center surround antagonism (*5, 6*). Application of Co^{2+} resulted in a hyperpolarization of the HPBC and a depolarization of the DPBC, associated with the loss of light response in both cells. During the early Co^{2+} exposure the transient "on" and "off" polarizations of these cells (arrows) were briefly exaggerated. With return to the normal perfusate, the light-evoked activity of both cell types did not recover to control response amplitudes and the membrane potentials were somewhat more depolarized than those observed before cobalt exposure. These effects were probably the result of cell deterioration, a common problem encountered with bipolar cell recordings.

To the extent that the principal effect of a brief exposure to Co^{2+} is to block synaptic transmission, the findings reported here are consistent with the view that a dark-released receptor transmitter depolarizes both horizontal cells and HPBC's but that it has a hyperpolarizing action on DPBC's. These findings do not support the idea that the DPBC is activated by a light-evoked transmitter release mechanism. If this were the case, Co^{2+} should abolish the light response but have a minimal effect on the membrane potential.

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11. The loss of light-evoked receptor activity has thus far been observed in recordings from receptors that are probably cones. The response of these cells is abolished within 6 to 10 minutes. Probable rod recordings have been maintained in Co^{2+} for periods up to 25 minutes with no detectable decrease in light-evoked response amplitude. Also, we have continuously perfused the isolated retina eyecup of the rabbit with 2 m*M* Co^{2+} and have noted the persistence of the rod-dominated PIII component for 45 minutes. Thus if rods are affected by Co^{2+}, an exposure of more than 25 minutes (mudpuppy) to 45 minutes (rabbit) is required.
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Horizontal Cell Potentials: Dependence on External Sodium Ion Concentration

Abstract. *The membrane potential of the horizontal cell of the axolotl is highly dependent on the extracellular concentration of sodium. Experimental results reported here are consistent with the suggestion that in the dark the receptors release a synaptic transmitter which increases primarily the sodium conductance of the postsynaptic membrane. Externally applied aspartate or glutamate depolarizes the horizontal cell membrane and eliminates the light response of the horizontal cell. However, it appears to increase the conductances of the postsynaptic membrane to several ions rather than just to sodium ions.*

One of the most useful techniques for investigating the ionic mechanism of neural potentials is to manipulate the ionic environment of a nerve cell while recording its membrane potential intracellularly. Recently this technique has been used to study the neurons in the vertebrate retina (*1–4*). We describe here our use of this technique to study the ionic mechanism of horizontal cell potentials in the isolated retina of the axolotl (*Ambystoma mexicanum*). The fact that the horizontal cell is a second-order neuron complicates the interpretation of results obtained by altering the ionic environment. Manipulations of ionic environment may influence receptor potentials and the synaptic interactions between receptors and horizontal cells, as well as the ionic mechanism of horizontal cells themselves. Therefore, we recorded intracellularly from both receptors and horizontal cells while we changed the ionic composition of the medium flowing over the receptor side of the retina.

In both rods and cones, illumination elicits a membrane hyperpolarization termed the receptor potential. Several studies have indicated that the receptor potential is caused by a decrease in the sodium conductance of the receptor membrane (*4, 5*). In horizontal cells, light elicits a hyperpolarization of the membrane (which we will call the horizontal cell potential), usually accompanied by a decrease in membrane conductance (*6*). It has been proposed (i) that in the dark, receptors release a transmitter which depolarizes the horizontal cells, and (ii) that the light-elicited hyperpolarization of the receptor terminals decreases transmitter release and thereby hyperpolarizes the horizontal cells (*7*). In support of this proposal, an increase in the external concentration of magnesium ions has been shown to hyperpolarize the horizontal cell membranes (*3, 8, 9*). This is the expected result if an increase in external magnesium ion concentration interferes with the release of transmitter in the photoreceptor synapse, as in the case of other chemical synapses (*10*).

For this study, young adult axolotls were dark-adapted for at least 8 hours and partially anesthetized by placing them on ice for an hour. The eye was removed and the eyeball hemisected under dim, red light. The eyecup was submerged in a pool of fresh, oxygenated solution in a bathing chamber. The retina was gently pulled away from the pigment epithelium and positioned receptor side up. The control bathing solution was composed of 109 m*M* NaCl, 2.4 m*M* KCl, 5 m*M* dextrose, 0.5 m*M* $MgCl_2$, 0.85 m*M* $CaCl_2$, 0.6 m*M* Na_2SO_4, 0.32 m*M* $NaHCO_3$, and 2.8 m*M*

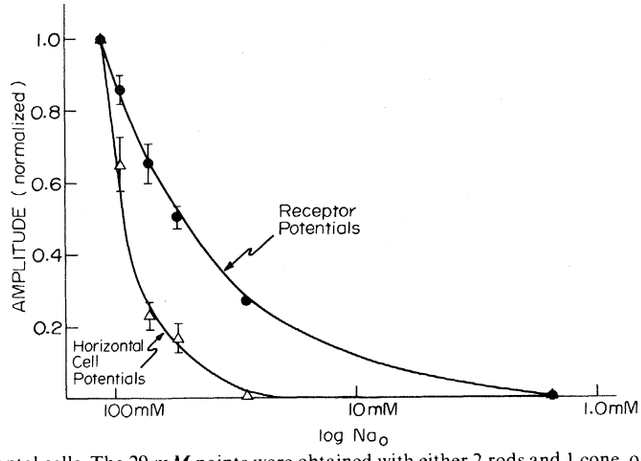

Fig. 1. Effect of decreasing Na_o on receptor and horizontal cell potentials. The horizontal cell potentials displayed much stronger dependence on Na_o than the receptor potentials. Data are from cells that recovered completely upon return to the control solution after being bathed in several different Na_o solutions. All points, except those for 29 m*M* Na_o (26 percent normal), were based on data obtained from either 14 rods and 3 cones, or 13 horizontal cells. The 29 m*M* points were obtained with either 2 rods and 1 cone, or 2 horizontal cells. For each cell, the amplitudes of the light responses were normalized with respect to the response amplitude for the same cell in the control bathing solution.

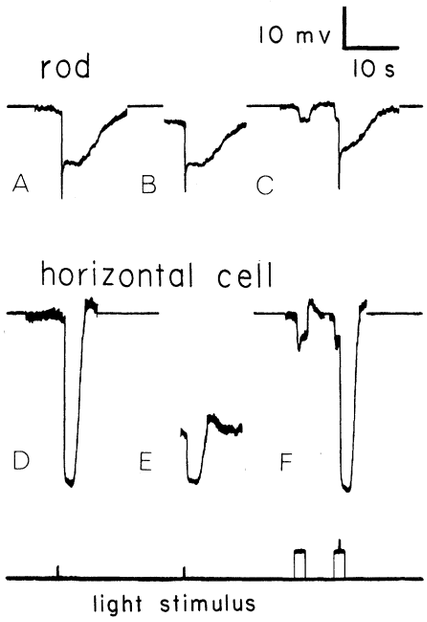

Fig. 2. Reduction of response magnitude induced by either decreased Na_o or equivalent background light. (A) Rod response to a 0.2-second test stimulus in the control solution. (B) In the 85 percent sodium solution, the rod was slightly hyperpolarized in the dark, and the response to a test stimulus was diminished. (C) Response of a rod to a 2.0-second equivalent background light (see text), followed by response to a test stimulus superimposed on the equivalent background light. (D) Horizontal cell response to a 0.2-second test stimulus in the control solution. (E) In the 85 percent sodium solution, the horizontal cell was greatly hyperpolarized in the dark, and the response to a test stimulus was greatly diminished. (F) Response of a horizontal cell to a 2.0-second equivalent background light, followed by response to a test stimulus superimposed on the equivalent background light. In the 85 percent sodium solution, the hyperpolarization of the membrane and the diminution of the response to the test stimulus were much greater than the amount of steady hyperpolarization and decrease in the light response due to the equivalent background light in the control solution.

HEPES buffer (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) adjusted to *p*H 7.8 with NaOH. The low sodium solutions were made by replacing NaCl with choline chloride. For the aspartate and glutamate experiments, 3 m*M* sodium aspartate or sodium glutamate was added to the control solution. The recording electrodes were filled with 4*M* potassium acetate. The microelectrode resistances were between 200 and 500 megohms, measured in the control solution. A 3*M* KCl-agar capillary tube, located downstream from the retina, was connected to an Ag-AgCl wire used as the reference electrode (*11*). The neurons (rods, cones, and horizontal cells) were identified on the basis of depth in the retina, wave shape of the light response, size of the receptive field, and spectral sensitivity (*12*).

We investigated the effect of decreasing Na_o (extracellular sodium concentration) to determine whether sodium might play a role both in maintaining the membrane potential of the horizontal cell in the dark and in producing the light-induced horizontal cell potential. When Na_o was decreased, the horizontal cell response diminished in size (Figs. 1 and 2, D and E) and the membrane potential hyperpolarized in the dark. However, the responses of the receptor cells, which synaptically drive the horizontal cells, were also dependent on Na_o (Fig. 1). If all the dependence of the horizontal cells on Na_o arose from the receptors, and if there were no large nonlinearities in synaptic transmission, one would expect the amplitudes of both the receptor and horizontal cell potentials to display similar dependence on Na_o. This was not the case. The horizontal cell potentials depended on Na_o much more strongly than the receptor potentials (Fig. 1).

There are at least two possible explanations for the observed difference in the dependence of the receptor and horizontal cell potentials on Na_o: either (i) the synaptic transmission is nonlinear (that is, the amount of transmitter released is not linearly related to the membrane potential of the receptors), or (ii) a sodium conductance change plays an important role in the production of horizontal cell potentials. We examined these two possibilities by comparing the reductions of response magnitude caused by steady hyperpolarizations induced by background light and by decreased Na_o (Fig. 2). In this analysis we assumed that the release of transmitter by the receptor is dependent on the receptor membrane potential, as in the case of neuromuscular junction (*13*). The dependence, however, need not be linear. In our preparation, it was much easier to record from rods than from cones; for this reason, we limited this part of our investigation to rods and rod-driven horizontal cells (*14*). After exchanging the control solution with a bathing solution containing slightly less than normal Na_o (85 percent normal), we measured the hyperpolarization of the rod membrane potential in the dark and the decrease in size of the receptor potentials (Fig. 2B). Then we determined, in the control solution, the intensity of a background light ("equivalent background light") that would produce equivalent amounts of both steady hyperpolarization and reduction in the receptor potentials (Fig. 2C). We next compared the effect on rod-driven horizontal cells of equivalent background light to the effect produced by the 85 percent Na_o solution. The hyperpolarization of the membrane and the reduction of the light-induced potentials were much larger in the 85 percent Na_o solution (Fig. 2E) than in the presence of the equivalent background light (Fig. 2F). The greater dependence of the horizontal cell membrane potential on Na_o cannot be attributed to nonlinearities in synaptic transmission, because the background light and the 85 percent Na_o solution had essentially the same effect on the receptor potentials. It is more likely that sodium is one of the ions important in maintaining the membrane potential of the horizontal cells and that a light-induced decrease in sodium conductance participates in the generation of horizontal cell potentials (*15*).

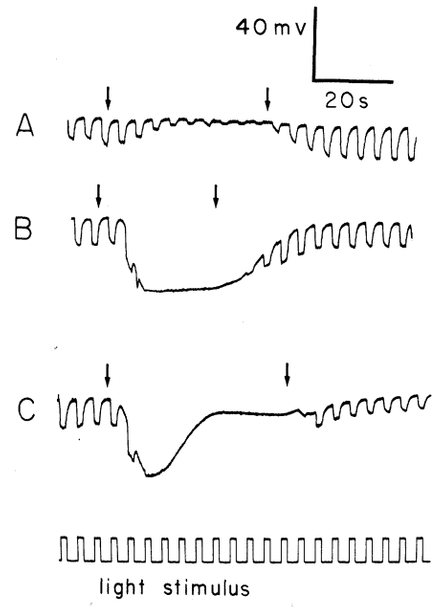

Fig. 3. Horizontal cell potentials in solutions containing aspartate. (A) Addition of 3 m*M* sodium aspartate to the control solution (first arrow) slightly depolarized the membrane and eliminated the light responses. Potentials recovered after the control solution was reintroduced (second arrow). (B) When the 1 percent sodium solution was introduced (first arrow), the membrane hyperpolarized and the light responses were eliminated. Potentials recovered in the control solution (second arrow). (C) In 3 m*M* aspartate plus 4 percent sodium (first arrow), the membrane initially hyperpolarized but subsequently depolarized almost to the dark potential level. Potentials recovered in the control solution (second arrow).

Our data are consistent with the following picture of horizontal cell potential generation. (i) The presynaptic terminals of the receptor release a transmitter in the dark, and the transmitter increases primarily the sodium conductance (g_{Na}) of the postsynaptic membrane. Thus, g_{Na}/g_i increases (where g_i is the conductance of each of the other permeable ions). Since the sodium equilibrium potential is expected to be more positive than the equilibrium potentials of the other permeable ions, the membrane depolarizes as g_{Na}/g_i increases. (ii) A light stimulus decreases

the amount of transmitter released by the receptors. Therefore, g_{Na}/g_i decreases and the membrane hyperpolarizes.

Externally applied aspartate and glutamate are believed to isolate the receptor component of the electroretinogram by depolarizing the horizontal cell membrane and eliminating the horizontal cell potential (*1, 16*). It has been suggested that in some species one of these amino acids might be the natural transmitter released from the photoreceptor terminal (*8, 17*). When we added 3 m*M* aspartate or glutamate to the control solution, the receptor potentials were unaffected. However, in these solutions, the horizontal cell membrane depolarized slightly and the light-evoked potentials disappeared (Fig. 3A). Figure 3B shows the hyperpolarizing effect of low Na_o solution on the horizontal cell membrane (see also Figs. 1 and 2), consistent with our suggestion that sodium is needed to maintain the horizontal cell membrane potential in the dark. If this suggestion is correct, and if aspartate and glutamate mimic the natural transmitter, their application should increase sodium conductance of the horizontal cell membrane. In a low Na_o solution, with or without aspartate or glutamate, the horizontal cell should be hyperpolarized relative to its level in the normal Na_o solution because of the much lower sodium equilibrium potential. The membrane should stay at this more negative level until sodium is reintroduced into the bathing solution. We found that the horizontal cell began to hyperpolarize and lose its ability to generate the horizontal cell potentials when bathed in a low sodium (4 percent normal) solution containing 3 m*M* aspartate (Fig. 3C). Instead of remaining hyperpolarized, the membrane potential gradually depolarized to a level similar to that obtained with aspartate in the normal Na_o solution. These results are incompatible with the idea of aspartate or glutamate increasing solely the sodium conductance of the horizontal cell membrane. The effect of these externally applied amino acids might be to cause an increase of conductance to several ions.

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Selenium in Fly Ash

Abstract. *Selenium, at concentrations exceeding 200 parts per million (ppm) (dry weight), has been found in white sweet clover voluntarily growing on beds of fly ash in central New York State. Guinea pigs fed such clover concentrated selenium in their tissues. The contents of the honey stomachs of bees foraging on this seleniferous clover contained negligible selenium. Mature vegetables cultured on 10 percent (by weight) fly ash–amended soil absorbed up to 1 ppm of selenium. Fly ashes from 21 states contained total selenium contents ranging from 1.2 to 16.5 ppm. Cabbage grown on soil containing 10 percent (by weight) of these fly ashes absorbed selenium (up to 3.7 ppm) in direct proportion (correlation coefficient* r = .89*) to the selenium concentration in the respective fly ash. Water, aquatic weeds, algae, dragonfly nymphs, polliwogs, and tissues of bullheads and muskrats from a fly ash–contaminated pond contained concentrations of selenium markedly elevated over those of controls.*

Fly ash is the material trapped by electrostatic precipitators in coal-burning, electric power–generating plants. It is estimated that 26 million metric tons of this material will be produced in this country during 1975 (*1*). A very small percentage of the fly ash produced is used in concrete, ceramics, and other products; the bulk of it is disposed of in sanitary landfills. Since fly ash contains many elements and may be quite alkaline, studies of its possible utility as a soil amendment in agriculture have been conducted (*2*).

In a recent paper (*3*) mature (over 91 cm tall) yellow sweet clover (*Melilotus officinalis*) that was growing on fly ash, 4.5 m in depth, in Lansing, New York, was found to contain 5.3 parts per million (ppm) (based on the dry weight of the entire aerial portion of the plant, stems plus leaves) of selenium, according to the method of Olsen (*4*). This clover was formulated at 45 percent into a diet and fed to guinea pigs for 90 days. Liver, kidney, and muscle tissues taken from these guinea pigs after they had been killed were found to contain, respectively, 5.6, 3.3, and 0.8 ppm (dry weight) of selenium. Corresponding control tissues from animals fed diets containing 45 percent yellow sweet clover (0.07 ppm of selenium) grown on soil (Howard gravelly loam, *p*H 7) contained, respectively, 0.6, 1.3, and 0.3 ppm of selenium.

We have recently collected mature white sweet clover (*M. alba*) growing (i) on fly ash (15 m deep) at a second landfill site in Lansing, New York, and (ii) on fly ash (23 m deep) in a landfill in Endwell, New York. The selenium content of the clover at these sites was, respectively, 14 and 69 ppm (dry weight) and that of the corresponding fly ashes was 22.9 and 21 ppm. The topmost 15-cm portions of the plants containing much less stem material than the rest of the plants showed up to five times these concentrations (more than 200 ppm of selenium in the clover from the Endwell site). The honey stomachs of bees found foraging on this clover (Endwell site) were found to contain less than 0.01 ppm of selenium. This result is probably understandable since the contents of the honey stomach consist entirely of carbohydrates, and selenium is typically associated with the protein fraction of plants. This sweet clover is presently being fed to lambs and pregnant goats, and the results of milk and tissue analysis will be reported elsewhere.

Deep beds of fly ash in landfills result after many years of ash disposal from the burning of coal. It is possible that deeper