to induce unresponsiveness to skin grafts of the same genotype have also been reported to occur in certain H-2 incompatible mouse strain combinations (*12*). Indeed, in this species, inoculation of both C57/A bone marrow and splenic cells is totally ineffective in inducing tolerance of A or C57/A skin grafts in lethally irradiated adult C57 recipients (*13*). Similar observations have been reported in cattle (*14*) and humans (*15*).

The occurrence of one or more antigens on skin (possibly determined by components of the major H-locus) which are not present or poorly represented in some or all of the cells of the lymphohematopoietic system (*13*) might well contribute to the now-established fact that skin is the most difficult organ to homograft successfully.

CLYDE F. BARKER
DAVID M. LUBAROFF
WILLYS K. SILVERS

Department of Surgery and
Immunobiology Research Unit,
Departments of Medical Genetics
and *Pathology, University of*
Pennsylvania School of Medicine,
Philadelphia 19104

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16. Supported by PHS grants AI-09275 and AI-07001. C.F.B. is a Markle scholar in academic medicine. W.K.S. is a PHS career development awardee. The technical assistance of J. Johnson, C. Stoffel, and G. Sawchuck is acknowledged. We also are indebted to Dr. R. E. Billingham for critical review of the manuscript.

23 February 1971; revised 22 March 1971 ■

Photoreceptors in Primitive Chordates: Fine Structure, Hyperpolarizing Receptor Potentials, and Evolution

Abstract. *Two species of primitive chordates have hyperpolarizing photoreceptor potentials, as vertebrates do. In* Salpa *the photoreceptive membrane is composed of microvilli, whereas in* Amaroucium *it is modified from cilia. There appears to be no functional correlation between fine structure of photoreceptive membrane and polarity of response to light.*

Most photoreceptors show one of two types of structural specialization at those regions of their cell membranes believed to contain photopigments—a folding of the plasma membrane into microvilli or into arrays of lamellae or disks modified from cilia (*1*). The microvilli-bearing photoreceptors which are common among invertebrates are associated with a depolarizing receptor potential, whereas vertebrate photoreceptors, whose outer segments are derived from cilia, respond to light with a hyperpolarizing potential (*2*). We have recently shown (*3*) that the ciliary-type photoreceptor of an invertebrate, *Pecten irradians*, is hyperpolarized by light. This response differs from that of vertebrate cones in two ways. It is associated with an increase, rather than a decrease, in membrane conductance, and it is linked to the generation of an "off" discharge in the axons of these cells. It is likely that a hyperpolarizing receptor potential also underlies the "off" responses of ciliary-type photoreceptors of other invertebrates (*4*). It is of some interest to determine if the relationship between the cell's fine structure and its response to light is real or simply a fortuitous correlation. To do this we have examined the membrane structure and electrical response of visual cells in the eyes of some primitive chordates, from the subphylum urochordata, whose receptors may be similar to those of higher chordates (vertebrates) (*5*). Our findings show that hyperpolarizing receptor potentials occur in both ciliary- and microvillar-type photoreceptors in chordates.

The tunicate *Salpa democratica* (class Thaliacea) has a lensless eye arising from the neural ganglion, which is directly accessible to light, being situated on the dorsal surface of the animal beneath its transparent tunic. The eye arises from a secondarily acquired portion of the nervous system and is thus not homologous with the eyes of other urochordates or those of vertebrates (*6*). Our electron microscopic observations show that the eye contains, in addition to a layer of pigment cells, several hundred elongated visual cells about 10 μm in diameter (*7*). These cells give rise to a randomly organized array of microvilli at one end (Fig. 1A), which in some sections can be seen to be in continuity with the cell's cytoplasm. No other types of neurons are present in the eye.

We made intracellular recordings from photoreceptor cells with high-resistance microelectrodes filled with KCl, using conventional recording methods and a Wheatstone bridge circuit to pass constant-current pulses across the cell membrane. The bridge was balanced after penetration to eliminate the voltage drop across the electrode, leaving the slower charging voltage drop across the cell membrane. White light from a 45-watt tungsten quartz-iodine bulb was focused to an evenly illuminated spot which covered the entire eye. For study of the response to light, the eye and the neural ganglion were removed from the animal and pinned in a small chamber under seawater.

Although the resting potentials of visual cells in the salp were low, about −10 mv, the response to a brief flash of light was a large hyperpolarization (Fig. 1B), which in some cells could be up to 70 mv in amplitude. The size of this response was graded with light intensity. The nature of the changes in membrane conductance during the receptor potential was studied by passing brief hyperpolarizing constant-current pulses across the membrane during darkness and illumination. The voltage drop caused by the test pulse across the cell membrane decreased in size during the response to light (Fig. 1C) indicating that the receptor potential is due to an increase in membrane conductance. Additional information about the response was obtained by passing steady currents across the membrane and observing the change in the amplitude and polarity of the receptor potential. From this data, a reversal potential of approximately — 80 mv is found. Thus, in the polarity of its receptor potential and underlying conductance mechanism, the salp photoreceptor is similar to the ciliary-type receptor of the scallop eye (*9*).

The tunicate *Amaroucium constellatum* (class Ascidiacea), in its free-swimming larval stage of development, has a single eye which degenerates upon metamorphosis of the "tadpole" larva to the adult form (*10*). This eye is believed to be homologous with the vertebrate median eye (*5, 6*), and in some ascidian species its photoreceptor cells show a distinct resemblance to vertebrate rods and cones (*11*). The fine structure will be described in greater detail elsewhere (*12*), but, in brief, electron microscopy shows that the photoreceptor cells have whorls of flattened membranes which are derived from a ciliary base and may be regarded as modified cilia. In Fig. 2A, the lamellar array of membranes can be seen to arise as elaborations of the plasma membrane of a cilium at the end of a photoreceptor cell.

For electrophysiological recording, individual tadpoles were caught immediately after hatching, during the period when they exhibit strong phototactic behavior (*10*). The tail and tunic were removed from the body of the tadpole, and the body was pinned under seawater. The experimental methods were the same as described above for the salp eye. It proved exceedingly difficult to record electrical activity from these preparations, possibly because the retinal cells, which lie close to the surface of the small animal, are often damaged by the removal of the thick tunic. Although rare, penetration of a visual cell was evidenced by a sudden drop in potential of — 5 to — 20 mv and the appearance of a response to light. Responses to flashes or steps of light (Fig. 2B) were always hyperpolarizing, but of small amplitude (2 to 7 mv), and they disappeared completely when the membrane potential was suddenly lost. This receptor potential cannot be due to extracellular current flow, since no responses could be recorded from the eye extracellularly with low impedance electrodes. It is possible that in the living animal the receptor potential may be considerably larger than shown in Fig. 2, B and C.

Measurements with constant-current pulses indicate that the hyperpolarizing response is associated with a decrease in membrane conductance, since the voltage drop across the membrane becomes larger during the response to light (Fig. 2C). If a linear current-voltage relation and the standard equivalent circuit (*13, 14*) for the photoreceptor membrane are assumed, the input conductance in darkness and during illumination can be used to pro-

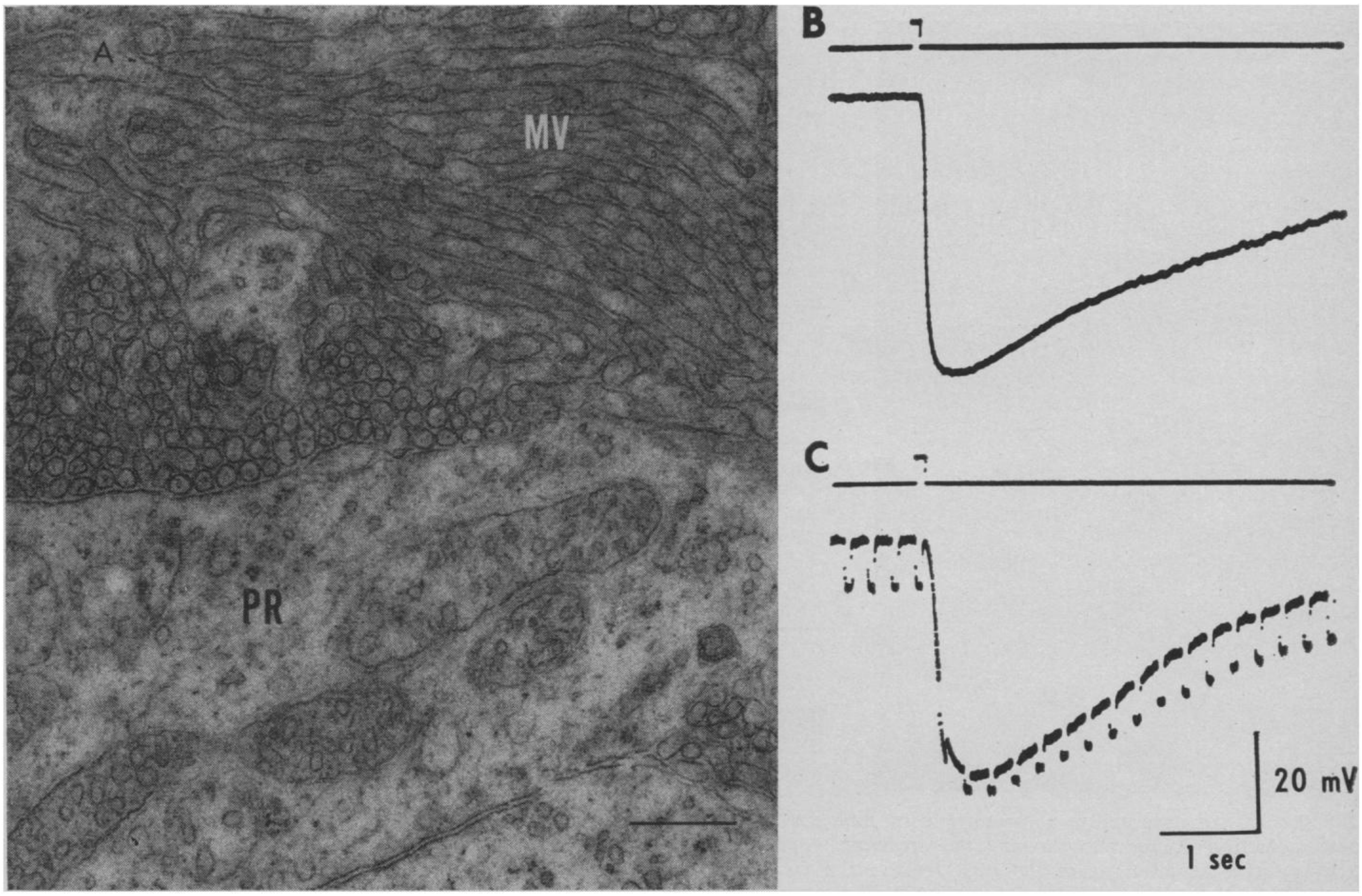

Fig. 1. Fine structure and response to light of *Salpa* photoreceptor. (A) High-power electron micrograph of microvillous region (*MV*) of photoreceptor cell body (*PR*). The microvilli are arranged in irregular arrays at the border of the cell. Bar indicates 0.5 μm. In this and in Fig. 2A, the eye was fixed in 6 percent glutaraldehyde and then treated with osmium tetroxide (*8*). (B) Response of cell to 100-msec light flash. Upper trace shows photocell monitor of light flash, and indicates zero potential level. The resting potential in this cell was — 10 mv, and the peak of the response reached —64 mv. (C) Increase in membrane conductance during the response to light is indicated by the decrease in the voltage drop produced by hyperpolarizing constant-current pulses of 0.12×10^{-9} ampere. Responses in B and C are from different cells.

vide an estimate of the reversal potential for the response. For this cell the estimated reversal potential is near zero. Thus, in its response to light and associated conductance change, the *Amaroucium* photoreceptor is similar to the vertebrate cone (*14, 15*).

Our results suggest that the apparent functional correlation between the fine structure of the photosensitive membrane of visual cells and the polarity of the receptor potential should be interpreted with care. Although it is true that all ciliary-type photoreceptors recorded from so far, both in vertebrates and invertebrates, give hyperpolarizing receptor potentials, this association may occur simply because they evolved from a common ancestral visual cell of this morphological type. The data from the salp photoreceptor shows, however, that a ciliary-type membrane specialization is not a necessary prerequisite for the generation of a hyperpolarizing response. It is likely that the ciliary and microvillous membrane specializations of photoreceptors represent separate evolutionary solutions to the same problem—that of providing a large surface area for supporting a large number of visual pigment molecules.

It would appear, however, that in the evolution of chordate photoreceptors selection has been for conveyance of visual information by membrane hyperpolarization (*16*). If the lower chordates represent variations on the development of vertebrate photoreceptors, then it seems that both microvillous and ciliary membrane specializations as well as both net increases and decreases in ionic conductance have been utilized in different chordates in the production of a common hyperpolarizing receptor potential. The evolutionary pathway for these responses remains unclear, but the possibility that the hyperpolarizing response originally preceded, and was linked to, the appearance of an "off" discharge in both primitive microvillous and ciliary-type photoreceptors, and was maintained to be utilized in its present form in chordates, is an intriguing idea.

A. L. F. Gorman

Laboratory of Neuropharmacology,
National Institute of Mental Health,
St. Elizabeths Hospital,
Washington, D.C. 20032

J. S. McReynolds

Laboratory of Neurophysiology,
National Institute of Neurological
Diseases and Stroke, National
Institutes of Health, Bethesda,
Maryland 20014

S. N. Barnes

Department of Anatomy,
University of Colorado Medical Center,
Denver 80220

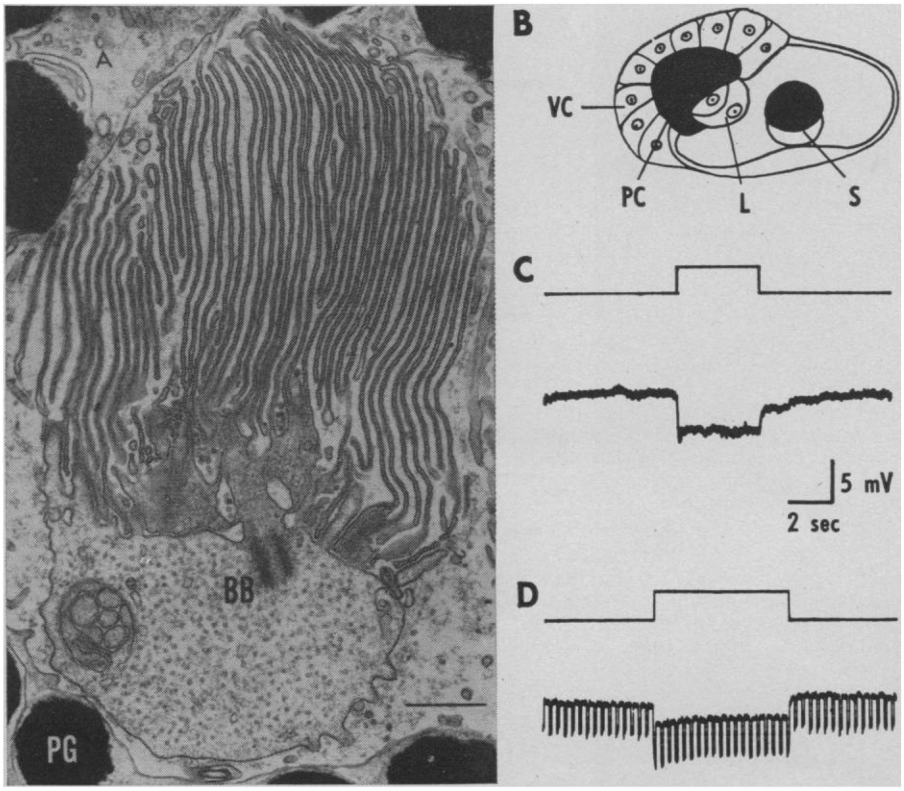

Fig. 2. Fine structure and response to light of *Amaroucium* photoreceptors. (A) High-power electron micrograph showing the array of lamellar photoreceptor membranes arising from the distal tip of the neck region of a visual cell; *BB*, basal body; *PG*, pigment granule. Bar indicates 0.5 μm. (B) Sensory vesicle of tadpole. The visual cells (*VC*) lie behind a cup of pigment cells (*PC*), but each receptor cell sends a narrow process or neck through the pigment layer toward the three-celled lens (*L*). The statocyst (*S*) is also shown. Modified from Grave (*10*). (C) Response of photoreceptor to illumination. Light monitor shown in upper trace. (D) Decrease in conductance during illumination is shown by the increase in voltage drop produced by hyperpolarizing constant-current pulses of 0.03×10^{-9} ampere. Responses in B and C are from different cells.

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6 January 1971