, little about resistance can be inferred from relative areas. Membrane capacity per unit area, however, is essentially constant and total capacity can be reliably determined from the total area. The series capacity can therefore be assigned with confidence to one or both of the proliferated membranes (cf. Waxman *et al.*, 1972; Kristol *et al.*, 1977).

Comparative data may provide some clue as to covering cell functions. In *Eigenmannia, Hypopomus* and *Electrophorus* the apical membrane of the covering cells is highly proliferated by llamellae rather than by a tubular reticulum (unpublished). The microvilli on the apical faces of the receptor cells are more numerous than in *Sternarchus*. In these fishes, the receptor cell appears more likely to have its own series capacity.

A secretory function of the covering cells is consistent with the large number of mitochondria they contain as well as with their large surface area. Mucous secreting cells occur in the walls of ampullary electroreceptors (Szamier & Bennett, 1974), whose canals open to the exterior, and secreted material is also present in the lumen of mormyrid tuberous organs that lack covering cells (Bennett, 1967). Some protection against the challenge of external solutions, hyposmotic and varying in ionic composition, may be essential for the sensitive operation of the receptor cells. The chloride cells of fish gills also have a tubular reticulum that is highly developed (Sardet *et al.*, 1977).

One of the original motivations for studying the tuberous electroreceptors was the correlation of intramembrane particle composition with electrical properties (Kristol *et al.*, 1977; Ne'eman *et al.*, 1980). However, no striking differences between apical and basal membranes of covering and receptor cells were noted, although apical and basal membranes are presumed to be of high and low resistance respectively. The large E face particles of the membrane of the receptor cells may represent channels responsible for its excitability. Also the postsynaptic membrane opposite the active zone exhibits an array of densely packed large particles which may represent receptor and/or channel molecules. Similar particles have been reported at ribbon synapses of the retina (Raviola & Gilula, 1975) and of other acoustico-lateralis receptors (Gulley & Reese, 1977; Luciano *et al.*, 1977).

The contact of receptor cell with afferent fibre is similar to that reported for other gymnotids (Wachtel & Szamier, 1966; Lissman & Mullinger, 1968; Szamier & Wachtel, 1970), although the regularity of the annular arrangement of active zones has not been previously described. These synapses are subject to a very high frequency input signal, the electric organ discharges, but postsynaptic impulses are generated at substantially lower frequencies (Scheich & Bullock, 1974; Hopkins, 1976). Since there are only electrically transmitting synapses between pacemaker nucleus and the neurogenic electric organ (Bennett, 1967; Pappas *et al.*, 1975; Tokunaga *et al.*, 1980), it was suggested that chemical synapses could not operate at the high frequency of discharge. At the sensory synapses a high frequency input can still be effective in causing transmitter release, probably because it is unnecessary for the output to follow the input one for one. A similar argument can be made in respect to auditory receptors responsive in the same and higher frequency ranges.

The postsynaptic vesicles at the sensory synapses have been repeatedly pointed out by others (Lissmann & Mullinger, 1968; Mullinger, 1969; Szamier & Wachtel, 1970). They may be used in release of a trophic substance required for the maintenance of receptor cell viability (Bennett, 1971b; Szamier & Bennett, 1974) or they may be used in sampling the extracellular milieu for retrograde transport to the somata in the sensory ganglion. The latter suggestion may be testable by intravascular or subcutaneous administration of HRP.

We did not find gap junctions between receptor cells and afferent fibres. Srivastava (1972) reported close appositions in this region which he interpreted as gap junctions. Our negative result with the freeze fracture technique, which is much more reliable for the definition of junctional relations, suggests that his conclusion was in error. We were able to obtain images similar to his in thin section, but under conditions where we believe that they were artefactual appositions resulting from inadequate fixation (cf. Bennett *et al.*, 1967). No gap junctions between neuroepithelial receptor cells and afferent terminals have been described definitively in any tissue. The earlier findings of Hamilton (1968) have not been confirmed by the freeze fracture technique (Gulley & Reese, 1977;

Luciano *et al.*, 1977). A further sensory synapse worth investigating in this respect is the large electroreceptor organ (Knollenorgan) in mormyrids. Physiological data indicate that transmission at this synapse is electrical (Bennett, 1971b).

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