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TABLE OF CONTENTS

FIGURE

ABSTRACT

The luminal surface of the Aplysia statocyst receptor cells is covered with many (approximately 700) true cilia, as shown by light and by transmission and scanning electron microscopy. The cilia have a 9 + 2 arrangement of internal filaments and are in intimate contact with the statoconia. There are 600-1000 statoconia, 6-20 μm in diameter, in each statocyst.

I. INTRODUCTION

The statocysts of gastropod molluscs are spherical organs specialized for sensing gravity. The wall of the sphere contains 13 ciliated mechanoreceptor cells, each with an axon projecting to the cerebral ganglia.[2,3,9] The lumen of the cyst is filled with fluid (statolymph) and many small stones (statoconia) which move under the influence of gravity. As an animal turns about a horizontal axis, receptor cells are activated when they come into a position low enough to be in contact with the statoconia.[8,9]

The statocyst receptor cells are sufficiently large that it is relatively easy to record from them using intracellular microelectrode techniques. They thus provide a useful model of ciliated mechanoreceptor cells which may have transducing mechanisms similar to those of vertebrate hair cells. In order to interpret physiological studies of the Aplysia statocyst,[8] it was necessary to have more information on the fine structure and organization of the cilia and their relationship to the statoconia. Therefore, detailed light and transmission and scanning electron microscopy (TEM and SEM, respectively) were performed to further study this organ.

II. METHODS

For light and TEM, statocysts from small (75 to 125 g) Aplysia californica were dissected out of a live preparation of the circumesophageal ring of ganglia and put directly into fixative. For SEM, the statocysts were exposed as they would be for penetration with microelectrodes[8] and the whole ring of ganglia fixed in this position. After 12 to 24 hours of fixation a block of tissue containing the cyst was cut away and this block was bisected down the center of the cyst. When the blocks were returned to the fixative, most of the statoconia were washed out of the cyst.

Intact statocysts that were to be examined by TEM were fixed with 2.5 percent glutaraldehyde in 250 mM of 2,4,6-trimethylpyridine (s-collidine) buffer (pH 7.4) and postfixed in 1 percent osmium tetroxide for 1 hour. Dehydration and epoxy-resin impregnation procedures as outlined by Luft[6] were used. Thick sections (1 μm) were cut and examined by light microscopy for orientation purposes and 60-nm thick sections were cut and stained with 0.2 percent lead citrate and were examined in a Siemens Elmiskop 1A electron microscope at 80 kV. Cross-sectioned statocysts to be examined by SEM were fixed with s-collidine buffered 2.5 percent glutaraldehyde, dehydrated through a graded series of ethanol and then ethanol-amyl acetate solutions, critical-point dried using liquid carbon dioxide, mounted on copper studs, and coated with a 25-nm thick layer of 60-40 gold-palladium. The specimens were examined using a JEOL model JSM-U3 scanning electron microscope operating at the accelerating voltage of 15 kV.

III. FINDINGS

The Aplysia statocysts are spherical, 200-300 μm in diameter, and are located bilaterally between the pedal and pleural ganglia. The statoconia found in the cyst lumen are ellipsoidal with diameters from 6 to 20 μm. By counting statoconia extruded from the cyst, we estimate that there are between 600 and 1000 in each cyst. In the intact preparation the statoconia can be seen to move independently of one another. They fall under gravity to occupy the lower third of the cyst lumen. As a dissected (unfixed) cyst is rotated, the statoconia are seen to roll down the cyst wall, taking several seconds to reach the bottom. The light micrograph of a section near the middle of a statocyst in Figure 1A shows numerous statoconia. The outlines of five receptor cells can also be seen.

The fine structure of the cilia and their insertion into the receptor cell surface can best be studied with TEM (Figure 1B, C). All cilia appear to possess a 9 + 2 arrangement of internal filaments (inset, Figure 1B). The cilia are 0.2 μm in diameter and 15 to 20 μm long (as measured from SEM, see below). The ciliary membrane is continuous with the cell plasma membrane. A bridge is formed between the filaments at a level of 0.2 to 0.3 μm above the cell surface. The central pair of filaments ends at this bridge (Figure 1B). Approximately 0.2 μm below the cell surface, filamentous lateral extensions of the basal body are frequently seen. (These appear similar to the "side roots" described by Wolff[9] in Pomacea.) The extensions can be up to 1 μm long. In adjacent cilia (some less than 1 μm apart), extensions can be seen pointing in either the same or opposite directions. In a section more nearly parallel to the cell surface (Figure 1C), lateral extensions can be seen to arise from the spiralling of the nine outer filaments in the basal body. In other sections parallel to the cell surface, lateral extensions have been seen to proceed in two or three directions. It seems possible that the lateral extensions point in all directions from the basal body although we do not have serial sections to verify this. In any case, the lateral extensions do not impart a clear morphological polarization to the individual cilia or their basal bodies.

The number of cilia, their arrangement, and relationship to the statoconia can best be seen in SEM (Figure 1D, E). In the SEM of a bisected statocyst in Figure 1D, the outlines of seven receptor cells can be seen. When viewed perpendicular to their surface, individual receptor cells are polygonal with dimensions as large as 100 x 160 μm. The luminal surfaces of the cells are covered with numerous cilia. The luminal surface of the cyst is made up almost completely of ciliated cells; 720 cilia

were counted on one receptor cell. The luminal surface area of this cell was approximately 9800 μm^2, giving approximately 1 cilium per 14 μm^2 of cell surface. If this cell is assumed to be a flat plate 10 μm thick, and the average cilium is taken to be 15 μm long, the cilia would account for 22 percent of the total membrane surface area

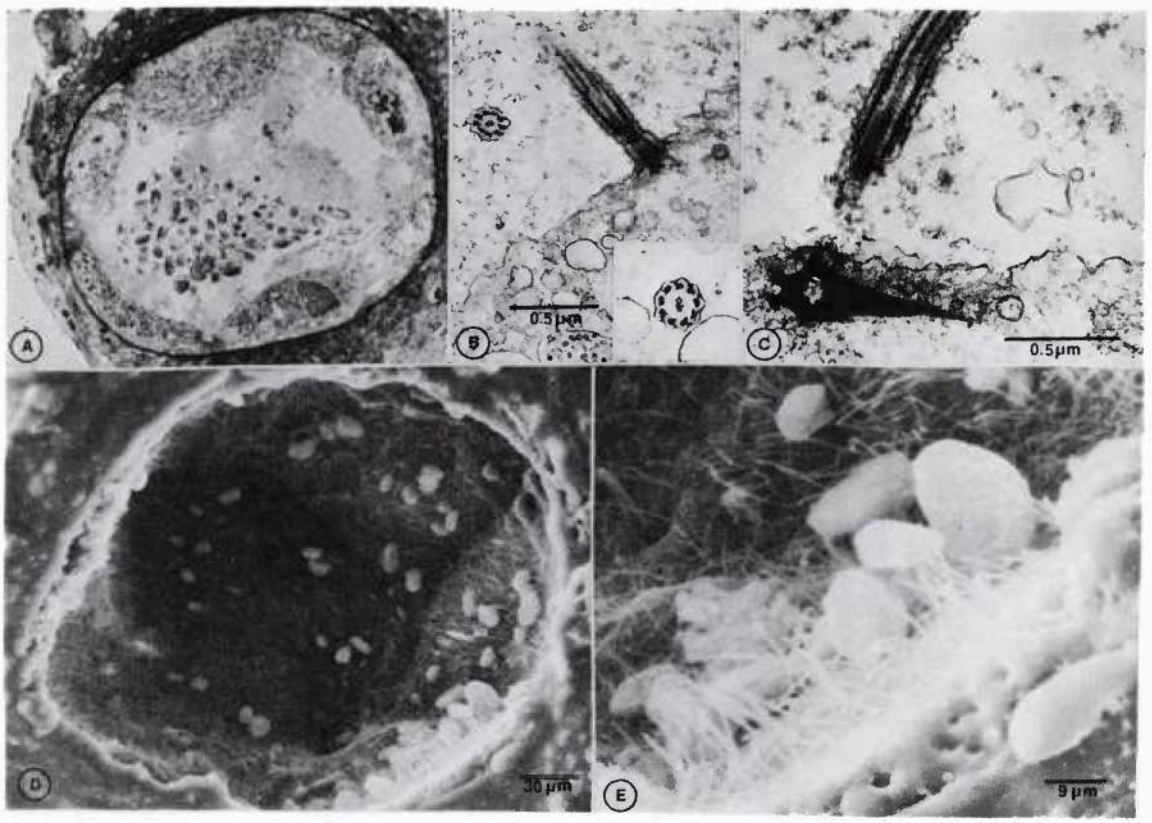

Figure 1. Photomicrographs of Aplysia statocyst. A. Dissected statocyst, as seen by light microscopy, showing several receptor cells. Numerous statoconia are noted within the lumen. Toluidine blue staining. Diameter of cyst is 200 μm. B. An emerging cilium from the surface of a receptor cell. Note the inset which shows the arrangement of nine peripheral and two central filaments. C. The basal body of a projecting cilium is observed in this photomicrograph. Note the lateral extension of the basal body. D. Scanning electron photomicrograph of a dissected statocyst showing numerous statoconia on the cilia-covered surface. E. Higher magnification of the statocyst's luminal surface, showing the close association of statoconia and cilia.

of the cell and 41 percent of the luminal surface. Most of the cilia appear to bend after they emerge from the cell wall, but it is not known whether this is a fixation artifact. There does not appear to be any order to the directions in which the cilia bend.

In considering the relationship of the statoconia to the cilia, it must be kept in mind that in the preparation of Figure 1D most of the statoconia have been washed out to reveal the cilia. The higher magnification SEM of Figure 1E illustrates that the statoconia do lie in intimate contact with the cilia, falling to the receptor cell surface. Due to the size of the statoconia and close packing of the cilia, it can be seen that when one statoconium falls it would strike several (probably 5-10) cilia nearly simultaneously.

Thus the Aplysia statocyst receptor cells are covered with true cilia over their entire luminal surface. There is probably no morphological polarization to individual cilia. If the lateral extensions seen in individual sections do indicate polarization, its effect for the whole cell would be neutralized by the differing directionality of adjacent cilia. In vertebrate lateral line organs,[4] the octopus statocyst[1] and inner ear hair cells,[5,7] a "basal foot" has been described projecting from the ciliary basal body in the direction of ciliary movement which excites the cell. This basal foot thus imparts both a morphological and functional polarization to the cells in which it is found. We have not observed basal feet in the basal bodies of the Aplysia statocyst cilia. Although the two clublike extensions on the left side of the basal body in Figure 1C might be interpreted as a double basal foot, it seems more likely that these are but two of the nine processes of outer filaments cut in oblique section. The basal foot described in other ciliated receptors with directional sensitivity emerges several tenths of a micrometer below the level of lateral extensions of the outer filaments.

The receptor cells are of sufficient extent that large rotations about a horizontal axis would cause a graded change in the number of cilia loaded by statoconia rather than simply a "loaded" or "nonloaded" situation. As a single statoconium strikes the cell surface, it would deflect several cilia nearly simultaneously.

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The luminal surface of the Aplysia statocyst receptor cells is covered with many (approximately 700) true cilia, as shown by light and by transmission and scanning electron microscopy. The cilia have a 9 + 2 arrangement of internal filaments and are in intimate contact with the statoconia. There are 600-1000 statoconia, 6-20 μm in diameter, in each statocyst.

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