klinokinesis or orthokinesis will prevail.

There is a simple explanation for the aberrant behavior of mutant d4-530 based on a model of membrane potential control of chemokinesis (*15*). A hyperpolarization of the wild type in sodium acetate relative to NaCl would account for a decreased frequency of avoiding reaction and an increased velocity in the attractant. The decreased frequency of avoiding reaction (klinokinesis) dominates and the animals are attracted. A larger hyperpolarization of mutant d4-530 in sodium acetate relative to NaCl would account for the even greater decrease in frequency of avoiding reaction and increase in velocity in sodium acetate. However, the increase in velocity (due to both the increased ciliary beat and the decrease in time spent backing in the avoiding reaction) dominates and the cells are repelled by orthokinesis. Preliminary experiments show that the wild type does hyperpolarize in sodium acetate and that the mutant hyperpolarizes to a greater extent (*16*).

The discovery of orthokinesis of mutant d4-530 has led to other examples of repulsion by orthokinesis and to the development of a model for membrane potential control of chemokinesis (*15, 16*). A mathematical model is needed to determine the contribution from velocity and frequency of avoiding reaction, and hence, the dominance of klinokinesis or orthokinesis.

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11. Measurement of frequency of avoiding reaction and velocity involve temporal rather than the spatial and temporal gradients that the animals experience in the T-maze. Instead of swimming in a changing concentration of attractant, the animals are suddenly transferred from a solution without attractant to one with attractant. It is not known to what extent the measured velocities and frequencies differ from those in the T-maze. The measurements are at least qualitatively useful because animals do show strong chemokinesis in response to step gradients in a modified assay (*16*), which simulates the rapid change of attractant concentration experienced in the temporal gradient ($I_{che}$ = 0.88 for sodium acetate versus NaCl). Animals were incubated 30 minutes in control solution before transfer to control or test solution. C. Kung and E. Gee have observed the frequency of avoiding reactions decrease with time to a basal level (personal communication). Therefore, the measured frequency of avoiding reaction in control solution may be an underestimate, making the real difference in frequency of avoiding reaction in NaCl and sodium acetate even greater. The incubation procedure was used for the following reasons: (i) cells had to be washed from culture fluid, which is undefined and more variable than control buffer; (ii) any solution transferred with cells would maintain the concentrations of all chemicals except chloride and acetate in transfer to test solution; (iii) cells show geotaxis in buffer and are easily collected with a minimum of solution at the top of tubes with buffer; and (iv) variable incubations up to 30 minutes prior to chemokinesis assays showed no significant change in $I_{che}$.
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## Amacrine Cells in *Necturus* Retina: Evidence for Independent γ-Aminobutyric Acid- and Glycine-Releasing Neurons

Abstract. *About one-half of on-off ganglion cells have inhibitory postsynaptic potentials (IPSP's) which are blocked by strychnine, while the remainder have IPSP's which are blocked by picrotoxin or bicuculline. These antagonists do not abolish light activity of the presynaptic inhibitory neuron, the amacrine cell. The existence of separate γ-aminobutyric acid- and glycine-releasing amacrine cells is implied by these results.*

The amacrine cells of the vertebrate retina constitute a class of nerve cells which lack axons and have dendrites that are both post- and presynaptic. As interneurons of the inner retina, these cells receive input from bipolar cells and, in turn, form a feedback synapse onto bipolar cell terminals; "feed forward" synapses are formed onto ganglion cells and other amacrine cells (*1*). Electrophysiological studies of the mud puppy retina have demonstrated that amacrine cells are "on-off" in that they respond to the onset and termination of a light stimulus with transient depolarizations (*2*). In addition, both the dendrites and the soma of these cells generate tetrodotoxin-sensitive impulse activity (*3*).

Miller and Dacheux (*4*) have demonstrated that the amacrine cells are inhibitory to on-off ganglion cells, through a chloride-dependent, hyperpolarizing inhibitory postsynaptic potential (IPSP). Since the amino acids glycine and γ-aminobutyric acid (GABA) have been implicated as possible transmitters of amacrine cells (*5*), we studied the effects of these agents as well as their antagonists on ganglion cells and amacrine cell responses. Our findings suggest that there are two populations of amacrine cells, one of which releases GABA and the other glycine. These cells appear to be about equal in number, but in most cases a single on-off ganglion cell receives input from only one type of amacrine cell. A very few cells apparently are influenced by both types.

The studies reported here were carried out in a perfused mud puppy eyecup preparation, which has been previously described (*4*). Intracellular recordings were first obtained while perfusing the eyecup with a normal Ringer solution. After impalement and stabilization of intracellular recordings, the perfusate was changed to a test solution.

Figure 1 shows recordings obtained from three different on-off ganglion cells. In most recordings only the excitatory postsynaptic potentials (EPSP's) and IPSP's are evident, since impulse activity was usually abolished by depolarization caused by injury from electrode penetration (*4*). This recording condition usually obscured the EPSP's but enhanced the IPSP's, which are the prominent responses of the recordings. The cell illustrated in Fig. 1a was exposed to strychnine ($10^{-5}M$) for 90 seconds, which enhanced the IPSP's and also made the EPSP's more apparent. After the exposure to strychnine, a solution containing picrotoxin ($10^{-5}M$) perfused the eyecup and completely abolished the IPSP's within 2 minutes, leaving on and

off EPSP's. These results were observed without any change in membrane potential.

The cell illustrated in Fig. 1b was exposed to strychnine, which abolished the IPSP's within 2 minutes, leaving large-amplitude EPSP's. Figure 1c shows a response which was enhanced and prolonged by picrotoxin administration, but a 60-second exposure to strychnine completely blocked the IPSP's. The decline in the EPSP response at 60 seconds in Fig. 1c was due to some deterioration of the recording. Stable recordings invariably show response enhancement from strychnine, picrotoxin, or bicuculline irrespective of whether the antagonist blocked the IPSP's. We have also studied the effect of bicuculline ($10^{-5}M$) and found the action of this agent indistinguishable from that of picrotoxin. Also, strychnine is an effective blocking agent at a concentration of $10^{-6}M$. At this concentration it seems unlikely that strychnine is blocking synaptic actions other than those mediated by glycine (*6*).

Our interpretation of the findings illustrated in Fig. 1 is that the IPSP's that were blocked by strychnine are mediated by glycine-releasing amacrine cells, while the responses that were blocked by picrotoxin or bicuculline depend on GABA-releasing amacrine cells (*7*). That amacrine cells may be segregated into different populations based on transmitter identity is consistent with the results of a number of uptake studies with labeled glycine and GABA. Uptake of tritiated glycine appears largely in amacrine cells in both frog and rabbit, while GABA uptake is also found in amacrine cells (*5*). In the frog, GABA uptake has also been reported in horizontal cells and a few bipolar cells (*5*).

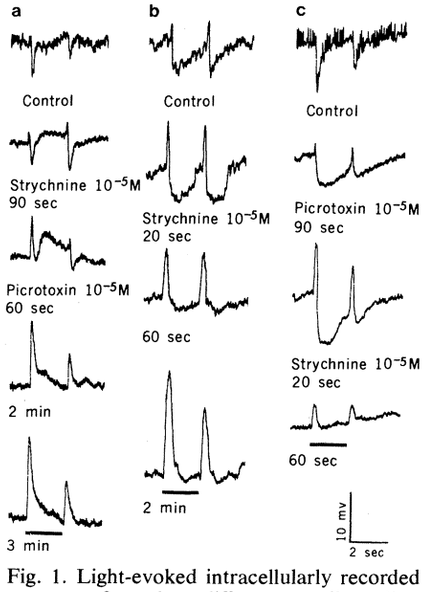


Fig. 1. Light-evoked intracellularly recorded responses from three different on-off ganglion cells. The responses of individual cells are displayed in vertical columns (a to c). All responses were evoked by diffuse light stimulation at the durations indicated. In all cases, the loss of the IPSP was not caused by changes in membrane potential, which was fairly stable during exposure to antagonists. The results are discussed further in the text.

In almost all cases, the IPSP's studied were sensitive to either strychnine or picrotoxin (or bicuculline). Of 43 cells studied, three showed some evidence of only partial IPSP block with one agent and complete block when both were added. At least one unit could not be blocked by either a glycine or a GABA antagonist (*8*).

To ascertain that the blocking action of the antagonists was localized to the ganglion cell and not mediated by suppression of amacrine cell responses, we studied the action of strychnine and picrotoxin (bicuculline) on amacrine cells. Figure 2, a and b, shows intracellular recordings from amacrine cells. The responses of these neurons consist of large-amplitude on and off EPSP's associated with impulse activity. Picrotoxin and strychnine enhance amacrine cell responses. Therefore, the action of these agents in blocking IPSP's must be at the level of the ganglion cell. One interesting difference between the amacrine cell enhancement of picrotoxin compared to that of strychnine is that picrotoxin also leads to a slowing of the declining phase of the on and off IPSP's. This suggests an additional role for GABA in modulating the wave form of amacrine cells to which glycine does not contribute. A similar difference between GABA and glycine was reported on the proximal negative response, a localized response of the inner retina, thought to reflect amacrine cell activity (*9*).

Since most ganglion cells appear to receive input from a GABA- or a glycine-releasing amacrine cell, we wondered whether the GABA and glycine receptors would be selectively distributed among the on-off ganglion cell population. The action of GABA and glycine on an on-off ganglion cell is illustrated in Fig. 2, c and d. In Fig. 2c, the preparation was perfused in normal Ringer solution. Between light stimuli, a brief current pulse ($0.1 \times 10^{-9}$ amp) was applied to the electrode and a bridge device to measure changes in input resistance. A brief exposure to GABA and glycine hyperpolarized the cell and decreased the input resistance (*10*). One criticism of the experiments represented in Fig. 2c is that the action of GABA or glycine may be mediated by activating neurons that are presynaptic to the ganglion cell (*11*). Therefore an on-off ganglion cell was made insensitive to light stimulation by previous treatment with $Co^{2+}$ (2 m*M*), which blocks chemically mediated synaptic transmission (*12*). Under these conditions, the application of both GABA and glycine resulted in hyperpolarization (Fig. 2d), suggesting that these cells have both GABA and glycine receptors. This latter result was obtained in most of our experiments. In a few cells, however, we were able to block either the GABA response or the glycine response in $Co^{2+}$. This finding suggests that some cells may have only one type of receptor and that the action of the other agent probably depends on activation of a cell presynaptic to the ganglion cell.

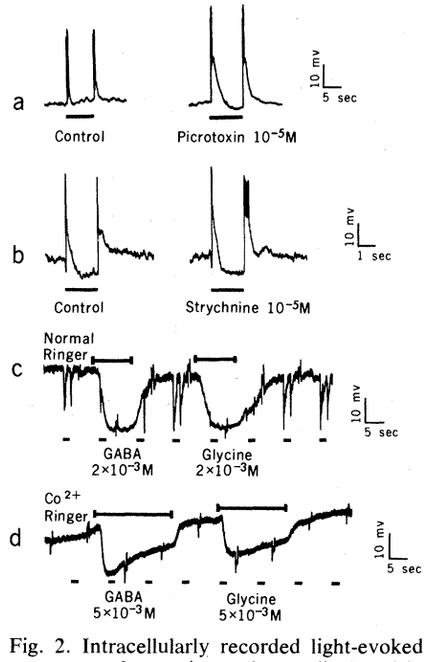


Fig. 2. Intracellularly recorded light-evoked responses of amacrine cells are displayed in (a) and (b). These responses consist of on and off EPSP's with impulse activity. (a) Picrotoxin enhanced and prolonged the declining phase of on and off EPSP's. (b) Strychnine slightly enhanced the EPSP's, but the wave form of the responses was less affected than with picrotoxin. (c) Intracellular recording from an on-off ganglion cell showing light-evoked IPSP's. (d) The light-evoked activity of an on-off ganglion cell was abolished by previous treatment with 2 m*M* $Co^{2+}$. The $Co^{2+}$ perfusate was maintained continuously while GABA and glycine were added. The action of both agents hyperpolarized the cell and resulted in a decrease in input resistance, as indicated by the more negative current pulse deflection during the action of GABA and glycine. The bars below the traces indicate the duration of a diffuse light flash, and the bars above the traces in (c) and (d) show the duration of exposure to a test solution containing GABA or glycine. The results are discussed further in the text.

The findings presented in Figs. 1 and 2 support the idea that, with respect to transmitters, two types of amacrine cells exist in the mud puppy retina. Each of these cells is inhibitory to on-off ganglion cells, but it is not certain whether there are significant physiological differences between the ganglion cells sensitive to strychnine and those sensitive to picrotoxin (or bicuculline). In the rabbit, intravenous injections of strychnine and picrotoxin had selective effects on the properties of ganglion cell receptive fields. Picrotoxin blocked motion selectivity, whereas strychnine blocked some features of other types of ganglion cells. Wyatt and Daw (*13*) concluded, as we do here, that different glycine- and GABA-releasing amacrine cells are required to explain these results.

Since most on-off ganglion cells are sensitive to both glycine and GABA, the fact that a particular cell is almost entirely influenced by one or the other implies a high degree of spatial separation in the operation of the two transmitters. It is possible that spatial separation could be based on differences in receptor distribution between dendrite and soma. Alternatively, isolation of synaptic elements by glial processes, aided by glial uptake of amino acid transmitters, could serve as a spatial buffering mechanism to maintain relative independence in transmitter systems. It seems unlikely, however, that the spatial separation of GABA and glycine action is complete. In all recordings, GABA and glycine antagonists enhanced light-evoked responses regardless of whether the agent abolished the IPSP's. Input resistance measurements show that this enhancement is associated with an increase in input resistance of the cell. It is possible, therefore, that both glycine and GABA are released in the dark, and light-evoked increases are superimposed on a continuous low level of transmitter release (*14*). A mechanism that could account for this release is suggested by experiments which demonstrated that amacrine cells are depolarized in the dark by an excitatory transmitter released by the hyperpolarizing bipolar cell (*15*). This possibility implies a subtle control system which is influenced by states of dark and light adaptation and regulates the efficiency of synaptic input to the ganglion cells.

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11. Both GABA and glycine affect amacrine and bipolar cells and one type of unidentified neuron. The action of GABA and glycine is differential with respect to bipolar cells. The depolarizing bipolar cell is more sensitive to GABA and the hyperpolarizing bipolar cell is more sensitive to glycine.
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14. Figure 1 shows that the initial action of GABA and glycine antagonists results in enhancement of EPSP's and IPSP's before any blocking action is evident. Since the intracellular recordings are probably from the soma of ganglion cells, it is possible to explain these results by assuming that the increased resistance reflects a block of continuous GABA or glycine action at the level of the soma, which initially enhances the EPSP's and IPSP's. As the antagonists diffuse to the dendrites, the primary site of synaptic input, IPSP block is obtained.
15. R. F. Miller and R. F. Dacheux [*J. Gen. Physiol.* **67**, 639 (1976)] suggested that both on-off amacrine cells and ganglion cells receive excitatory input from depolarizing and hyperpolarizing bipolar cells. The onset of a light stimulus results in an EPSP in the ganglion cell mediated by the depolarizing bipolar cell, followed by an amacrine-mediated IPSP. At the termination of a light stimulus the sequence is repeated, but the EPSP at off is mediated by the hyperpolarizing bipolar cell.
16. Supported by NIH grant EY 00844.
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## Interactions Between Rod and Cone Systems in the Goldfish Retina

Abstract. *Signals from both the rod and the cone receptor systems converge upon the same retinal ganglion cell, but only one or the other of these systems appears to be effective at any particular level of adaptation. In this report we provide evidence that the change from one receptor system to the other is not simply due to the two systems having nonoverlapping dynamic ranges; rather, there is a distance-dependent interaction between the two systems.*

It is generally accepted that in the vertebrate retina cones mediate vision under conditions of high background luminance, while rods are responsible for vision under dark adapted conditions. However, the way that the visual system accomplishes the transition from one receptor system to the other is not completely understood. One mechanism that probably plays a part in the switch from rod to cone vision is rod saturation (*1*). However, with an active process such as a nonlinear inhibitory interaction (*2*) between the two receptor mechanisms, increased cone activity could reduce the contribution of the rod system.

Experiments investigating the nature of the interaction between rods and cones have yielded inconsistent results. Whitten and Brown (*3*) found that the range of light intensities that evoked responses from both rod and cone systems in the monkey retina was greatly increased by introduction of barbiturate anesthetic, which seems to depress lateral interactions in the retina. The effects of barbiturate anesthesia are very complex, however, and it is possible that the increased dynamic range of the rods was due directly to the drug, rather than being a result of release from inhibition. Other evidence for an inhibitory interaction comes from studies of monkey ganglion cell responses (*4*), but similar experiments performed on cats failed to reveal anything except a linear summation of rod and cone signals (*5*). Psychophysical studies of rod-cone interaction have also yielded equivocal results, with some experimenters claiming evidence for an inhibitory rod-cone interaction (*6*) and others concluding that the two systems act independently of each other (*7*).

A nonlinear inhibitory interaction between rods and cones in the retina should be apparent in ganglion cell responses, since the two systems have combined at or before this level in the