diac abnormalities (*3, 21*) and, less often, mental retardation (*22*). The transcriptional data that have been presented for the DMD gene thus far account for the skeletal and cardiac muscle defects, but do not explain the mental retardation. When more is understood about the DMD and surrounding Xp21 loci, the mental retardation seen in some DMD boys may be explained. Further insights into the basic biochemical defect in DMD must await specific protein studies with antibodies directed against the DMD protein product, analyses of the specific mutations giving rise to the DMD phenotype, and cell-based expression studies.

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11. The number and positions of the RNA samples on the Northern blots that were reported previously (*6*) were incorrectly assigned. The human DMD gene transcription attributed to expression in small intestine and lung was instead due to expression in human fetal heart. Later experiments have shown that the expected 14-kb human DMD transcript is present in fetal heart at levels equivalent to those found in fetal skeletal muscle.
12. The cDNA library was constructed using a modification of the oligo(dT) primed, ribonuclease H procedure of U. Gubler and B. J. Hoffman [*Gene* **25**, 263 (1983)], with modifications suggested by Stratagene's librarian, H. Short, including a Sepharose 4B exclusion column to eliminate Eco RI linkers and cDNA molecules shorter than 600 bp. We used the vector λ gt10 [T. V. Huynh, R. A. Young, R. W. Davis, in *DNA Cloning*, D. M. Glover, Ed. (IRL Press, Washington, DC, 1985), vol. 1, pp. 49–78].
13. Mouse genomic locus size was calculated as follows; mouse genomic DNA, digested with Hind III, was fractionated on a 10-cm, 0.7% agarose gel, transferred to nitrocellulose (*24*) and hybridized with five overlapping subfragments of MC2-6 (Fig. 2). Two additional, weakly hybridizing Hind III fragments were observed but are not evident in this figure. The single-copy genomic fragments detected by the five probes are 22 kb, 12 kb, 11 kb, 8.5 kb, 8 kb, 5.9 kb, 5 kb, 4.9 kb, 4.3 kb, 3 kb, 2.1 kb, and 1.1 kb. These fragments total more than 90 kb and represent a minimum estimate of the genomic size as there are probably many more Hind III genomic DNA fragments lying between those detected by our probes (*6*).
14. Hydropathy measurements and plots, protein secondary structure predictions, NBRF (National Biomedical Research Foundation) protein sequence database searches, and lowered stringency homology searches between myosin and the DMD protein were done on the BIONET (Palo Alto, CA) or the Howard Hughes Medical Institute (Harvard Medical School, Boston, MA) computer systems. Both the University of Wisconsin Genetics Computer Group (U-WISC) and Intelligenetics (BIONET) software packages were used. α helix–β sheet propensities were calculated according to P. Y. Chou and G. D. Fasman [*Biochemistry* **13**, 222 (1974)]. Hydropathy measurements were calculated according to Kyte and Doolittle [*J. Mol. Biol.* **157**, 105 (1982)]. Figure 4 shows the hydropathy plot output of the U-WISC PEPPLOT program.
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Depolarization Without Calcium Can Release γ-Aminobutyric Acid from a Retinal Neuron

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Calcium influx is often an essential intermediate step for the release of neurotransmitter. However, some retinal neurons appear to release transmitter by a mechanism that does not require calcium influx. It was uncertain whether depolarization released calcium from an intracellular store or released transmitter by a mechanism that does not require calcium. The possibility that voltage, and not calcium, can regulate the release of transmitter was studied with pairs of solitary retinal neurons. Horizontal and bipolar cells were isolated from fish retinas and juxtaposed in culture. Communication between them was studied with electrophysiological methods. A horizontal cell released its neurotransmitter, γ-aminobutyric acid, when depolarized during conditions that buffered the internal calcium concentration and prohibited calcium entry. The speed and amount of material released were sufficient for a contribution to synaptic transmission.

CALCIUM-TRIGGERED EXOCYTOSIS operates to release transmitters at neuromuscular junctions, between peripheral neurons, and in the brain. Synapses that utilize this mechanism are often recognized by two features: first, an aggregation of vesicles marks each presynaptic site and second, Ca^{2+} is required for transmitter release. Exceptions to this ubiquitous mechanism may now have been identified in the distal retina (*1, 2*). Studies of its anatomy and physiology indicate that both photoreceptors and horizontal cells make synapses that operate differently.

Photoreceptors make two morphological types of synapse (*3*): one has an aggregation of vesicles at a release site, the other is vesicle poor. Isolated photoreceptors release transmitters by two mechanisms (*2*): one requires extracellular Ca^{2+}, the other continues in the absence of Ca^{2+}. Moreover, one component of the normal synaptic transmission from photoreceptors to postsynaptic cells requires extracellular Ca^{2+} (*4*), while another component functions without extracellular Ca^{2+} (*5*).

Complementary observations have been made for horizontal cells. They make synapses that lack vesicles (*6*) and release the

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transmitter GABA (γ-aminobutyric acid) when depolarized in a medium that lacks Ca^{2+} (*1, 7, 8*). Release has been suggested to involve a membrane carrier that also functions in GABA uptake. During hyperpolarization the carrier would move GABA into a cell; during depolarization the carrier would cycle backward and move GABA outward. However, the evidence that intracellular Ca^{2+} was not involved was indirect and doubt remained as to whether neurons use more than one mechanism for releasing transmitter. I now report that depolarization alone can regulate the release of GABA and provide evidence that the effect of depolarization is exerted by control of a membrane carrier.

The catfish (*Ictalarus punctatus*) retina has two types of horizontal cells that are large and suitable for electrophysiological and optical experiments. One receives input from cones and avidly accumulates GABA; the other receives input from rods and does not accumulate GABA (*9*). When retinas are dissociated, each type is easily distinguished by either visual (Fig. 1A) or electrophysiological criteria (*10*).

"Cone" horizontal cells were loaded with the Ca^{2+}-sensitive dye fura-2 (*11*) and penetrated with a micropipette. A single-micropipette voltage clamp (*12*) was used to measure the current-voltage relation while a cell was bathed first in a Ca^{2+}-rich medium (5 m*M* Ca^{2+}; Fig. 2A, curve 1) and afterward in a Ca^{2+}-poor medium (less than 10 μ*M* Ca^{2+}; Fig. 2A, curve 2). The Ca^{2+} current was reduced at least 20-fold by removing external Ca^{2+}.

Internal Ca^{2+} concentration was estimated from the fluorescence of intracellular fura-2. When a cell was bathed in the Ca^{2+}-rich medium, a long depolarizing step to −10 mV produced a Ca^{2+} current that slowly declined (Fig. 2C, trace 1). The decline has been attributed to an inactivation of the current produced as Ca^{2+} enters the cell and raises the internal concentration (*13*). As expected, the fluorescence of fura-2 declined as Ca^{2+} entered and increased the cytoplasmic Ca^{2+} concentration (Fig. 2C, trace 2). In contrast, a very different behavior occurred when the same cell was bathed in a Ca^{2+}-poor medium. A long depolarizing step produced a current that was at least 20-fold smaller (trace 3) and, of particular importance, the cytoplasmic Ca^{2+} concentration detected by fura-2 fluorescence remained constant (trace 4). Comparison of the records indicates that the decrease in fluorescence (and increase in Ca^{2+} concentration) observed during trace 2 was due to Ca^{2+} entry without a component that could be attributed to the release of Ca^{2+} from an internal store.

Thus horizontal cells can be depolarized and the internal Ca^{2+} concentration kept constant if the external Ca^{2+} concentration is reduced approximately 50 times (from 5 m*M* to less than 10 μ*M*). In subsequent experiments the external free Ca^{2+} concentration was reduced another 50-fold (to less than 20 n*M*) by the addition of 0.1 m*M* BAPTA [1,2-bis(2-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid (*14*)] to the external medium. In addition, the use of patch pipettes (*15*), rather than micropipettes, allowed the cytoplasm to be exchanged with a Ca^{2+} buffer containing 10 m*M* EGTA [ethylene glycol bis-(2-aminoethyl ether)-*N,N,N',N'*,-tetraacetic acid], 1 m*M* BAPTA, and 1 m*M* Ca^{2+}.

Bipolar cells of the goldfish (*Carassius auratus*) are large retinal neurons that readily detect the presence of GABA. The membrane currents of dissociated bipolar cells were recorded with a whole-cell voltage clamp through a patch pipette sealed to the synaptic ending (Fig. 1B). Two preliminary findings determined the final procedure. First, intrinsic membrane current became noisier as membrane voltage was hyperpolarized. Second, a bolus of GABA "puffed" over the cell from a nearby extracellular pipette produced a large current carried by Cl^-. Large responses to GABA with a minimum of intrinsic noise were subsequently obtained by filling the patch pipette with a Cl^--free solution, bathing the cells in a Cl^--rich solution, and holding the membrane voltage near zero. When GABA

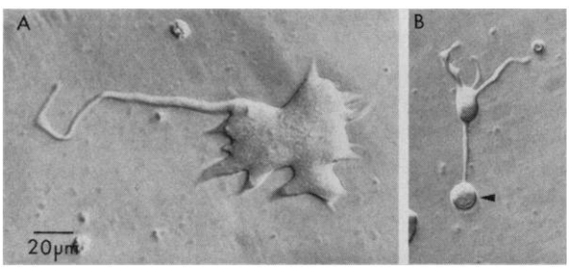

Fig. 1. (**A**) A solitary, catfish "cone" horizontal cell. An irregular, flattened soma has multiple pyramidal extensions. Often a single process extends, gradually tapers, and ends abruptly. (**B**) A solitary, goldfish bipolar cell. Multiple dendrites (two to six) arise from one pole of an ellipsoidal soma. At the opposite pole, a single process extends 5 to 40 μm to terminate in spheroidal, synaptic bulb (arrowhead). Cells were dissociated by a method similar to that described by Bader *et al.* (*12*). Catfish cells were plated into dishes whose bottom was a cover slip modified by the covalent attachment of concanavalin A (*28*). One day later, goldfish cells were plated into the same dishes. Cell pairs were usually studied 1 to 8 hours later.

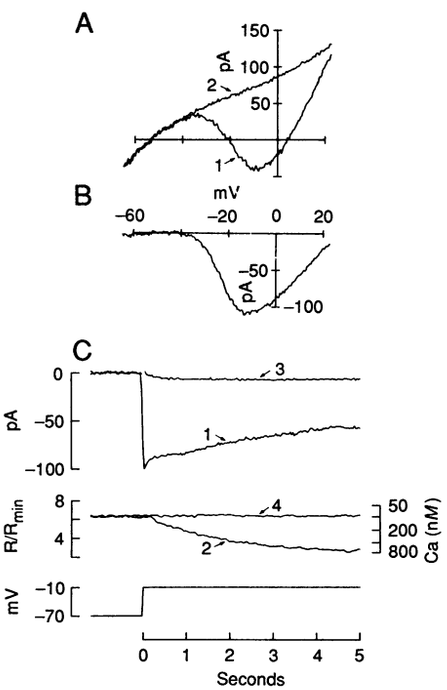

Fig. 2. Depolarization without a change in cytoplasmic Ca^{2+} concentration. (**A**) A cell was penetrated with a micropipette and membrane voltage controlled with a single-micropipette voltage clamp (*12*). A cell was bathed first in a medium containing 5 m*M* Ca^{2+} and 5 m*M* Mg^{2+} and then in one containing less than 10 μ*M* Ca^{2+} and 10 m*M* Mg^{2+}. The current-voltage relation was measured in each medium (traces 1 and 2). Trace 2 is determined primarily by the passive, resistive properties of the membrane. (**B**) The difference between traces 1 and 2 is the Ca^{2+} current. (**C**) A cell was superfused with a Ca^{2+}-rich medium. A depolarizing voltage step produced a Ca^{2+} current (trace 1). The fluorescence of fura-2, measured simultaneously (*29*), declined as Ca^{2+} entered the cell (trace 2). Next, the external solution was changed to a Ca^{2+}-poor, Mg^{2+}-rich medium. Depolarizing the same cell did not produce a Ca^{2+} current (trace 3) and did not produce a change in fluorescence (trace 4). (Depolarizing the membrane to +20 mV also did not produce a change in fluorescence.) Similar results were obtained from 11 cells. Cells were superfused with a medium containing 120 m*M* NaCl, 10 m*M* CsCl, 5 m*M* KCl, 5 m*M* $CaCl_2$, 5 m*M* $MgCl_2$, 2 mM Hepes, 5 m*M* glucose, and 1 μm of tetrodotoxin (*p*H 7.4) or a similar solution containing less than 10 μ*M* Ca^{2+} (measured by atomic absorption spectroscopy) and 10 m*M* $MgCl_2$. Cesium suppressed a voltage-activated, inwardly rectifying current carried by K^+; tetrodotoxin suppressed a voltage-activated Na^+ current. In (A) membrane voltage was changed from −65 to 25 mV at 2.7 mV/msec. Four trials were averaged for each trace. In (C), voltage steps were repeated at 22-second intervals; average responses for 12 presentations were calculated. Ca^{2+} currents were measured after linear leak subtraction. Membrane resistance was measured for steps from −70 to −40 mV. Current records were low pass–filtered with 3 db at 40 Hz; fluorescence records were low pass–filtered with 3 db at 5 Hz.

opened Cl^- channels, negatively charged ions flowed into the cell and a large outward (positive) current was recorded. Used in this way, bipolar cells were excellent GABA detectors.

Dissociated catfish and goldfish retinal cells were cocultured in dishes whose volume could be reduced to approximately 150 μl. Before an experiment, a dish was perfused (at 0.5 ml/min) with a calcium-poor solution for at least 15 minutes. The medium contained 10 m*M* Mg^{2+} to block Ca^{2+} channels and 0.1 m*M* BAPTA to chelate trace amounts of Ca^{2+}. A catfish "cone" horizontal cell and a goldfish bipolar cell could often be identified within a single microscopic field. A patch pipette was sealed onto each cell. The pipette with the attached bipolar cell was gently lifted from the dish bottom and the dangling bipolar cell moved against the horizontal cell. Then the electrical properties of both cells were controlled and observed with whole-cell voltage clamps.

Depolarizing a cone horizontal cell produced an outward current in an apposed bipolar cell. For the example shown in Fig. 3A, the membrane voltage of the horizontal cell was depolarized to a series of new values. The response of the bipolar cell increased with the size of a depolarizing step. The relation between horizontal cell membrane voltage and bipolar cell membrane current is shown in Fig. 3B. Five cell pairs showed a similar relation.

Communication between a horizontal and a bipolar cell depended on depolarization of the horizontal cell and was best when the cells were apposed (Fig. 4). Communication was consistently observed between cone horizontal and bipolar cells but not observed with three pairs formed between rod horizontal and bipolar cells.

Cone horizontal cells in teleost retinas accumulate GABA (*9*). The amount of GABA in the retina as a whole is equivalent to a uniform concentration of 3 m*M* (*16*). Therefore, the normal concentration within cone horizontal cells must be much greater. To prevent the depletion of GABA from a cell during a whole-cell voltage clamp, the patch pipette sealed to the horizontal cell contained 10 m*M* GABA. Consequently,

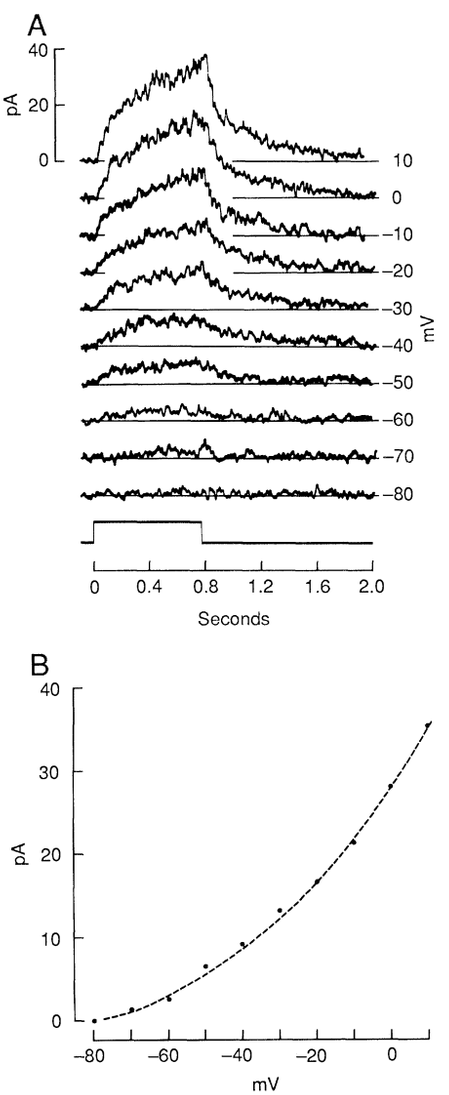

Fig. 3. Transmission from a horizontal to a bipolar cell during conditions that keep the cytoplasmic Ca^{2+} concentration constant. The extracellular medium lacked Ca^{2+} and contained the Ca^{2+} chelator BAPTA. The solutions in patch pipettes included a Ca^{2+} buffer. (**A**) Membrane currents were recorded from a bipolar cell. During each trace the voltage of an apposed horizontal cell was stepped (as indicated by the timing trace) from −80 mV to a new value (written at the right of each trace). Membrane voltage of the bipolar cell was kept at −10 mV. (**B**) The bipolar cell's membrane current, measured at the end of each step, is plotted against the voltage of the horizontal cell maintained during the step. (Hyperpolarizing the horizontal cell from −80 to −120 mV did not produce a current change in the bipolar cell.) Similar results were obtained with five cell pairs. The dish was perfused with a medium containing 120 m*M* NaCl, 10 m*M* CsCl, 5 m*M* KCl, 10 m*M* $MgCl_2$, 0.1 m*M* BAPTA, 2 m*M* Hepes, 5 m*M* glucose, 1 μm of tetrodotoxin, and a *p*H of 7.4. Patch pipettes were sealed to a horizontal and a bipolar cell. The seal resistance was always greater than 5 Gohm. The pipette sealed to the horizontal cell contained 95 m*M* KCl, 10 m*M* NaCl, 10 m*M* CsCl, 1 m*M* $CaCl_2$, 10 m*M* EGTA, 1 m*M* BAPTA, 2 m*M* Hepes, 10 m*M* GABA, and a *p*H of 7.0. The pipette sealed to the bipolar cell contained 105 m*M* Cs-methanesulfonate, 1 m*M* Ca-gluconate, 10 m*M* EGTA, 1 m*M* BAPTA, 2 m*M* Hepes, and a *p*H of 7.0. The bipolar cell was lifted from the bottom of the dish and pressed against the horizontal cell. The intracellular voltage of each cell was controlled with a whole-cell voltage clamp. Records were low pass–filtered with 3 db at 40 Hz. It should be noted that the chloride gradient across the bipolar cell membrane was arranged so that communication during depolarization of a horizontal cell or the exogenous application of GABA produced an outward current. In contrast, a gap junction or artifactual coupling between the pipettes would produce an inward current.

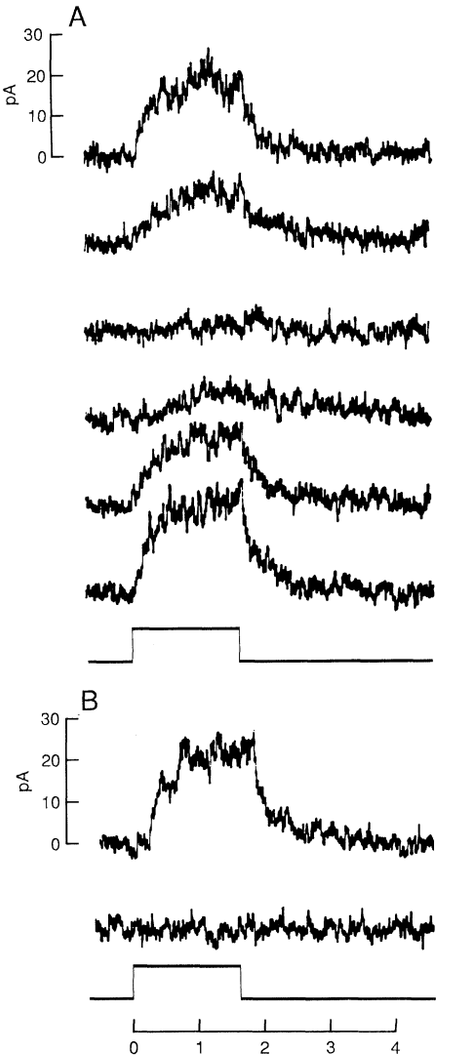

Fig. 4. Communication requires the close apposition of horizontal and bipolar cells. (**A**) The top trace is the membrane current recorded from a bipolar cell while it was apposed to a horizontal cell whose membrane voltage was stepped from −45 mV to 45 mV. The next lower trace was recorded after the bipolar cell was lifted a short distance away from the horizontal cell and the voltage step repeated. The next lower trace was recorded after the distance was further increased (the maximum separation was 10 to 20 μm). Subsequent traces were recorded as the bipolar cell was lowered back onto the horizontal cell. (**B**) The upper trace was recorded while the cells were apposed. Afterwards, the pipette attached to the horizontal cell was withdrawn to sever its connection with the cell and leave a small membrane patch occluding its tip. The pipette was returned to its original position. Now, when the patch was depolarized, the bipolar cell did not respond (lower trace). The bipolar cell membrane voltage was held at −11 mV. Records were low pass–filtered with 3 db at 20 Hz. Similar results were obtained with five cell pairs.

GABA was available and a likely candidate for mediating communication between a horizontal and bipolar cell. Four series of experiments indicated that GABA was released. (i) High concentrations of GABA similarly desensitized responses to both GABA and the released compound. (ii) GABA and the released compound both opened a Cl^- channel; responses were absent when cells were superfused with a medium that lacked Cl^- and immediately reappeared when the external medium was changed to contain Cl^-. (iii) GABA and the released compound gated channels with similar open time and amplitude; power density spectra were similar for the currents recorded from a bipolar cell while the apposed horizontal cell was continuously depolarized and afterwards while 1 μM GABA was continuously applied from a nearby pipette. [This test required that the GABA pipette be carefully positioned so that the mean amplitude of the GABA-induced current matched the mean amplitude of the signal produced by the horizontal cell (*17*).] (iv) The effect of the released compound was decreased approximately 50% by 10 μM picrotoxin, a noncompetitive antagonist of GABA's ability to open GABA-sensitive Cl^- channels. A similar dose appears to be required to block GABA-mediated transmission in the intact teleost retina (*18*). Because the released compound opened a channel whose permeant ion, amplitude, and mean open time were the same as the channel opened by GABA, and the released compound had an effect that was desensitized by exogenous GABA and was decreased by a pharmacological antagonist of GABA action, the simplest conclusion is that the released compound was itself GABA.

Like many other small molecules, GABA can be transported into select cells by a Na^+-dependent carrier. The carrier is believed to transport either two Na^+ with one GABA molecule (*19*) or two Na^+ and one Cl^- ion with one GABA molecule (*20*). In either case, since GABA has no net charge at neutral *p*H, the carrier would be electrogenic. The net direction of GABA flow would depend on the GABA concentration on each side of the membrane, the Na^+ (and perhaps Cl^-) concentrations, and membrane voltage. Because the carrier translocates a net positive charge, depolarization would increase release and simultaneously decrease uptake [see (*1*)].

GABA carriers have a specific pharmacology. Nipecotic acid (*21*) binds GABA carriers while having less affinity for GABA receptors linked to pores (*22*). In some cells, nipecotic acid binds to the carrier and is translocated quickly. The result is to make the carrier cycle and thereby increase an exchange efflux of GABA. In other cells, for example, fish horizontal cells (*8*), nipecotic acid binds to the carrier but is translocated slowly. The result is to "freeze" the carrier and to block both influx and efflux. A puff of 10 μM nipecotic acid reduced communication between a horizontal and a bipolar cell (Fig. 5A). A higher concentration (100 μM) produced a more dramatic effect and completely blocked communication (Fig. 5B). Additional experiments support the view that nipecotic acid preferentially acts upon the release process. Nipecotic acid and GABA were puffed onto solitary bipolar cells. Nipecotic acid at 10 μM did not alter the current produced by 2 μM GABA (Fig. 5C). Nipecotic acid at 100 μM reduced the effect of GABA approximately 30 to 40% (Fig. 5D). The partial reduction of the GABA-induced current produced by 100 μM nipecotic acid should be compared to the complete block of communication between horizontal and bipolar cells produced by the same concentration. Therefore, nipecotic acid appears to have two effects. Low concentrations interfere with the release of GABA but do not affect the post-synaptic receptor. This can occur if nipecotic acid binds the carrier, prevents the transport of GABA, and thereby stops the release of GABA during depolarization. High concentrations of nipecotic acid allow an additional direct action on bipolar cells. This can be explained by a weak mimetic effect of nipecotic acid for GABA at the post-synaptic receptor.

Consistent results were obtained with three cell pairs formed between rod horizontal and bipolar cells. GABA was not released from rod horizontal cells even after GABA diffused into a rod horizontal cell from a patch pipette, a result that is easily explained if rod horizontal cells lack a GABA carrier (*9*). Depolarization cannot induce carrier-mediated release when a carrier is not present.

Another type of Ca^{2+}-independent transmitter release is the nonquantal leakage of acetylcholine from motor neuron terminals at neuromuscular junctions (*23*). Unlike the release of GABA from horizontal cells, acetylcholine leakage is not influenced by elec-

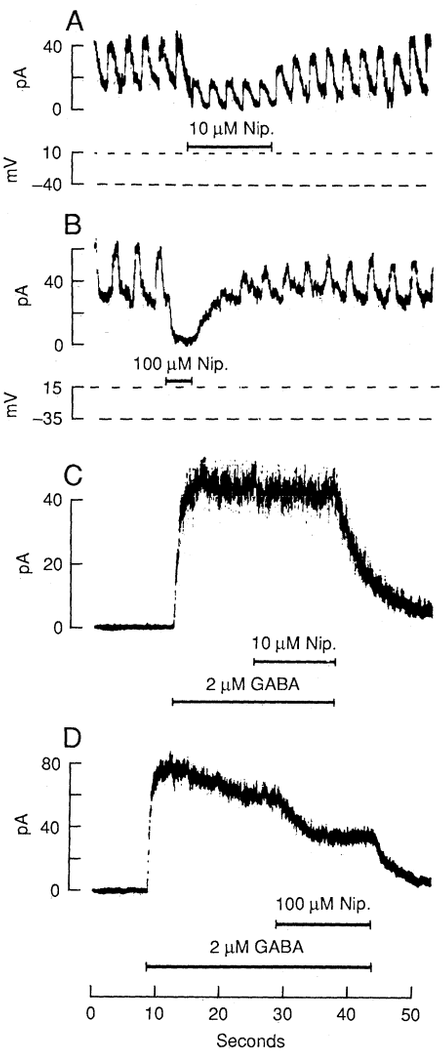

Fig. 5. Nipecotic acid acts upon horizontal cells to inhibit transmitter release. (**A**) A pipette containing 10 μM nipecotic acid was positioned near a cell pair. The horizontal cell's membrane potential was stepped from −40 to 10 mV at regular intervals. A puff of nipecotic acid (indicated by the bar) reduced the amplitude of current steps recorded from the bipolar cell. The bipolar cell's membrane potential was maintained at −15 mV. Record was low pass–filtered at 10 Hz. A similar result was obtained with four cell pairs. (**B**) A pipette containing 100 μM nipecotic acid was positioned near a cell pair. The horizontal cell's membrane potential was stepped from −35 to 15 mV at regular intervals. A puff of nipecotic acid (indicated by the bar) reduced the amplitude of current steps recorded from the bipolar cell. The bipolar cell's membrane potential was maintained at −14 mV. Record was low pass–filtered at 10 Hz. A similar result was obtained with six cell pairs. (**C**) Two pipettes were positioned near a bipolar cell. One pipette contained medium with 2 μM GABA; the other pipette contained medium with 2 μM GABA and 10 μM nipecotic acid. GABA at 2 μM produced an outward current and increased membrane current noise; 10 μM nipecotic acid produced no additional effect. The bipolar cell's membrane potential was maintained at −8 mV. Record was low pass–filtered at 40 Hz. A similar result was obtained with eight cells. (**D**) Two pipettes were positioned near a bipolar cell. One pipette contained medium with 2 μM GABA; the other pipette contained medium with 2 μM GABA and 100 μM nipecotic acid. Nipecotic acid at 100 μM reduced the GABA-induced current by approximately 40%. The bipolar cell's membrane potential was maintained at −7 mV. Record was low pass–filtered at 40 Hz. A similar result was obtained with seven cells.

trical activity (*24*). Significantly, although motor neurons have a carrier for choline, they do not appear to have a carrier for acetylcholine. The implication is that the leakage of acetylcholine is not mediated by a carrier.

The effect of voltage-dependent GABA release observed with dissociated cells is sufficiently large to contribute to synaptic transmission. The effect of release from cells in tissue may be even larger. The responses observed with dissociated cells probably underestimate the effects that occur in the intact retina. Two cells pushed together in a dish do not reconstitute the intimate contact that can occur in tissue. Diffusion of a transmitter into the large extracellular volume of a petri dish attenuates responses. The restricted extracellular space of tissue would retard diffusion and should magnify the type of responses observed during the present experiments.

Carrier-mediated release should depend on both membrane voltage and the Na^+ concentration. Although depolarization is sufficient to release GABA, changes in internal Na^+ concentration should have an added effect. Horizontal cells have sodium pores that are opened by transmitters (*25*). Constant transmitter input during darkness could raise the internal Na^+ concentration. Synaptic input may increase carrier-dependent release by both depolarizing the membrane and loading the cytoplasm with Na^+. Although the large horizontal cells of the catfish may not be susceptible to this influence, the smaller horizontal cells in other vertebrates might be more affected.

Aside from neurons, glial cells also have voltage-dependent carriers. However, the physiology of glial cells should preclude their carriers from normally operating to release transmitters. Glial cells are held at a hyperpolarized potential by a large K^+ conductance and should not release transmitters in the absence of significant changes in membrane voltage. The voltage-dependent release of transmitters from glia may be important during pathological states such as spreading depression.

The possible roles of carrier-dependent and vesicular release of transmitters in the outer retina are illustrated in Fig. 6. Horizontal cells make frequent synaptic contacts with cone photoreceptors and, depending on the species, fewer contacts with bipolar cells and infrequent contacts with other cells. The morphology of the contacts made with each cell type has been repeatedly investigated (*26*). Relatively infrequent synapses made onto bipolar cells have a presynaptic collection of vesicles. Exocytosis at these sites is presumably regulated by Ca^{2+} entry and a rise in the cytoplasmic Ca^{2+} concentration. The invaginating contacts made with photoreceptors lack vesicles. These vesicle-poor contacts between horizontal cells and photoreceptors may be characteristic of carrier-mediated release. Photoreceptors, like horizontal cells, also use two mechanisms for releasing transmitter (*2*) and have two morphological types of synapse (*3*).

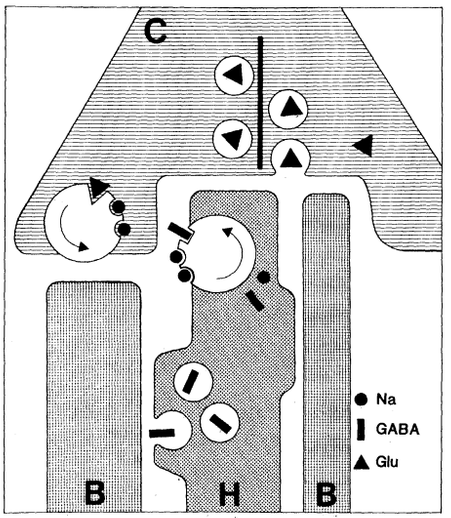

Fig. 6. The possible roles of carrier-dependent and vesicular release of transmitters in the outer retina. Cone photoreceptors make two morphological types of synapse and have two mechanisms for releasing their transmitter, which is probably glutamate. Part of glutamate's release is mediated by a voltage-sensitive, Na^+-cotransport carrier. The distribution of carrier molecules is not yet certain. They may be distributed diffusely in the surface membrane or be concentrated at vesicle-poor basal contacts. In addition, a major part of glutamate's release is by Ca^{2+}-dependent exocytosis at contacts where vesicles crowd about an intracellular ribbon (indicated by a dense bar in the drawing) situated opposite two or three postsynaptic processes. Horizontal cells can also make two types of synapse. GABA is the transmitter used by one type of horizontal cell in teleost, reptilian, and amphibian retinas. The predominant mechanism for GABA release utilizes a voltage-sensitive, Na^+-cotransport carrier. Frequent vesicle-poor contacts made with cones may be sites of carrier-mediated release. In addition, there are, in some species, infrequent morphological synapses characterized by an aggregation of vesicles. In the drawing C is a cone, H is a horizontal cell, and B indicates bipolar cells. Horizontal and bipolar cell process are drawn at a ribbon synapse; a bipolar cell is drawn receiving a basal contact.

It is not clear whether carrier-mediated release operates only at discrete foci or is diffusely distributed throughout the cell surface membrane. Communication always occurred when a cone horizontal cell was apposed to a GABA-sensitive bipolar cell. This may indicate that release is diffusely distributed. However, no attempt was made to observe foci or "hot spots" for release.

The electrical responses of horizontal and photoreceptor cells are well suited to control carrier-mediated release. These cells do not normally produce action potentials. They are depolarized during darkness. A flash of light produces a hyperpolarizing wave that can last several hundred milliseconds. A slow, graded response may be needed to regulate carrier-mediated release. In contrast, cells with regenerative, relatively rapid action potentials may require the quick mobilization of transmitter that is characteristic of vesicle release. Of course, photoreceptor and horizontal cells may use both processes to advantage. Since carrier-mediated release and Ca^{2+} entry differ in their dependence on membrane voltage (compare Figs. 2B and 3B), vesicular and carrier-mediated release should behave differentially to changes in membrane voltage. Calcium-dependent release should be silent at potentials more hyperpolarized than −40 mV while carrier-mediated release should operate over the entire range of horizontal cell response.

Synaptic mechanisms discovered in the retina may serve as models for other brain regions. Most central synapses use glutamate or GABA as transmitters. Carrier-mediated release of these compounds could have a widespread role in the nervous system and may contribute to synaptic transmission in many brain regions. Tissue slices and synaptosomes release glutamate and GABA by both a Ca^{2+}-dependent and Ca^{2+}-independent mechanism when depolarized by an elevated potassium concentration (*27*). The Ca^{2+}-dependent component has been attributed to the activity of a synaptic mechanism; the Ca^{2+}-independent component has usually been ignored. Experiments with retinal cells now demonstrate that a release mechanism that does not require Ca^{2+} influx can have a role in synaptic transmission (*5*) and that depolarization can directly control transmitter release without a change in intracellular Ca^{2+} concentration.

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inwardly rectifying K^+ current. Rod horizontal cells are larger with a flatter soma composed of three to five broad, radiating parts. Rod horizontal cells have a passive resistance of 6 to 10 Gohm. They have a simpler mixture of voltage-activated currents and lack both the voltage-dependent Na^+ current and the inward rectifier.
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29. A cell was bathed for 50 minutes in the Ca^{2+}-rich medium containing an added 5 μM fura-2/AM and then superfused with medium lacking dye. A cell was illuminated during 6-second periods timed to coincide with the voltage steps. Illumination was restricted to the impaled cell by a diaphragm in the light path. The wavelength of the incident light was either 360 or 380 nm (10-nm half-bandwidth). The fluorescence emitted at 500 nm (40-nm half-bandwidth) was measured with a photomultiplier tube (Thorn-EMI, England, type 9924B) operated for photon counting. When a cell was illuminated with 360-nm light and depolarized in a medium containing Ca^{2+}, the fluorescence intensity remained constant as expected for an isoasbestic wavelength. When a cell was illuminated with 380-nm light and depolarized in a Ca^{2+} medium, the fluorescence intensity declined. The average intracellular Ca^{2+} concentration was estimated from the equation $[Ca^{2+}] = K_d(R_{max} - R)/(R - R_{min})$ in which $R = F_{380}/F_{360}$ where F_{380} is the fluorescence measured for an exciting light of 380 nm and F_{360} is the fluorescence for an exciting light of 360 nm; R_{max} is a similar ratio measured for standard solutions containing 5 mM EGTA; R_{min} is the ratio for standard solutions containing 1 mM Ca; and K_d is 170 nM (*14*). The ratio of the maximum to minimum signal was 9.34 at 380 nm and 1.04 at 360 nm. Therefore, R/R_{min} had a range from 1.0 to 9.3. Fluorescence was corrected for the background detected in a nearby, blank microscopic field. Cells that had not been incubated in fura-2/AM had no detectable autofluorescence.
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Blockade of "NMDA" Receptors Disrupts Experience-Dependent Plasticity of Kitten Striate Cortex

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Intracortical infusion of the "*N*-methyl-D-aspartate" (NMDA) receptor blocker D,L-2-amino-5-phosphonovaleric acid (APV) renders kitten striate cortex resistant to the effects of monocular deprivation. In addition, 1 week of continuous APV treatment (50 nanomoles per hour) produces a striking loss of orientation selectivity in area 17. These data support the hypothesis that crucial variables for the expression of activity-dependent synaptic modifications are a critical level of postsynaptic activation and calcium entry through ion channels linked to NMDA receptors.

AT THE TIME OF FIRST EYE OPENING in the newborn cat, most cells in kitten striate cortex are responsive to visual stimulation through either eye (*1*). During a critical period of development (the second and third months after birth in cats) these binocular connections are readily modified by visual experience (*2*). For example, a week of monocular deprivation during the critical period leaves few neurons in visual cortex responsive to stimulation of the deprived eye. This decrease in synaptic efficacy has been attributed to competition between the afferents from the two eyes, and it appears that the activation of cortical neurons is one necessary condition for such modifications to occur (*3*). However, the postsynaptic depolarization required for experience-dependent modification apparently differs from the activation threshold of Na^+-dependent soma spikes: the "modification threshold" is reached only if there is sufficient cooperativity between retinal and nonretinal inputs to cortical cells (*4*). It has been proposed that this cooperativity is required for the activation of postsynaptic Ca^{2+} conductances and that the resulting Ca^{2+} fluxes serve as a trigger for synaptic modifications (*5*).

The "*N*-methyl-D-aspartate" (NMDA) receptor could evaluate cooperativity between converging afferents of different origin and gate Ca^{2+} conductances accordingly (*6*). Activation of this receptor by the endogenous ligand, presumably an excitatory amino acid neurotransmitter, opens a channel that is permeable to Ca^{2+} ions, but only if the membrane is concurrently depolarized sufficiently to eliminate a voltage-dependent block of the channel by Mg^{2+} ions (*7–10*). That NMDA-mediated processes might be crucial for ocular dominance plasticity is indicated by the fact that NMDA receptors exist in the membranes of cortical neurons (*11*) and the fact that NMDA receptor activation is involved in the induction of activity-dependent long-term changes of synaptic transmission both in the hippocampus (*12*) and in the visual cortex (*13*).

To determine whether NMDA receptors are involved in developmental plasticity we continuously applied the selective NMDA receptor blocker D,L-2-amino-5-phosphonovaleric acid (APV) (*14*) to the striate cortex of kittens as they were monocularly deprived. Animals (age 4 to 5 weeks) were fitted with osmotic minipumps connected to 27-gauge cannulae inserted 2 mm below the cortical surface near the area centralis representation of area 17 (Table 1). These pumps delivered either 5 or 50 mM APV to the striate cortex of the left hemisphere at a rate of 1 μl (50 nmol) per hour for 1 week. The opposite hemisphere either received an infusion of saline or was left undisturbed, and served as control. At the same time, one eye was deprived of vision by monocular lid suture. After 7 days, the animals were prepared for a standard neurophysiological assay of ocular dominance and orientation selectivity (*15*). Recordings were taken from both hemispheres at a distance of between 3 and 6 mm from the infusion site. Assuming an approximately exponential dilution of the drug with increasing distance, we estimate that the concentrations of APV during the infusion were between 10^{-4} and $10^{-6}M$ at the cortical loci sampled (*16*).

Two kittens were reared in complete darkness before minipump implantation and monocular exposure to light (Table 1). In these animals infusion of 50 mM APV had three consequences (Fig. 1, A and B). (i) The ocular dominance profile in the treated hemispheres appeared to be completely unaffected by the monocular experience, as evidenced by the absence of an ocular dominance shift. (ii) In the drug-treated cortex there was a virtually complete absence of neurons with normal orientation selectivity. Most neurons had large receptive fields and responded to nonoriented visual stimuli presented to either eye. (iii) The neurons in the experimental hemispheres were less respon-

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