EVIDENCE FOR EXTRACELLULAR SPACE IN THE RHABDOME OF THE HONEYBEE DRONE EYE

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INTRODUCTION

In the retinula cell of the honeybee drone, the response to light, recorded with intracellular mico-electrodes, consists of a graded depolarization. This potential change is probably caused by an increase in membrane permeability and by an ionic current flowing from the extracellular space into the retinula cell (1). In the drone, eight retinula cells are grouped together to form an ommatidium. The center of the ommatidium is occupied by the rhabdome, a compact arrangement of microvilli formed by the retinula cells (2). The rhabdome is thought to contain the photo pigment and, therefore, to initiate the response to light (3). In studying the distribution of diffusion tracers within the ommatidium, our purpose was to determine whether there are grounds to believe that the flow of current responsible for depolarization of the retinula cell passes through the membrane of the microvilli. The possibility that this was the case has been denied by a number of authors (4, 5), who have pointed out the compact arrangement of the microvilli and the presence of desmosomes around the rhabdome.

MATERIAL AND METHODS

Heads of the drones are halved by a frontal section passing through both eyes, parallel to the longitudinal axis of the ommatidia. The halved heads are fixed in 5% phosphate-buffered glutaraldehyde, pH 7.4, 740 milliosmols (mOsM) (6). After brief rinsing in phosphate buffer 0.2M, they are postfixed in 2% phosphate-buffered osmium tetroxide, pH 7.4, 340 mOsM (7), then dehydrated in alcohol and embedded in Epon (8). In addition, some heads were stained in block with uranyl acetate (10). Fig 3*c*. shows a section obtained from such a block. All sections are stained with lead hydroxide (9). The penetration of ferritin into the ommatidia was investigated according to the method of Huxley (11). The drone heads are immersed in a solution containing approximately 18% of purified ferritin in 0.2M phosphate buffer, pH 7.3, for 90 min at 4°C. They are then fixed and dehydrated as described above. The lanthanum solution is prepared according to the method of Revel and Karnovsky (12). The drone heads are immersed in a mixture of equal amounts of 6% cacodylate-buffered glutaraldehyde and 1% lanthanum hydroxide, pH 7.4, 840 mOsM for 2 hr and 30 min. After being briefly washed in 0.2M cacodylate buffer containing 1% lanthanum hydroxide, they are postfixed in a solution composed of equal parts of 1% cacodylate-buffered osmium tetroxide and 1% lanthanum hydroxide, pH 7.3, 320 mOsM. for 60 min. The relatively high osmolarity of the glutaraldehyde fixative did not affect the dimensions of the microvilli, which remained unchanged in an isotonic 3% glutaraldehyde fixative and in the 5% fixative described above. Glutaraldehyde was preferred in high concentration since it provided better tissue preservation.

RESULTS

The arrangement of the retinula cells in an ommatidium is shown in Fig. 1. Two of the eight cells differ from the others, owing to their relatively small size and to their clear cytoplasm, which contains but a small number of organelles. The ommatidium is surrounded by pigment cells rich in glycogen. The rhabdome, in the center of the ommatidium, is formed mainly by the six large retinula cells (Fig. 2). A junctional complex resembling a desmosome is formed near the rhab-

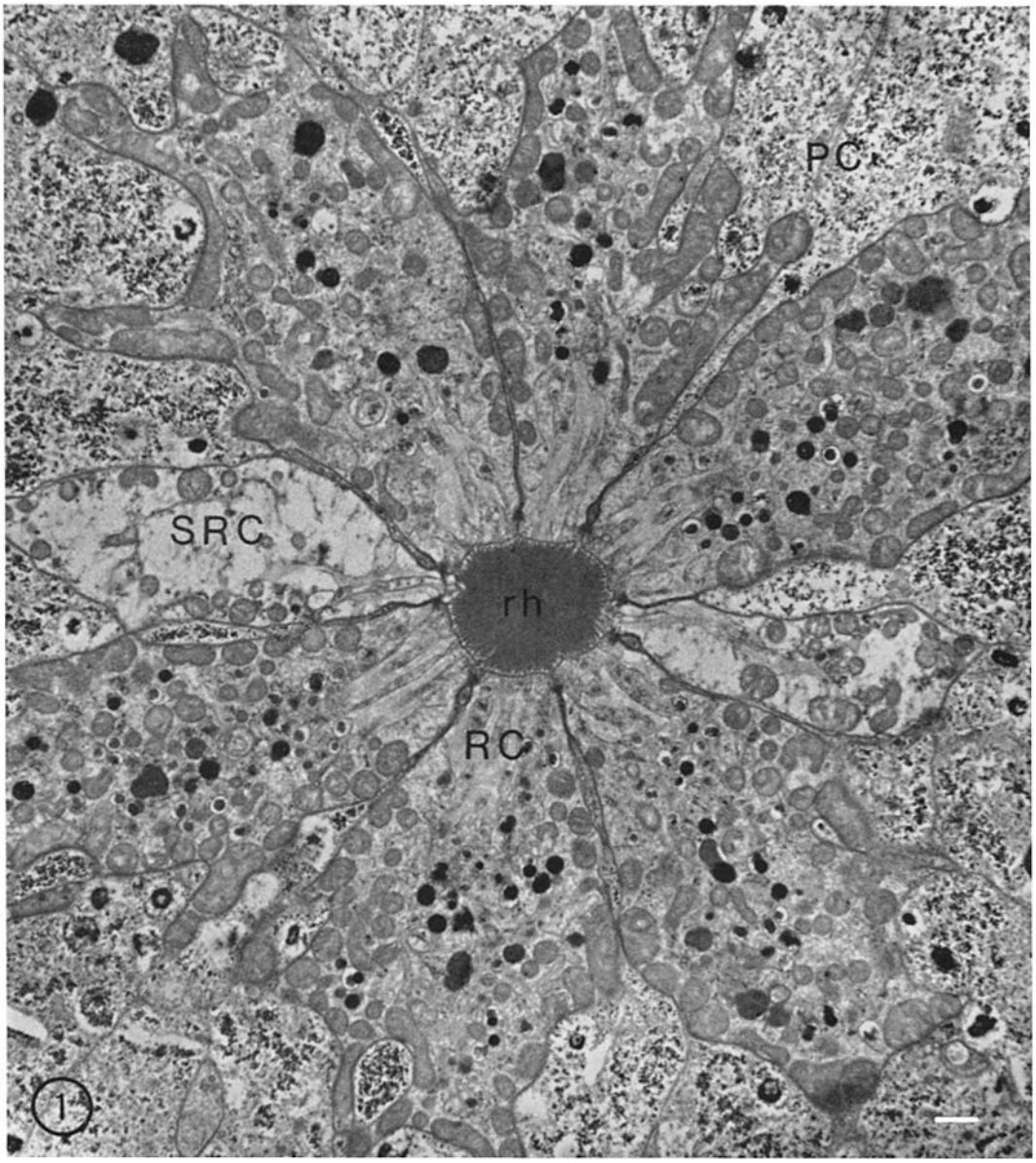

FIGURE 1 Cross-section of an ommatidium, showing the arrangement of the retinula cells. The level of the section involves the peripheral (corneal) third of the ommatidium. The six large retinula cells (*RC*) are of irregular shape and contain numerous mitochondria, as well as pigment granules of various sizes. The cytoplasm of the two small facing retinula cells (*SRC*) is slightly clearer. The retinula cell cytoplasm near the rhabdome (*rh*) is devoid of organelles and contains intracellular channels. The ommatidium is surrounded by pigment cells (*PC*), rich in glycogen and containing large pigment granules. × 5,700.

dome between each retinula cell and its adjacent cell (Figs. 2 and 3). Behind four of the eight junctional complexes, a membrane clearly outlines an ovoid structure containing microtubules (Fig. 2). These structures probably represent extensions of the crystalline cone, as Waddington and Perry (13) suggested for *Drosophila*. The microvilli forming the rhabdome are approximately 800 A in diame-

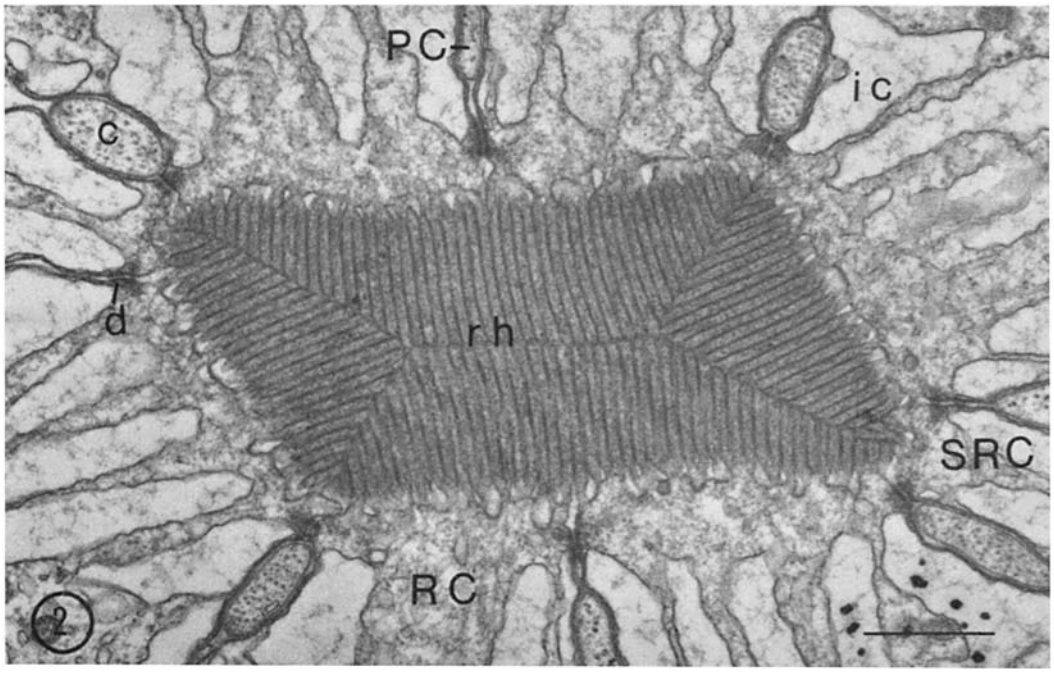

FIGURE 2 Center of the ommatidium. The rhabdome (*rh*) consists of microvilli formed mainly by the six large retinula cells (*RC*), the small cells (*SRC*) contributing only a few microvilli. Near the rhabdome, each retinula cell forms a junctional complex with its adjacent cells (*d*). The four extensions of the crystalline cone (*c*) are found just beyond the junctional complexes. Extensions of two pigment cells (*PC*) are wedged deeply in between two retinula cells. This figure clearly shows intracellular channels (*ic*). × 17,000.

ter. They are attached to the cell surface by a narrow pedicle, 400 A across. Conspicuous extracellular space can be seen between the pedicles (Fig. 3*a*). The microvilli are outlined by a thin membrane similar to that bounding the retinula cell cytoplasm (Fig. 3*a*). In sections of blocks stained with uranyl acetate, this membrane shows the typical trilaminal unit membrane structure (Fig. 3*c*). Most of the microvilli are seen to touch each other, with mere apposition of their adjoining membranes. Staining with uranyl acetate was rather uneven. In some places, the membranes were heavily stained: uranyl acetate was deposited on the outer leaflet and was sometimes found in the triangular spaces comprised between the microvilli. This gave a picture similar to that of blocks treated with lanthanum. In other regions, staining was absent. In this case, the trilaminar structure of the membrane bounding the microvilli was not discernible.

The microvilli are embedded in a substance of appreciable electron opacity (Fig. 3*b* and *c*). The presence of this substance and the fact that the thickness of a section corresponds approximately to the diameter of one microvillus may account for the poor contrast at the borderlines of the microvilli in longitudinal sections. A similar difference between longitudinal and transverse views is seen in Fig. 3 of the paper by Eguchi and Waterman (14) on the fine structure of the eye of the crab, *Libinia*.

Sections obtained from blocks treated with ferritin and lanthanum show that both substances fill the space between the pigment and retinula cells; the two tracers penetrate the junctional complex and diffuse into the extracellular space at the base of the microvilli (Figs. 4 and 5*a*). Lanthanum, but not ferritin, can be traced between the microvilli, up to their tips, both in longitudinal (Fig. 5*a*) and in transverse sections (Fig. 5*b*). Lanthanum seems also to infiltrate the external leaflet of the unit membranes (Fig. 5*b*). Thus the regions of contact between the microvilli described in uranyl acetate—stained sections appear as narrow dark lines. Each microvillus seems to be separated from the black deposit of lanthanum by a clear rim.

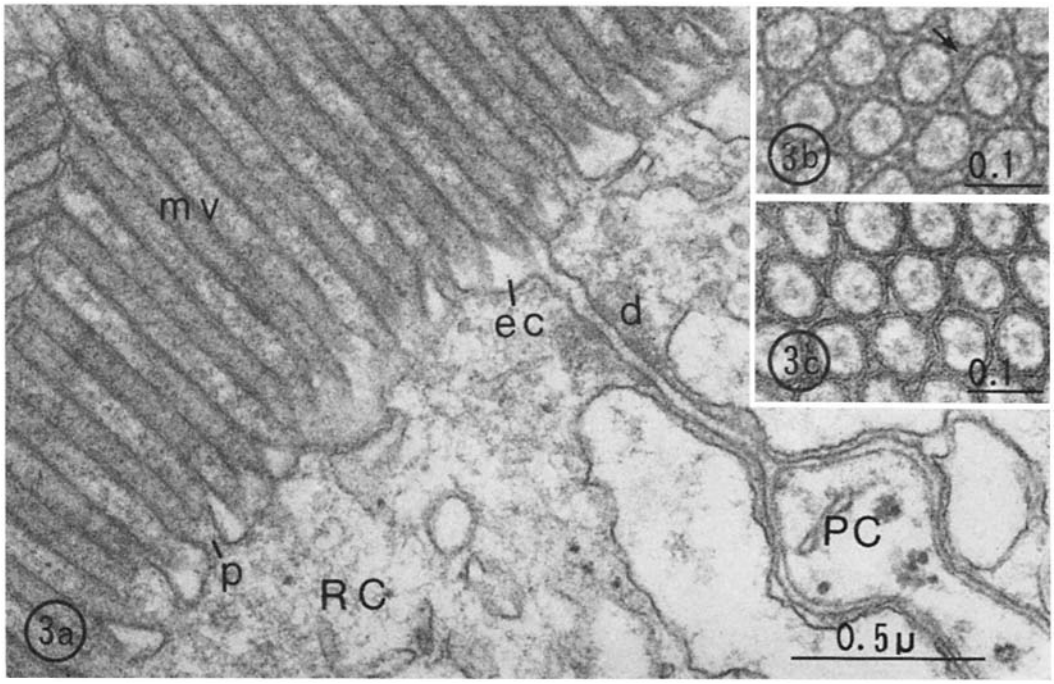

FIGURE 3*a* Rhabdomeric region of two retinula cells. A narrow pedicle (*p*) attaches the microvilli (*mv*) of the rhabdome to the retinula cells (*RC*). Within the rhabdome, extracellular space (*ec*) is clearly discernible between the pedicles of the longitudinally cut microvilli. The junctional complex (*d*) consists of symmetrical cytoplasmic densifications and of a strip of condensed material in the extracellular space. The extension of a pigment cell (*PC*) can be seen just beyond the junctional complex. × 50,000.

FIGURES 3*b* and 3*c* Transverse sections of the microvilli at high magnification. Fig. 3 *b* comes from a block not stained with uranyl acetate and shows the lack of a unit membrane structure at the periphery of the microvilli. Arrow indicates small dots between microvilli. Fig. 3*c* represents a section of a uranyl acetate-stained block revealing the trilaminar unit membrane structure and the points of contact between adjacent microvilli. Both figures, × 110,000.

Owing to the infiltration of the external leaflet, this line can be considered as the central part of the unit membrane. It should also be noticed that small, clear dots between the microvilli are outlined by lanthanum (Fig. 5*b*). These dots (tubules?) seem similar to those observed in rhabdomes not treated with lanthanum (arrow Fig. 3*b*); however, they were not observed in uranyl acetate—stained sections (Fig. 3*d*).

DISCUSSION

Studies by Huxley (11) and by Revel and Karnovsky (12) have shown that, in muscle, ferritin and lanthanum seem to penetrate freely into the extracellular space by mere diffusion from the bathing medium. If this applies for the drone eye as well, the regions of the ommatidium rendered opaque by these substances can be considered accessible to the extracellular fluid surrounding an ommatidium.

In view of the fact that ferritin did not penetrate as deeply into the rhabdome as lanthanum, the question might arise whether the space filled by lanthanum only is also extracellular space. In fact, since lanthanum was applied during and after fixation, its deposit between the microvilli could be due to an artifact. This does not seem probable, however, for sections of tissue fixed without lanthanum clearly show triangular zones comprised between three adjacent microvilli, which can safely be considered extracellular.

As noted above, these spaces are filled with a dense intercellular substance. The different distribution of ferritin and lanthanum might be explained by the presence of this substance which

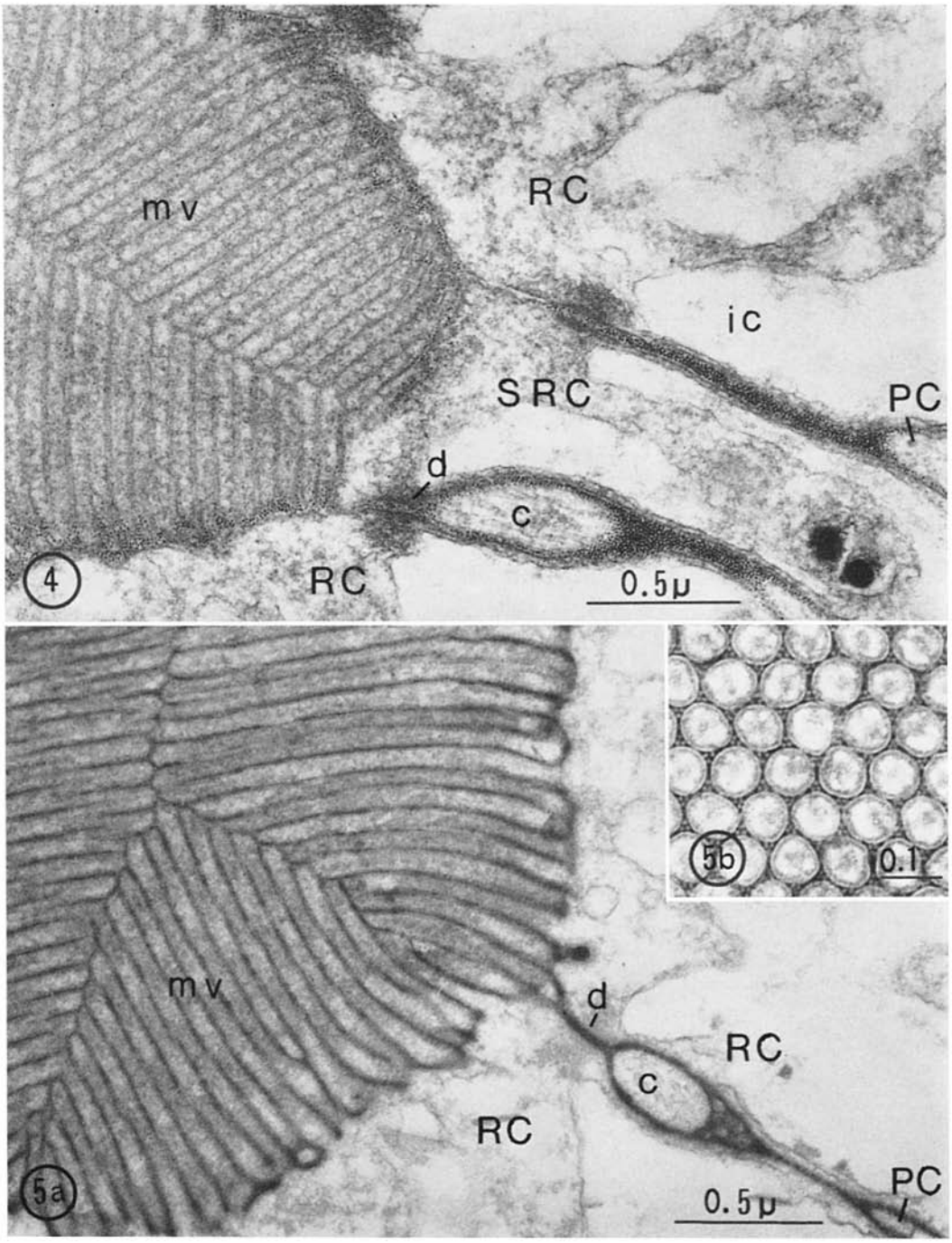

FIGURE 4 Central area of an ommatidium treated with ferritin. The space separating the different cells of the ommatidium (*RC*, *SRC* and *PC*) is filled with ferritin, which also surrounds the extension of the crystalline cone (*c*). Ferritin is found within the junctional complex (*d*) and between the pedicles of the microvilli (*mv*). The retinula cell cytoplasm and the intracellular channels (*ic*) contain no ferritin at all. × 43,000.

FIGURE *5a* Central area of an ommatidium treated with lanthanum. Like ferritin, lanthanum fills the space separating the cells of the ommatidium, surrounds the extension of the crystalline cone, and penetrates within the junctional complex. Unlike ferritin, lanthanum is seen as a narrow, electron-opaque strip surrounding each microvillus. Labels as in Fig. 4. × 42,000.

FIGURE *5b* Transverse section at high magnification of the rhabdome infiltrated with lanthanum. × 94,000.

could, in fact, form a fine network not accessible to ferritin but to lanthanum. Revel and Karnovsky (12) have already demonstrated that lanthanum is capable of penetrating spaces as small as 20 A wide. Another possibility is that lanthanum, unlike ferritin, has a particular affinity for the intercellular substance found between the microvilli. Doggenweiler and Frenk (15) showed that lanthanum, in ionic form at least, has properties for staining cell membranes and their external coat. Such staining may have occurred in our experiment, and lanthanum might not have penetrated freely between the rhabdomeric microvilli. At present, it cannot be determined which of these processes is responsible for the uneven distribution of lanthanum and ferritin.

Our results indicate that in the eye of the honeybee drone an extracellular space extends from the periphery of the ommatidium to the tips of the rhabdomeric microvilli. Although ferritin did not penetrate deeply into the rhabdom it is probable that ions, other than lanthanum ions, can gain access between the microvilli. In this case, the results presented here are in favor of the hypothesis which was stated in the Introduction and which proposed that the light-induced depolarization of the retinula cell is caused by an ionic current flowing into the microvilli from the extracellular space.

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