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6. The constituents of the artifical CSF in millimoles per liter were: NaCl, 127.7; KCl, 2.6; $CaCl_2$, 1.3; $MgCl_2 \cdot 6H_2O$, 0.9; $NaHCO_3$, 21.0; Na_2HPO_4, 1.3; and glucose, 3.4. The solution was prepared in pyrogen-free glassware and passed through a sterilized 0.22-μm Millipore filter.
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Inactivation of Horizontal Cells in Turtle Retina by Glutamate and Aspartate

Abstract. *Glutamate and aspartate completely suppress the activity of horizontal cells but only partially affect the response of receptor cells to light. The changes observed in the receptor responses are consistent with the interruption of a synaptically mediated process rather than with a direct action on the receptor membrane.*

In vertebrates the electroretinogram consists of three major components: the a wave, attributed primarily to the activity of photoreceptors; the b wave, related to postsynaptic activities; and the c wave, presumably generated in the pigment epithelium (*1*). Acidic amino acids such as glutamate and aspartate modify the electroretinogram by eliminating the b wave and leaving a vitreous-negative component called PIII (*2*). The PIII component is believed to reflect the electrical activity of photoreceptors not complicated by the activity of other retinal elements.

Electroretinograms from retinas treated with glutamate and aspartate have been used for studying the photoreceptor response when intracellular measurements are too difficult or impossible (*3*). Consequently, many conclusions about the functional properties of photoreceptors are based on extracellularly recorded responses from retinas treated with one of these amino acids. These conclusions depend on the assumption that glutamate and aspartate selectively block the response of the more proximal retinal cells without altering the receptor response.

To test the validity of this assumption, we studied the effect of glutamate and aspartate on the intracellularly recorded response of receptors and horizontal cells in the perfused retina of the turtle (*Pseudemys scripta elegans*). This research is a continuation of our studies on skate retina (*4*).

The turtle eye was removed and cut along a medial lateral axis. After the vitreous chamber was drained, the eyecup was mounted in a chamber where oxygenated and buffered Ringer solution continuously flowed over the vitreous side at 4 to 5 cm^3/min. The ionic composition of the Ringer solution used was similar to that of cerebrospinal fluid of the turtle (*5*). Test solutions contained glutamate or aspartate substituted for equimolar amounts of sodium chloride. Intracellular recordings were made by conventional methods with high-impedance (200- to 500-megohm) glass microelectrodes filled with 4*M* potassium acetate.

The retina was stimulated by white light from a tungsten quartz iodine lamp (*6*) run from a regulated 7-amp d-c supply. The reduced image of a circular diaphragm was focused on the retina with a diameter that could be varied between 60 and 2000 μm. The light intensity was attenuated by a pair of

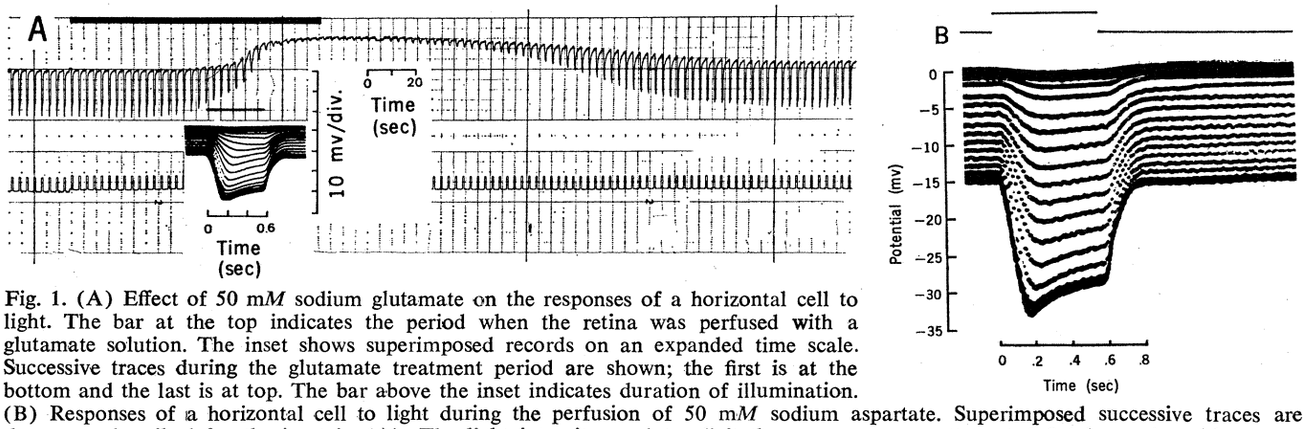

Fig. 1. (A) Effect of 50 m*M* sodium glutamate on the responses of a horizontal cell to light. The bar at the top indicates the period when the retina was perfused with a glutamate solution. The inset shows superimposed records on an expanded time scale. Successive traces during the glutamate treatment period are shown; the first is at the bottom and the last is at top. The bar above the inset indicates duration of illumination. (B) Responses of a horizontal cell to light during the perfusion of 50 m*M* sodium aspartate. Superimposed successive traces are shown, as described for the inset in (A). The light intensity used to elicit the responses was attenuated 2.4 log units with respect to the total available energy. The area of retina illuminated by the spot was 500 μm. The zero level of membrane potential is arbitrary and indicates the final depolarization reached by the membrane during perfusion of aspartate. The upper bar at the top indicates the illumination period.

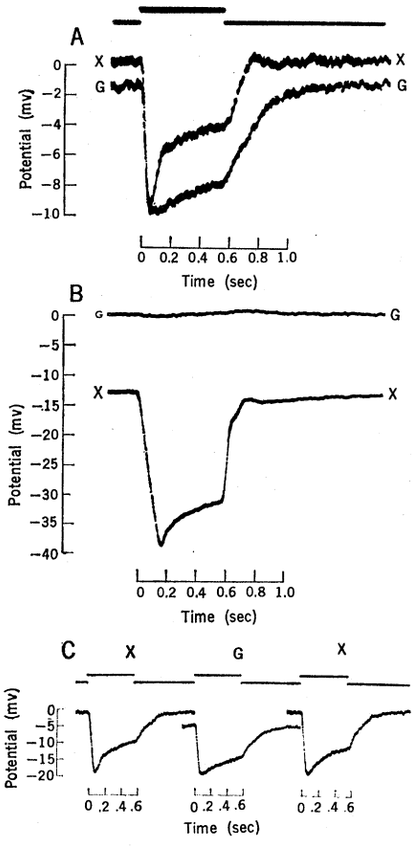

Fig. 2. (A and B) Effects of sodium glutamate on the activity recorded from a receptor (A) and from a horizontal cell (B). Responses to light were obtained during perfusion of control Ringer solution (*X*) and during subsequent perfusion with 50 m*M* sodium glutamate (*G*). The light intensity used was attenuated 2 log units with respect to the maximum available energy; the area illuminated was 1000 μm in diameter. The raised bar at the top indicates duration of illumination. (C) Recovery of photoreceptor responses after treatment with 100 m*M* sodium glutamate. The records were obtained during perfusion with control Ringer solution (*X*, left); after the retina was perfused for 15 minutes with 100 m*M* sodium glutamate (*G*, center); and after the glutamate-treated retina was again perfused with control solution for 15 minutes (*X*, right). Light intensity was attenuated by 2 log units; the diameter of the spot was 500 μm. The raised bars at the top indicate periods of illumination.

counterdriven neutral density wedges. The irradiance of the unattenuated light of wavelength between 4000 and 8000 Å was about 4.5×10^3 μw/cm^2. Receptor and horizontal cell responses were identified by the criteria of Baylor and Fuortes (*7*) for the turtle.

The effects of 50 m*M* sodium glutamate on the response of a horizontal cell are shown in Fig. 1A. Shortly after the test solution was substituted for the control Ringer solution, the membrane became depolarized and the response to light was gradually reduced. After the retina was returned to the control solution, both the membrane potential and the response to light recovered.

The effects produced by 50 m*M* sodium aspartate are shown in Fig. 1B. As with glutamate, the cell became depolarized and the response to light decreased concurrently. This is shown by a series of superimposed records; the first record (at bottom) is the normal response. The final record (at top) shows a depolarization of approximately 15 mv in darkness and the elimination of the response to light.

The effects of glutamate on intracellular records from a photoreceptor and a horizontal cell in the same preparation are shown in Fig. 2. The receptor (Fig. 1A) can produce a response to light when the horizontal cell is depolarized and unresponsive (Fig. 2B). Also, the drug produces a moderate hyperpolarization of the receptor membrane and alters the response to light by decreasing the ratio between the amplitudes of the peak and the plateau (Fig. 2A).

In many cases these changes could be reversed when the preparation was again perfused with normal fluid (Fig. 2C). Identical results were obtained with aspartate.

The peak relative amplitudes of responses to increasing intensities of light were measured for a receptor perfused with normal Ringer solution and with aspartate or glutamate. In both conditions the range of light intensities to which the receptor responded was approximately the same.

Our results indicate that aspartate and glutamate are effective in suppressing the activity of horizontal cells, whereas the photoreceptors are still able to signal light changes over a normal range of light intensities. However, the receptor response is appreciably modified by these agents. The more significant change appears to be in the shape of the hyperpolarizing response to a step increase in light covering a large area of retina. With these stimuli, the ratio between peak and plateau amplitudes decreases because the delayed depolarization that is present in the normal response of turtle cones is eliminated. This delayed depolarization has been ascribed to a feedback on the photoreceptor from horizontal cells (*8*). Because glutamate and aspartate block the horizontal cell response, the feedback on the receptors is removed; consequently, the delayed depolarization of the photoreceptor response is eliminated. Hence, the effects of low concentrations of glutamate and aspartate on the photoreceptor responses may be ascribed to interruption of a synaptically mediated process rather than to a direct action on the receptor membrane.

The mechanism by which these agents selectively act on the postsynaptic elements is not clear. If the interpretation that a depolarizing transmitter is released by receptors in darkness (*9*) is correct, our results could be obtained if these agents caused a depolarization of the horizontal cell membrane to an equilibrium level by acting on either the postsynaptic receptor sites or the entire horizontal cell membrane. The present experiments do not distinguish between these possibilities. However, they do show that glutamate and aspartate abolish the horizontal cell response and leave a receptor response unmodified by the feedback from horizontal cells (*10*).

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