of the indicator MLV-MSV on plating alone yielded no foci but on plating together with the fixed dilution of Rad LV yielded 2, 1, 1, 0, and 2 foci, respectively, on five separate plates. The probability was high that these foci arose solely from cooperative infection of the MLV-MSV and Rad LV. On day 6 after infection, the cells were frozen and thawed and the cell-free fluids from individual dishes were tested for focus-forming capacity. Each of the five dishes which contained foci yielded enough virus to ensure confluent focus transformation on plating on either Swiss or C57BL mouse embryo cells.

The finding of nonreplicating, virion-associated helper activity in Rad LV stocks emphasized the potential existence of defective murine leukemia viruses whose biological activity could become apparent only after coinfection of a leukemia virus–infected cell with sarcoma virus (*3*). We have observed a degree of synergism between MLV-MSV and other leukemia virus strains also. An infection of 3T3 cell cultures with MLV alone gave less than 10^4 detectable leukemia virus helper units after 2 to 3 days of infection regardless of the number of infecting viral particles; whereas a cooperative infection of 1 leukemia virus helper unit per cell and 0.1 competent MSV per cell yielded, together with 10^4 focus forming units of MSV, more than 2×10^5 helper units of MLV on day 2. Leukemia virus titer was determined by the induction of helper activity in Swiss mouse embryo fibroblasts after 14 days by terminal dilutions of this MSV-LV yield. Our findings are compatible with the hypothesis that Rad LV is defective and that synergistic interactions occur between the sarcoma and leukemia viruses within a cell. If leukemia virus potentiation by sarcoma viruses is a general phenomenon, then a sarcoma virus infection of cells which do not overtly exhibit viral particles might elicit cryptic leukemia viruses. The utilization of this rapid assay of Rad LV could extend explorations of Rad LV tissue culture defectiveness, its manner of replication in vivo, and possibly its mode of neoplastic transformation.

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Hyperpolarizing and Depolarizing Receptor Potentials in the Scallop Eye

Abstract. *Depolarizing and hyperpolarizing responses to light were recorded intracellularly from different cells in the scallop retina. Both types of potentials appear to be primary effects of light on photoreceptor cells.*

The eye of the scallop *Aequipecten irradians* (*1*) contains two retinal layers of cells whose axons give rise to separate branches of the optic nerve (Fig. 1A). The visual cells in the distal retinal layers have a ciliated photoreceptor structure while the cells of the proximal layer possess microvilli (*2*). Although early microscopic work reported interconnections between the two retinal layers (*3*), more recent light and electron microscopic studies reveal no evidence for synaptic connections (*2, 3*). In examining the electrical responses of the two branches of the optic nerve, Hartline (*4*) found that the fibers from the proximal retinal cells discharged only upon illumina-

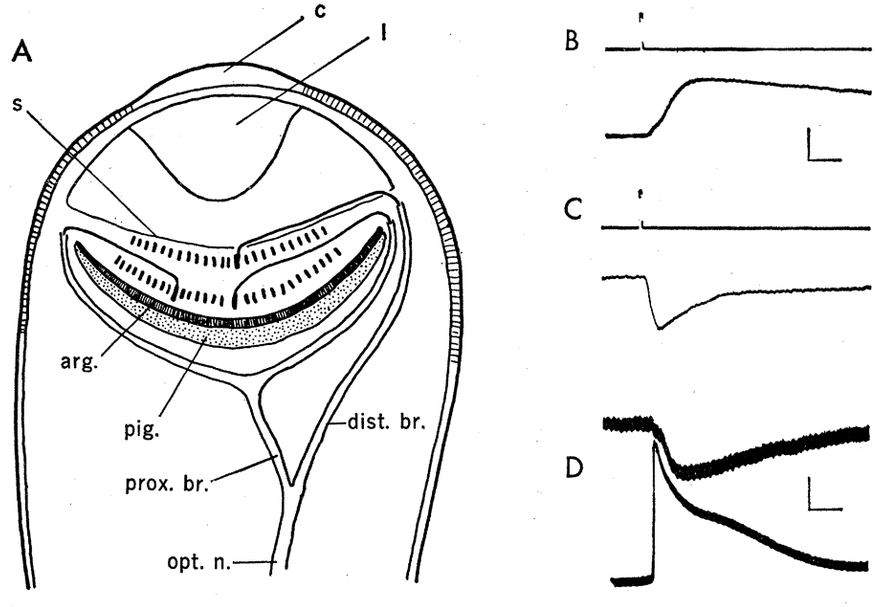

Fig. 1. Responses of visual cells in the scallop eye. (A) Schematic diagram of eye (after Dakin, 1928). In the retina one cell from the distal layer and two from the proximal layer are shown enlarged with their axons running to the respective branches of the optic nerve. Behind the retina are the argentea (*arg.*) and pigment layer (*pig.*). The proximal (*prox. br.*) and distal (*dist. br.*) branches join behind the eye to form the main optic nerve (*opt. n.*); (*c*), cornea; (*l*), lens; and (*s*), septum. (B and C) Intracellular recordings of depolarizing (B) and hyperpolarizing (C) responses to flashes of light. Photocell output monitoring light flash shown above each response. Calibration: 10 mv, 100 msec. (D) Simultaneous recording from extracellular electrodes located in proximal (upper trace) and distal (lower trace) regions of the retina. Light flash (not shown) at same time as in (B) and (C). Calibration: 0.5 mv, 100 msec for upper trace; 1 mv, 100 msec for lower trace.

tion ("on" response), while those from the distal retina gave a burst of firing upon reduction of illumination ("off" response). Land (see *5*) has shown that a pattern of moving stripes, which forms an image only on the distal cells, elicits only "off" responses in the optic nerve.

From the above evidence, one might expect that intracellular recording would reveal different types of responses from cells in the two layers. Cells in the scallop retina which hyperpolarize during illumination have been described in a recent abstract (*6*); however, no mention was made of cells that are depolarized. In order to determine the mechanism of the "on" and "off" responses in the scallop eye, we recorded from visual cells in the two layers of the retina, using both intracellular and extracellular techniques.

The eyes of the scallop are situated on the ends of short stalks in the mantle. Each eye consists of a cornea, a lens, a fibrous septum, and double-layered retina. The proximal surface of the retina is loosely covered by a reflecting argentea and a pigment layer (Fig. 1A). The eye was severed from its stalk under seawater and pinned through the remaining connective tissue with its corneal surface down. Careful removal of the argentea and pigment layers provided a full exposure of the proximal surface of the retina for microelectrode exploration and light stimulation. Potentials were recorded between a micropipette filled with 3*M* KCl and a reference electrode in the seawater bath that covered the preparation. Micropipettes of 80 to 100 megohms were used for intracellular recordings, and lower resistance pipettes of 1 to 5 megohms for recording extracellular responses. The microelectrode was connected through a capacity-compensated electrometer to a d-c amplifier. The stimulating light was obtained from a 15-watt tungsten filament and focused to a spot that covered the entire retina (about 750 μm in diameter). All responses discussed in this report are to brief (10 msec) flashes of light of fixed intensity, in dark-adapted eyes. The preparations gave stable responses to light for several hours.

Intracellular recordings were difficult to obtain and cells were usually held for less than 1 minute; this is probably related to the small size of the cells [4 to 5 μm in diameter (*2*)]. Penetration of a cell was evidenced by a sudden drop in potential to a level of 20 to 60 mv inside negative. Two types of units could be distinguished in the same eye according to their location in the retina and their response to illumination. One type, which was always encountered near the proximal surface, responded to the light flash with a depolarizing potential change (Fig. 1B). The second type, found more distally in the retina, responded with a hyperpolarizing potential change (Fig. 1C). No significant differences in resting potential were noted in the two cell types. The two types of responses showed a marked difference in latency: the onset of the hyperpolarizing potential was generally 15 to 25 msec after the beginning of the flash; that of the depolarizing potential, 30 to 80 msec. The hyperpolarizing responses also had a much faster time-to-peak (see Fig. 1, B and C). When spikes were seen in cells hyperpolarized by light, firing was absent during hyperpolarization, and, with flashes of longer duration, a burst of impulses occurred as the potential returned to the resting level. However, in most cells action potentials were not recorded and, when present, disappeared soon after penetration.

Potentials recorded with extracellular microelectrodes support the hypothesis that the depolarizing responses arise from cells in the proximal layer and the hyperpolarizing responses from cells in the distal layer. When the electrode was in the proximal portion of the retina the response was essentially a slow, negative-going potential. This response gradually changed to a larger, positive-going potential as the electrode was advanced more distally. The responses from the distal part of the retina were again of shorter latency and time-to-peak. Figure 1D shows responses to a single light flash recorded simultaneously from two different depths in the retina; the response shown in the upper trace was recorded from an electrode located near the proximal surface, and that in the lower trace was obtained from a more distal electrode. The difference in polarity and time course of the two extracellular responses indicates that they arise from two different sources that are segregated into separate layers in the retina.

Our results show the existence of two groups of cells in the scallop retina which respond to light with potentials of opposite polarity. From their position in the retina and their response to illumination, the depolarizing type probably corresponds to Hartline's "on" cells (proximal cells) and the hyperpolarizing type to his "off" cells (distal cells). It seems well established histologically that the axons in the proximal and distal nerve arise directly from the proximal and distal cells, and that there are no additional neurons in or near the retina (*2, 3*). Hyperpolarization of the distal cells inhibits impulse activity when present, and causes a transient increase in firing on return to darkness, whereas depolarization of the proximal cells presumably causes firing of their axons during illumination. Our finding that the hyperpolarizing response has a shorter latency, together with the optic nerve responses to moving stripes (*5*) and lack of anatomical evidence for synaptic connections in the retina (*2, 3*), makes it unlikely that this response results from synaptic activity. It appears that two independent types of photoreceptors that give opposite responses to light are present in the scallop retina. Hyperpolarizing receptor responses to light may be a more general phenomenon than heretofore believed, since they have also been recorded from vertebrate cones (*7*). It will be of interest to see if a common mechanism underlies both types of response.

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