Temporal Contrast Enhancement via GABA_C Feedback at Bipolar Terminals in the Tiger Salamander Retina

CUN-JIAN DONG AND FRANK S. WERBLIN
Department of Molecular and Cell Biology, Division of Neurobiology, University of California at Berkeley, Berkeley, California 94720

Dong, Cun-Jian and Frank S. Werblin. Temporal contrast enhancement via GABA_C feedback at bipolar terminals was prevented, by either removing Ca^{2+} from the Ringer solution or blocking Ca^{2+} channels with Co^{2+}. This suggests that the Cl\textsuperscript{-} current is Ca^{2+}-dependent. In those bipolar cells whose axon terminals were cutoff during slicing no Cl\textsuperscript{-} current was observed, indicating that this current is generated at the synaptic terminals. The Cl\textsuperscript{-} current consists of a predominant synaptic component that can be blocked by the non-N-methyl-D-aspartate (NMDA) glutamate receptor antagonist 6-cyano-7-nitroquinolxaline-2,3-dione (CNQX) or by the y-aminobutyric acid-C (GABA_C) receptor antagonist picrotoxin. There also exists a relatively small nonsynaptic component. Thus both glutamatergic and GABAergic transmission were involved in the generation of this Cl\textsuperscript{-} current, suggesting that it is mediated by a recurrent feedback to bipolar cells. Picrotoxin, which blocks both GABA_C receptors at BC terminals and GABA_A receptors on the dendrites of ACs and GCS, converted the light-elicited voltage response in most ON-OFF ACs and GCs from transient to sustained. Bicuculline, which blocks only the GABA_A receptors, did not prolong the transient response in ON-OFF ACs and GCs. This suggests that a negative feedback mediated by the GABA_C receptor on the bipolar terminals is responsible for making these responses transient. After the GABAergic feedback was blocked with picrotoxin the light-elicited voltage responses (recorded under current clamp) were more sustained than the current responses (recorded under voltage clamp) to the same light stimuli. This suggests that a voltage-dependent conductance converts the relatively transient current responses to more sustained voltage responses. Our results imply a synaptically driven local GABAergic feedback at bipolar terminals, mediated by GABA_A receptors. This feedback appears to be a significant component of the mechanism underlying temporal contrast enhancement in ON-OFF ACs and GCs.

INTRODUCTION

The vertebrate visual system responds best to stimuli whose intensities change with time, such as moving or flashing targets. The ON-OFF neurons in the visual pathway express this sensitivity by responding mainly at the onset and offset of the light stimulus. ON-OFF responses are first found in the visual pathway at the level of retinal amacrine and ganglion cells and are believed to be generated by synaptic interactions between bipolar and amacrine cells (Burkhardt 1972; Dowling and Werblin 1969; Toyota and Fujimoto 1984; for review see Dowling 1987). However, the exact neural mechanisms that underlie the conversion of light responses from relatively sustained at bipolar cells to transient in ON-OFF amacrine and ganglion cells are still not clear. Over the last 25 yr, a number of hypotheses have been proposed to address this question. One leading hypothesis is that the transient responses are generated by a negative feedback from amacrine cells to bipolar cell terminals that are presynaptic to transient retinal amacrine (ACs) and ganglion cells (GCs) (Burkhardt 1972; Dowling 1968; Toyota and Fujimoto 1984; Werblin et al. 1988). This hypothesis receives strong support from the findings that reciprocal synaptic aspases exist between processes of bipolar and amacrine cells (Dowling and Werblin 1969; Vaughn et al. 1981; Witkovsky and Dowling 1969; Wong-Riley 1974) and that the receptors for y-aminobutyric acid (GABA) , which is thought to be the transmitter mediating the feedback, have been identified at the bipolar axon terminals (Feigenspan et al. 1993; Karschin and Wassle 1990; Lukasiewicz et al. 1994; Maguire et al. 1989; Matthews et al. 1994; Qian and Dowling 1995; Tachibana and Kaneko 1987). Studies of the calcium dynamics in bipolar cells have demonstrated that exogenously applied GABA inhibits depolarization-induced calcium entry at the axon terminals (Lukasiewicz and Werblin 1994; Wells and Werblin 1995).

However, despite the compelling evidence listed above, direct demonstration of the feedback signal synthetically generated by excitation of a single bipolar cell, which is crucial to the feedback hypothesis, is still lacking. Furthermore, the physiological function of this proposed feedback has not been well established. The feedback is believed to make the postsynaptic response more transient, but this has not been unequivocally demonstrated. Previous studies have shown that blocking the GABA-gated chloride channels with picrotoxin prolonged both the transient proximal negative response (Burkhardt 1972), which is thought to reflect the activity of amacrine cells and the transient ganglion cell light response recorded extracellularly (Backstrom 1981). Picrotoxin also prolongs transient excitatory postsynaptic potentials (EPSPs) in mudpuppy GCs (Belgium et al. 1984). But it not known whether the picrotoxin effect was due to blockade of the feedback or feed-forward pathway or both.

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because the same GABAergic amacrine cells that form reciprocal synapses with bipolar cells have been shown to make feed-forward synapses with other amacrine cells and ganglion cells (Vaughn et al. 1981).

We attempted to demonstrate the synaptically generated feedback signal in the bipolar cell and to determine the importance of the feedback compared with the feed-forward in producing the transient responses in amacrine and ganglion cells. Direct excitation of a single bipolar cell with a depolarizing voltage pulse elicited a recurrent feedback current at its axon terminal. This feedback signal is mediated by chloride ions primarily through activation of GABAc receptors at the bipolar terminal. Blocking the feedback converted most ON-off amacrine and ganglion cells into sustained cells. Our results demonstrate a synaptically driven GABAergic feedback at the axon terminals of bipolar cells, which provides the first direct physiological evidence for the feedback hypothesis. This feedback appears to be the primary mechanism responsible for generation of the transient responses in amacrine and ganglion cells. Some of the results reported here have already appeared in an abstract (Dong and Werblin 1997).

METHODS

Preparations and cell identifications

All recordings were made from bipolar, amacrine, and ganglion cells in the tiger salamander retinal slice preparation (Werblin 1978; Wu 1987). The procedures of making the preparation and electrical recording have been described in detail elsewhere (Barnes and Werblin 1986). Briefly, after an animal was rapidly decapitated and double pithed, one of its eyes was enucleated and anterior was carefully removed. A small square section (about 4 mm²) was cut from the remaining eye cup and placed vitreal side down onto a small piece of Millipore filter. The sclera and retinal pigment epithelium layer were pulled away, leaving the retina adhering to the Millipore filter with the photoreceptor side up. The retina and filter were then sliced at 150-μm intervals. After that, the slices were transferred to the recording chamber and were positioned so that the cells on the cut surface could be viewed and recorded with patch electrodes.

The cells were initially identified by the location of their soma in the inner nuclear layer and ganglion cell layer. The cell identities were confirmed by their characteristic light responses and by Lucifer yellow staining (1 mg/ml intracellular solution). Figure 1 illustrates a Lucifer yellow filled on-center bipolar cell in a tiger salamander retinal slice. The dissection of eyes and position of patch electrodes were carried out under dim red light to preserve the light response.

Bathing medium, electrode solutions, and drug application

The bathing medium (salamander Ringer) contained (in mM) 108 NaCl, 2.5 KCl, 2 CaCl₂, 1 MgCl₂, 5 glucose, and 5 N-2-hydroxyethylpiperazine-Ν'-2-ethanesulfonic acid (HEPES), adjusted to pH 7.8 with NaOH. Two different intracellular solutions were used for bipolar cell recordings. The high chloride solution (ECl = 0 mV) consisted of (in mM) 116 CsCl, 5 HEPES, 4 ATP-Mg, and 0.5 GTP-Na₃, adjusted to pH 7.5 with CsOH. The low chloride solution (ECl = −60 mV) contained 90 K-gluconate, 16 CsCl, 10 HEPES, 4 ATP-Mg, and 0.5 GTP-Na₃, adjusted to pH 7.5 with CsOH. For amacrine and ganglion cell recordings, the intracellular solution contained: 96 K-gluconate, 10 KCl, 0.5 CaCl₂, 5 ethylene glycol-bis(β-aminoethyl ether)-Ν',Ν',Ν',Ν'-tetraacetic acid (EGTA), 10 HEPES, 4 ATP-Mg, and 0.5 GTP-Na₃, adjusted to pH 7.5 with KOH.

CNQX (6-cyano-7-nitroquinazoxaline-2,3-dione) and picrotoxin were obtained from Research Biochemicals (Natick, MA). Bicuculline methiodide was obtained from Sigma (St. Louis, MO). All drugs were dissolved directly into the bathing medium and ultrasonicated for 10 min before use. The control and test bathing media were driven by gravity and delivered to the recording chamber through a six-way valve at a rate of about 3 ml/min.

Electrophysiological recording, light stimulation, and data analysis

Whole cell recordings (Hamill et al. 1981) were performed in a plexiglass perfusion chamber mounted on a fixed stage Nikon microscope. A Zeiss ×40 water-immersion objective with Hoffman modulation contrast optics (Modulation Optics, Greenvale, NY) was used to view the cells on the surface of the slice. Responses were amplified with a List EPC-7 (Medical Systems, Greenvale, NY) patch-clamp amplifier. Patch electrodes were fabricated from borosilicate glass tubing (TW-150F-4, World Precision Instru-
ments, New Haven, CT) with a Flaming-Brown horizontal puller (model P-87, Sutter Instruments, Novato, CA). The resistance of the patch electrodes was between 5 and 10 MΩ in the normal bathing medium when filled with the pipette solution whose composition is given above. Patch electrodes were used without fire polish or coating. The electrode series resistance ranged from 8 to 20 MΩ and was not compensated, as the series resistance compensation often caused deterioration of the recordings. Membrane potentials were corrected for liquid junction potential.

Full-field light stimulation was provided by the trans-illuminator built into the microscope. Stimuli were either 550 nm (6 × 10¹² quanta/cm²/s) or white light whose intensity was adjusted to elicit responses of the same amplitude as those elicited by 550 nm stimuli. The results obtained with both types of stimuli were nearly identical although most experiments were carried out using the dim white light flashes. The duration of the light stimuli was set by a Uniblitz shutter that was controlled by a 486 computer.

The software used to generate voltage commands, acquire data, control the duration of the light stimulation, and analyze data were developed in this lab (Grant and Werblin 1994). The responses and stimuli were digitized by a Data Translation DT2828 A/D interface and recorded on an IBM compatible PC. The whole cell currents were low-pass filtered at 3 kHz and digitized at 5 kHz. Statistical data are expressed in the text as mean ± SE.

**RESULTS**

**Depolarization-elicited tail currents in bipolar cells**

According to the feedback hypothesis (Burkhardt 1972; Dowling and Werblin 1969; Toyota and Fujimoto 1984; Werblin et al. 1988), transmitter release from the bipolar terminals is made transient by a negative feedback from amacrine cells. To test this we briefly depolarized a single bipolar cell stepping from −50 to −10 mV under whole cell patch clamp. The holding potential was chosen to be −50 mV, which is about 10 mV more negative than the threshold of calcium channel activation in bipolar cells (Maguire et al. 1989), so that transmitter release from the terminal was negligible at the holding potential. Brief depolarization of a bipolar cell should cause glutamate release from its terminals, thereby exciting its postsynaptic cells, including GABAergic amacrine cells. We looked for a chloride tail current at the termination of the depolarizing step because both GABA_A and GABA_C receptors that gate chloride channels have been identified at the synaptic terminals of the bipolar cells in the tiger salamander retina (Łukasiwickicz et al. 1994). GABA released from amacrine cells after bipolar depolarization should act on these GABA receptors and open the associated chloride channels.

The normal E_Cl in bipolar cells is estimated to be between −60 and −70 mV (Tachibana and Kaneko 1987; Yamashita and Wassle 1991). At the termination of the depolarizing pulse there is ∼10–20 mV of driving force for chloride ions so an increase in chloride conductance should elicit an outward current at the terminal. However, because there might be significant electrotonic decay along the thin axon (Fig. 1) connecting the terminals to the recording site at the cell body, the feedback signal generated at the terminals may not be easily detected. To enhance the magnitude of the chloride current we used a high intracellular chloride solution for which E_Cl = 0 mV. This reversed and increased the driving force for chloride ions at the end of the depolarizing voltage step to −50 mV and resulted in a larger inward chloride tail current.

With high chloride concentration in the pipette, a relative large inward current (tail current) was recorded in most bipolar cells at the termination of the depolarizing pulse (Fig. 2). The largest currents were recorded ∼2 min after establishing a whole cell recording as E_Cl at the terminal shifted to 0 mV. The magnitude of these currents slowly decayed over the next 5–10 min, probably because of wash out of some important intracellular components critical to function of ion channels. Reducing tip size of patch electrodes slowed rundown of tail current.

**Tail current is calcium-dependent**

The tail currents appeared to be activated by synaptic mechanisms initiated by an increase in [Ca²⁺], because they disappeared after the preparation was bathed in zero calcium Ringer (Fig. 2B), or in Ringer containing 4 mM cobalt that blocked the voltage-gated calcium current (Fig. 2A).

**Tail current is mediated by chloride ions and is confined to the bipolar cell axon terminal**

The top current trace in Fig. 3A was from a typical cell with terminals, revealed by Lucifer yellow staining and recorded with a high chloride intracellular solution (E_Cl = 0 mV).
FIG. 3. A: tail current originates at bipolar cell terminals and is carried by chloride ions. Top and middle: recorded from bipolar cells with and without synaptic terminals by using electrodes filled with a high chloride intracellular solution ($E_{\text{Cl}} = 0 \text{ mV}$). Bottom: a cell with terminals but recorded with an electrode filled with a low-chloride intracellular solution ($E_{\text{Cl}} = -50 \text{ mV}$). All cells were held at $-50 \text{ mV}$ and were briefly depolarized to $-10 \text{ mV}$ for 25 ms, as indicated by upward deflection of horizontal line above current traces. Width of gray area indicates time period over which tail currents were integrated to calculate charge transfer across cell membrane (in units of pico-coulombs, pC). This charge transfer was used as a quantitative comparison of size of tail currents among cells in above 3 different groups. Integration started 10 ms after termination of depolarizing pulse to exclude capacitance transients and lasted for 200 ms. B: averaged charge transfer in 3 different cell groups. Error bars show means ± SE. Inset: light-elicited current response from cell with no terminal whose tail current is shown in A, middle.

A prominent tail current was observed in most bipolar cells recorded under these conditions (Fig. 4). However, no tail current was seen in three other bipolar cells whose axon terminals had been cutoff during slicing, even though the characteristic Landolt Club and some of the dendrites were preserved, as revealed by Lucifer yellow staining. Figure 3A (middle) shows the current response from one of these cells whose light response is shown in Fig. 3B (inset), which identifies it as an ON-bipolar cell and indicates that the cell was still functioning. This rules out the possibility that lack of the tail current was due to the fact that cell was dead. In six other bipolar cells, a low chloride intracellular solution ($E_{\text{Cl}} = -50 \text{ mV}$) was used so that there was no driving force for chloride ion on the termination of the depolarizing pulse (cells were held at $-50 \text{ mV}$). No tail current was observed in these cells (Fig. 3A, bottom).

To make a quantitative comparison of the size of the tail currents among cells in the three different groups, charge transfer (in the unit of picocoulomb, pC) across the cell membrane was calculated by integrating the tail current over 200 ms during which the tail current was most prominent. The integration started 10 ms after the termination of the depolarizing pulse (indicated by the width of the gray area in Fig. 3A) to exclude the capacitive transients. Average values for the three groups are plotted in Fig. 3B. In cells filled with a high chloride intracellular solution the average charge transfer was $22.71 ± 4.03 \text{ pC} (n = 19)$. Virtually no tail current was recorded in cells without the axon terminals or in cells with terminals but with zero driving force for chloride ions ($E_{\text{Cl}} = -50 \text{ mV}$). The average charge transfer for these two groups was $0.21 ± 0.05 \text{ pC} (n = 3)$ and $0.23 ± 0.15 \text{ pC} (n = 6)$, respectively. These experiments show that the tail current is produced at the terminals and that a driving force for chloride ions is required for generation of the current. This suggests that the current was carried by chloride ions.

**Variation of the tail current**

The amplitude of the tail current (indicated by the size of the charge transfer) varied considerably among all the different bipolar cells. Figure 4 shows the charge transfer during 200 ms period after the termination of the voltage pulse in 19 bipolar cells with intact axon terminals and recorded with electrodes filled with a high-chloride intra-
feedback to bipolar cell synaptic terminals (Yang et al. 1991).

Picrotoxin prolongs transient responses in ON-OFF amacrine and ganglion cells

The normal $E_{C1}$ is estimated to be between $-60$ and $-70$ mV (Tachibana and Kaneko 1987; Yamashita and Wassle 1991), more negative than either the threshold for calcium-channel activation or the dark membrane potential in bipolar cells, which are both about $-40$ mV (Hare and Owen 1990; Maguire et al. 1989). GABAergic feedback to the bipolar cell terminal should therefore hyperpolarize the terminal and truncate glutamate release, leading to transient responses in the postsynaptic cells (ACs and GCs). In the above experiments, we have demonstrated a GABA-mediated feedback signal generated at the bipolar cell terminal when we used a high chloride intracellular solution. To determine whether or not GABAergic feedback can produce transient responses in the postsynaptic cells at physiological values of $E_{C1}$ the, light responses in ON-OFF ACs and GCs were recorded and compared in the absence and presence of PTX (50 $\mu$M), which blocks the feedback (see Fig. 5). In most third-order neurons tested (3 of 4 amacrine cells and 8 of 9 ganglion cells), PTX made the responses much more sustained, as shown for two amacrine cells in Fig. 6 and a ganglion cell in Fig. 7. These results indicate that at physiological values of $E_{C1}$, GABAergic mechanisms can play a major role in generation of transient responses in the third-order neurons.

In about half of the third-order neurons tested (6 of 13 cells), the kinetics of the light-elicited ON responses in the presence of PTX (Fig. 6A) resembled those of depolarizing bipolar cells to a large-field stimulus in normal Ringer (Wu 1985): the light response decayed...
gradually after reaching a peak amplitude at light onset. In ~40% of the cells (5 of 13), PTX made the light-elicited ON responses even more sustained (Fig. 6C and Fig. 7C) than those of the ON-center bipolar cells (Wu 1985). This result is surprising because blocking the GABAergic feedback alone should not make the postsynaptic responses more sustained than that of the bipolar cells. One explanation is that outward rectification in the third-order neurons serves to compress the voltage response at depolarized levels obscuring the slowly decaying phase of the response (see Fig. 8).

**Feedback pathway mediated by GABA_B receptors at bipolar cell terminals is responsible for the transient responses in ACs and GCs**

The specific GABA_A receptor antagonist bicuculline (BIC) was used to infer the site of the PTX action. PTX...
Fig. 8. The input resistance of the cell (the slope of the curve in Fig. 8A) was high at potentials more negative than −50 mV, but decreased abruptly as the cell was depolarized to potentials more positive than −40 mV. At −20 mV, the upper limit of the physiological response range of ACs and GCs, the input resistance was less than one-fourth the value measured at the dark potential (−67 mV in this cell). Similar results were obtained in both amacrine and other ganglion cells. In five cells (2 ACs and 3 GCs), the average values of the input resistance at −90, −60, and −30 mV are 1.44 ± 0.13, 1.65 ± 0.14, and 0.39 ± 0.07 GΩ, respectively. Because of the compressive effect of the outward rectifier in ACs and GCs the voltage responses may be better measures of the kinetics of signal processing at the inner retina than the membrane currents.

**DISCUSSION**

A brief depolarization of a single bipolar cell can elicit chloride tail current. This current is generated at the bipolar terminal because no current exists if the terminal is missing (Fig. 3). The current is calcium mediated because it is blocked by low calcium and cobalt (Fig. 2). The current is synaptic because it is blocked by either CNQX or picrotoxin (Fig. 5). The current is mediated at GABA<sub>C</sub> receptors because its effects are not blocked by BIC (Fig. 6). These results suggest the neural circuit shown in Fig. 9. Depolarization blocks both GABA<sub>C</sub> receptors on bipolar cell terminals and GABA<sub>A</sub> on cone photoreceptor terminals (Wu 1991) and on ACs and GCs dendrites (Łukasiwicz et al. 1994). BIC acts predominantly at the GABA<sub>A</sub> receptors. In 8 of 11 ON-OFF third-order neurons tested, BIC had no effect on the light-elicited voltage responses. In the remaining three cells (1 AC and 2 GCs), BIC enhanced the voltage responses slightly, but the responses remained quite transient as shown in Fig. 6C. BIC does not prolong the transient responses of ON-OFF ACs and GCs shown in Figs. 6 and 7, which indicates that PTX acts mainly on GABA<sub>C</sub> receptors on the bipolar terminals. This conclusion is further supported by current recordings obtained under voltage clamp. In Fig. 6, B and D and 7D the contribution from the GABA<sub>A</sub> receptor mediated feed-forward pathway to the light-elicited current responses was eliminated: the cells were voltage clamped at −60 mV, which was the value of $E_{Cl}$ set by adjusting the internal solution, thereby eliminating driving force for Cl<sup>−</sup> ions. Under these conditions BIC had no effect on the current responses in 9 of 11 cells, as expected. But PTX still significantly enhanced the current responses in 18 of 19 cells. This result can best be explained by a PTX blockade of feedback inhibition mediated by GABA<sub>C</sub> receptors at the bipolar cell terminals.

**Outward rectifying membrane conductance at ACs and GCs may enhance sustained responses**

After the GABAergic feedback was blocked with PTX in most amacrine and ganglion cells, the voltage response was more sustained than the current response to the same light stimuli (see Figs. 6 and 7). This transient-to-sustained conversion could be mediated by the compressive effect of the outward rectifying membrane conductance of these cells. We tested this possibility by injecting currents of different magnitude and polarity under current clamp and measuring the resulting displacement of the membrane potential over a wide potential range spanning the physiological response range. The results from a typical ganglion cell are shown in Fig. 8A. The input resistance of the cell (the slope of the curve in Fig. 8A) was high at potentials more negative than −50 mV, but decreased abruptly as the cell was depolarized to potentials more positive than −40 mV. At −20 mV, the upper limit of the physiological response range of ACs and GCs, the input resistance was less than one-fourth the value measured at the dark potential (−67 mV in this cell). Similar results were obtained in both amacrine and other ganglion cells. In five cells (2 ACs and 3 GCs), the average values of the input resistance at −90, −60, and −30 mV are 1.44 ± 0.13, 1.65 ± 0.14, and 0.39 ± 0.07 GΩ, respectively. Because of the compressive effect of the outward rectifier in ACs and GCs the voltage responses may be better measures of the kinetics of signal processing at the inner retina than the membrane currents.

**FIG. 9.** A possible neural circuit that could underlie generation of temporal contrast enhancement in retina. PC, photoreceptor; BC, bipolar cell; AC, narrow field GABAergic amacrine cell; GC, ganglion cell. + and −: excitatory and inhibitory synapses mediated by glutamate and GABA, respectively.
tion of the bipolar cell releases glutamate that excites GABAergic ACs. These ACs in turn release GABA back onto the synaptic terminals of the excited bipolar cell to hyperpolarize the terminals and thereby truncate glutamate release. Opening the feedback loop, by blocking either the glutamatergic output from the bipolar cell or GABAergic input to the bipolar cell, eliminates the feedback signal. These results demonstrate a local, synaptically driven GABAergic feedback signal at the bipolar terminal and provide the first direct physiological evidence for the feedback hypothesis.

**Justification for using a high chloride intracellular solution**

As noted earlier, we used a high-chloride intracellular solution to enhance the feedback signal by increasing the driving force for chloride ions. We believe that the feedback signal, measured as the tail current, is not an artifact of high chloride intracellular solution. The signal (tail current) appears to be synaptically generated based on the following criteria. First, the signal disappeared after synaptic transmission was abolished by removing extracellular calcium or by blocking calcium channels (Fig. 2), indicating that a depolarization-induced increase in intracellular calcium is a prerequisite for generation the feedback signal. Second, the feedback signal was eliminated by interrupting either the glutamatergic or GABAergic transmission (Fig. 5). However, an important question remains: at physiological intracellular chloride concentration, is the feedback at bipolar terminal strong enough to produce transient responses in the postsynaptic cells? Our results suggest that feedback is sufficient because blocking this feedback with PTX converted the responses of the ON-OFF amacrine and ganglion cells from transient to sustained (Figs. 6 and 7).

**Feedback is the primary mechanism responsible for the transient responses in ACs and GCs**

A number of different mechanisms have been reported that could contribute to generation of transient responses in ACs and GCs. In dissociated mouse retinal bipolar cells, Kaneko et al. (1989) observed a transient calcium current at the synaptic terminals in response to a depolarizing voltage step. They suggested that this transient $I_{Ca}$ might be a presynaptic mechanism that enhances transient responses in ACs and GCs. Lukasiewicz et al., (1995) found in the salamander retina that desensitization of $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptors on the ganglion cells can shape transient light-induced excitatory postsynaptic currents (LEPSCs). Another mechanism that could truncate transmitter release from bipolar terminals is the calcium-dependent chloride conductance (Okada et al. 1995). Because the physiological $E_C$ in bipolar cells is estimated to be between −60 and −70 mV (Tachibana and Kaneko 1987; Yamashita and Wassle 1991), more negative than the threshold of calcium channel activation, which is about −40 mV (Lukasiewicz and Werblin 1994; Maguire et al. 1989), this chloride conductance could provide local negative feedback at the terminals when intracellular calcium concentration rises.

The contributions from the factors mentioned above are also evident in our experiments. When GABAergic feedback was blocked in the presence of PTX, the LEPSCs recorded from most third-order cells were still relatively transient (see Fig. 7D). This shows that GABAergic feedback is not the only mechanism that is involved in generation of the transient responses in ACs and GCs. However, in the tiger salamander, GABAergic feedback appears to be the primary mechanism because after this feedback is blocked with PTX the voltage response (which is the physiological response used in retinal signal transmission) of ON-OFF cells resembled that of sustained cells (Figs. 6 and 7).

**GABA$_C$ receptors are more prevalent than GABA$_A$ receptors at bipolar terminals**

Bipolar cells have both GABA$_A$ and GABA$_C$ receptors (Feigenspan et al. 1993; Lukasiewicz et al. 1994; Qian and Dowling 1995). The inability of bicuculline to convert the response from transient to sustained (Figs. 6 and 7) in ACs and GCs suggests that the GABA$_C$ receptor plays a dominant role in mediating the negative feedback. This is consistent with recent findings in salamander bipolar cells that inhibition of calcium entry at the axon terminals by GABA is mediated predominantly by the GABA$_C$ receptor (Lukasiewicz and Werblin 1994; Wellis and Werblin 1995) and that in the majority of bipolar cells the GABA-elicited chloride current is predominantly mediated by GABA$_C$ receptors (Lukasiewicz et al. 1994).

GABA receptors have been identified on cone photoreceptor terminals (Kaneko and Tachibana 1985) and are thought to mediate the negative feedback from horizontal cells. In the salamander, the GABA receptor on cone synaptic terminals are believed to be type A since the feedback-elicited depolarization of cone photoreceptors can be blocked by bicuculline (Wu 1991). We show that bicuculline had little effect on the transient responses in most amacrine and ganglion cells (Figs. 6 and 7). This suggests that blocking negative feedback at cone terminals does not contribute to PTX effect on the response kinetics of the third-order neurons.

**Feedback versus feed-forward**

Previous studies have demonstrated that those amacrine cells that make reciprocal synapses with bipolar cells are also presynaptic to other amacrine cells and ganglion cells (Vaughn et al. 1981; Wong-Riley 1974). Thus these amacrine cells can provide feed-forward as well as feedback inhibition. The feedback pathway is mediated primarily by the GABA$_C$ receptor on the bipolar terminals. The feed-forward pathway, on the other hand, is mediated mainly by the GABA$_A$ receptor on amacrine and ganglion cells, because the GABA-elicited chloride currents in those cells can be completely blocked by specific GABA$_A$ receptor antagonists (Dong et al. 1994; Lukasiewicz et al. 1994).

Blocking the GABA$_A$ receptors with bicuculline had only a small effect on the transient responses in most amacrine and ganglion cells (Figs. 6 and 7). This suggests that the feed-forward pathway contributes very little to generation of the transient responses in those cells. This finding is rather surprising in view of the fact that almost all amacrine and
ganglion cells receive strong GABA<sub>A</sub> receptor mediated inhibition in the tiger salamander retina (unpublished observations). What is the function of this feed-forward inhibition? Because those GABAergic amacrine cells that are part of the autofeedback loop (Fig. 9) are likely to be transient cells themselves the feed-forward inhibition provided by the same cells would be transient as well (strongest at the onset of the light stimulus and decays as the stimulus persists). Furthermore, the GABA<sub>A</sub> receptor desensitizes rapidly. One possible physiological role for this GABA<sub>A</sub> receptor mediated inhibition might be to expand the dynamic range of ganglion cell light responses by neutralizing the large transient excitatory synaptic currents at the light onset and offset. Alternatively, the GABAergic inputs in amacrine and ganglion cells may be involved in direction and orientation detection (Ariel and Adolph 1985; Bonaventure et al. 1985).

**Variation in the size of the feedback signal**

The size of the tail current varied considerably from cell to cell (see Fig. 4). This variation can be partly attributed to the fact that some part of the synaptic terminals may be cutoff during slicing. However, the tail current size was not always consistent with the degree of axonal arborization revealed by the Lucifer yellow staining. In the two bipolar cells that showed virtually no tail current in Fig. 4, the staining of the axon terminals was as robust as other cells that had large tail currents. It is likely that some bipolar cells receive little or no feedback inhibition because some bipolar cells must provide sustained output to drive sustained amacrine and ganglion cells. It is also possible that weak or no tail current is caused by a selective damage of GABAergic amacrine cells during slicing.

**Autofeedback versus network feedback**

Our experiments describe a type of autofeedback where the signal is elicited by the excitation of the cell from which it is recorded. It is most likely mediated by the reciprocal synapses between bipolar and amacrine cells (Vaughn et al. 1981; Wong-Riley 1974). Because those GABAergic amacrine cells that form reciprocal synapses with bipolar cells also make synapses with other bipolar cells (Vaughn et al. 1981), the feedback signals recorded at the terminals of one bipolar cell under normal light stimulation can contain two components: one from the autofeedback and the other from the network feedback. This network feedback might mediate inhibition similar to that carried out by horizontal cells in the outer retina, except that the space constant for the GABAergic amacrine cells is quite narrow (Luksasiewicz and Werblin 1990). This feedback has been implicated in a nonclassical form of local edge enhancement (Werblin et al. 1996).

**Strength of the feedback and transition between transient and sustained responses may be physiologically regulated**

Because the feedback is mainly mediated by the GABA<sub>A</sub> receptor (Figs. 6 and 7) whose activity is modulated by endogenous neuroactive substances, such as dopamine (Dong and Werblin 1994; Wellis and Werblin 1995) and zinc ions (Dong and Werblin 1995, Qian and Dowling 1995), the findings of this study suggest that the strength of the feedback, and therefore the degree of the transience of the responses of the retinal output neurons (ganglion cells), as well as the activities of their postsynaptic cells in the visual pathway, can be modulated. Thus, the dichotomy of sustained versus transient GC responses may not be as rigid as previously thought: the kinetics of the light response may change significantly in the same cell under different physiological conditions.

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Address for reprint requests: F. S. Werblin, Dept. of Molecular and Cell Biology, Division of Neurobiology, 145 LSA, University of California at Berkeley, Berkeley, CA 94720.

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