Inhibition to retinal rod bipolar cells is regulated by light levels

Running head: Regulation of rod bipolar cell inhibition

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8 figures
Number of pages: 35
Number of words: Abstract – 250

Author contributions:
E.D.E conceived the study and wrote the paper. E.D.E., R.E.M. and J.S.K. performed the experiments and analyzed the data.
Abstract

The retina responds to a wide range of light stimuli by adaptation of retinal signaling to background light intensity and the use of two different photoreceptors: rods that sense dim light and cones that sense bright light. Rods signal to rod bipolar cells that receive significant inhibition from amacrine cells in the dark, especially from a rod bipolar cell activated GABAergic amacrine cell. This inhibition modulates the output of rod bipolar cells onto downstream neurons. However, it was not clear how the inhibition of rod bipolar cells changed when the rod signaling was limited by an adapting background light and cone signaling becomes dominant. We found that both light-evoked and spontaneous rod bipolar cell inhibition significantly decreases with light adaptation. This suggests a global decrease in the activity of amacrine cells that provide input to rod bipolar cells with light adaptation. However, inhibition to rod bipolar cells is also limited by GABAergic connections between amacrine cells, which decrease GABAergic input to rod bipolar cells. When we removed this serial inhibition, the light-evoked inhibition to rod bipolar cells remained after light adaptation. These results suggest that decreased inhibition to rod bipolar cells after light adaptation is due to decreased rod pathway activity as well as an active increase in inhibition between amacrine cells. Together these serve to limit rod bipolar cell inhibition after light adaptation, when the rod pathway is inactive and modulation of the signal is not required. This suggests an efficiency mechanism in the retina to limit unnecessary signaling.

Keywords: Retina, light, GABA, glycine, patch-clamp
Introduction

The retina responds to light stimuli that vary by 10 orders of magnitude, using several different mechanisms of light adaptation. One way that the retina achieves this is to have two different photosensors – rod photoreceptors that respond to dim light and cone photoreceptors that respond to brighter light. The information from photoreceptors is separated into parallel pathways: ON cone bipolar cells (BC) that respond to the onset of bright light, OFF cone BCs that respond to the offset of bright light, and rod BCs that respond to the onset of dim light.

These BC pathways receive inhibitory input from amacrine cells (ACs) to their axon terminals that shapes their response to light (Dong and Werblin 1998; Eggers and Lukasiewicz 2006b; Sagdullaev et al. 2006). However, previous recordings of light-evoked inhibition from BCs (Eggers and Lukasiewicz 2006a; Pang et al. 2004) were made in dark-adapted retinas where rod signals are most active, it is not clear what roles cone pathways vs. rod pathways play in this inhibition.

Rod signals have a significantly slower timecourse than cone signals (Ashmore and Copenhagen 1980; Copenhagen et al. 1983; Schnapf and Copenhagen 1982), so we might expect that transitioning from rod-mediated to cone-mediated inhibition would speed up the timecourse of inhibition. Rod BCs receive large reciprocal inhibition onto GABA receptors from the GABAergic A17 AC, the only known AC to feed input solely to rod BCs (Figure 1) (Chavez and Diamond 2008; Eggers and Lukasiewicz 2010; Hartveit 1999). However, over half of the synapses onto rod BC terminals come from non-reciprocal connections (Kim et al. 1998; Strettoi et al. 1990). These glycinergic and GABAergic AC inputs come at the onset of a light stimulus, (Eggers and Lukasiewicz 2006a; Pang et al. 2004) and thus are presumably activated by ON
cone BCs, and would be active in light-adapted retinas. In dark-adapted retinas a large proportion of the inhibition to rod BCs is mediated by the GABA_C receptor, with smaller proportions mediated by the GABA_A and glycine receptors, but it is not clear how this changes with light adaptation (Eggers and Lukasiewicz 2006a; b).

Other rod-mediated inhibitory signals are transmitted through the OFF cone BC pathway. OFF cone BCs receive glycinergic inputs when rods are active through the rod BC-activated glycinergic AII ACs (Grunert and Wässle 1996; Haverkamp et al. 2003; Strettoi et al. 1994). Inputs from AII ACs are likely responsible for the large glycinergic currents seen in OFF cone BCs (Eggers et al. 2007; Ivanova et al. 2006), although they do not necessarily set the threshold for rod responses in the OFF pathway (Arman and Sampath 2012). However, dark-adapted OFF cone BCs also receive GABAergic inhibition and potentially glycinergic inhibition from other ACs (Eggers et al. 2007; Ivanova et al. 2006). It is not known how the inhibition in light-adapted conditions varies from this.

Additional components that can modulate BC inhibition are inhibitory connections between retinal ACs. BC inhibition is suppressed by GABA_A receptor mediated connections between ACs (Eggers and Lukasiewicz 2006a; 2010; Eggers et al. 2007; Roska et al. 1998; Zhang et al. 1997). Blocking GABA_A receptors in the retina causes a large increase in inhibition to rod BCs mediated by GABA_C receptors (Eggers and Lukasiewicz 2006a; 2010). These connections serve to limit the spatial extent of inhibition to BCs (Eggers and Lukasiewicz 2010), but it is not known how they change between rod and cone dominant conditions.
In this study we address these questions about how retinal inhibition changes with light adaptation by recording light–evoked and spontaneous inhibition from BCs in dark and light adapted conditions. Surprisingly we found that rod BC inhibition is almost gone after the application of a rod-adapting background, while the OFF BC inhibition shows no significant change, suggesting a selective suppression of rod BC input. The retina potentially minimizes unnecessary signaling by suppressing rod BC inhibition in conditions where rod photoreceptors are not significantly active. This could be part of a smooth transition between light intensities.

**Methods**

*Preparation of retinal slices*

Animal protocols were approved by the University of Arizona Institutional Animal Care and Use Committee (IACUC). As described previously (Eggers and Lukasiewicz 2006a) C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME, USA), aged 35-60 days, were euthanized using carbon dioxide and eyes were enucleated. The cornea and lens were removed and the eyecup was incubated in cold extracellular solution (see Solution and drugs), containing 800 units/mL of hyaluronidase for 20 minutes. The retina was removed from the eyecup, trimmed approximately square to remove the peripheral retina and mounted onto 0.45 μm nitrocellulose filter paper (Millipore Billerica, MA, USA). The filter paper-mounted retina was sliced into 250 μm thick slices and placed onto vacuum grease on glass cover slips after rotating 90°. Slices were taken from the central portion of the retina. All dissection and recording procedures were performed under infrared illumination to preserve the light sensitivity of the preparations.
Solutions and drugs

Extracellular solution used as a control bath and for dissection contained (in mM) 125 NaCl, 2.5 KCl, 1 MgCl₂, 1.25 NaH₂PO₄, 20 Glucose, 26 NaHCO₃, 2 CaCl₂ and was bubbled with a mixture of 95%/5% O₂/CO₂. The pipette intracellular solution contained (in mM) 120 CsOH, 120 Gluconic Acid, 1 MgCl₂, 10 HEPES, 10 EGTA, 10 TEA-CL, 10 phosphocreatine-NA₂, 4 Mg-ATP, 0.5 Na-GTP, 50µM Alexa Fluor 488 (Invitrogen, Carlsbad, California, USA) and was adjusted to pH 7.2 with CsOH. To block connections between amacrine cells, 20 µM SR95531 was used to block GABAₐ receptors. Antagonists were applied to the slice by a gravity-driven superfusion system (Cell Microcontrols, Norfolk, VA) at a rate of ~1mL/minute. Unless otherwise indicated, all chemicals were purchased from Sigma-Aldrich (St. Louis, Missouri, USA).

Whole-cell recordings

Glass cover slips containing retinal slices were placed in a custom chamber. The preparation was heated to 32° by temperature controlled thin stage and inline heaters (Cell Microcontrols, Norfolk, VA). Whole-cell patch recordings were made from BCs and ACs from retinal slices as described previously (Eggers and Lukasiewicz 2006b). To isolate inhibitory currents, BCs were clamped at 0 mV, the reversal potential for currents mediated by non-selective cation channels. To isolate excitatory currents, ACs were clamped at -60 mV, the reversal potential for currents mediated by chloride channels. Electrodes, with resistances of 5-7 MΩ were pulled from borosilicate glass (World Precision Instruments, Sarasota, Florida, USA) using a P97 Flaming/Brown puller (Sutter Instruments, Novato, California, USA). Liquid junction potentials of 20 mV were corrected at the beginning of each recording.
Light-evoked IPSCs (L-IPSCs) and spontaneous IPSCs (sIPSCs) were recorded from BCs. Light-evoked excitatory postsynaptic currents (L-EPSCs) were recorded from A17 ACs. Responses were filtered using a 6 KHz 4-pole low pass Bessel filter on an Multi-clamp 700B patch-clamp amplifier (Molecular Devices, Sunnyvale, California, USA) and digitized at 10 KHz with a Digidata 1140A data acquisition system (Molecular Devices, Sunnyvale, California, USA) and Clampex software (Molecular Devices, Sunnyvale, California, USA). Prospective rod and OFF BCs and A17 ACs were identified by their soma location in the upper INL for rod BCs, mid INL for OFF BCs and lower INL for A17 ACs and shape as oval for BCs or large and round for A17 ACs. BC (Ghosh et al. 2004) and AC (Menger and Wässle 2000; Nelson and Kolb 1985; Singer and Diamond 2003) morphology was confirmed by dendrites and axon location and shape at the end of the recording by imaging the Alexa fluorescence using an Intensilight fluorescence lamp and Digitalsight camera operated by Elements software (Nikon Instruments, Tokyo, Japan).

**Light Stimulation**

To evoke L-IPSCs, full-field light stimuli were generated using a light-emitting diode (LED, $\lambda_{\text{peak}} = 525$ nm) that was projected through the camera port of the microscope onto the retinal tissue. The maximum intensity of the light was $9.5 \times 10^5$ photons/µm²/sec, measured with an optometer (Gamma Scientific) and the duration of all light stimuli was 30 ms. Light intensity was controlled by varying the current through the LED. The relationship of current through the LED to light intensity was calibrated at many current levels and the appropriate LED stimulus was chosen to give the desired light intensity. Background light adaptation was applied for 5 minutes before light-adapted responses were recorded.
Data analysis and Statistics

L-IPSC and L-EPSC traces from a given response condition were averaged using Clampfit software (Molecular Devices, Sunnyvale, California, USA) and the charge transfer (Q), peak amplitude, time to peak and decay to 37% of the peak (D37) were measured in each condition. Q was measured over the length of the response, using the same time parameters in each condition for the same cell. This was typically 1 second. For the kinetic parameters (time to peak and D37) average traces were replaced with a 100-fold decimation, substituted with an average of those points to smooth out small fluctuations due to spontaneous activity. For intensity-response curves, L-IPSCs were normalized to the response to the maximal light stimulus in dark-adapted conditions. The normalized data were plotted versus the log_{10} of the stimulus intensity.

sIPSC data were analyzed using Clampfit software. A sIPSC template was calculated for each data file, using the average of >10 prototypical events from the recording. The software used this template to automatically detect spontaneous events. These events were manually accepted or rejected based on rigid criteria. Events that were used to calculate the frequency were rejected if they appeared to be noise. Events that were used to calculate the average peak amplitude were rejected if they appeared to be noise or were overlapping. Frequency was calculated by dividing the number of events recorded by the recording time. The distributions of sIPSC amplitude values were compared using the Kolmogorov-Smirnov test (K-S).
For each cell, a normalized data value of the % of response to maximal light stimulation in the dark was calculated. Paired Student’s t-tests were used to compare values between conditions for the same cell. Standard Student’s t-tests were used to compare values between different cells. Differences were considered significant when \( p \leq 0.05 \). All data are reported as mean ± standard error of the mean (SEM).

**Results**

*Switching from rod to cone pathways decreases rod BC inhibition*

To determine how inhibition to rod BCs varies with light intensity we recorded L-IPSCs in response to stimuli of increasing intensities with no background light (dark-adapted) and compared these responses to L-IPSCs after a background light (950 photons/\( \mu \text{m}^2/\text{sec} \), light-adapted) of an intensity that maximally activates the rods was applied (Wang and Kefalov 2009). This is an intensity that severely desensitizes and potentially saturates mouse rods, although they may be able to adapt to brighter backgrounds to retrieve a small amount of rod response (Naarendorp et al. 2010), possibly through rod-cone coupling. In rod BCs, L-IPSCs are a combination of currents mediated by glycine, GABA\(_A\) and GABA\(_C\) receptors (R) (Eggers and Lukasiewicz 2006a; b). Rod BC L-IPSCs at the highest light intensity tested were almost completely absent after light adaptation (Figure 2A). At all intensities tested above the adapting background, the rod BC L-IPSC charge transfer (Q) was significantly decreased by light-adaptation (n=6, \( p<0.01 \), Figure 2B). This suggests that inhibition to rod BCs potentially comes primarily from rod-dominant sources, like the A17 AC that makes a GABAergic feedback circuit with rod BCs. This is supported by our data (not shown) that only 29.2 ± 2.3% of the L-IPSC to rod BCs (n=3, \( p<0.01 \)) remains after the application of DHT (50 \( \mu \text{M} \)), a drug that inactivates
A17 ACs (Chavez et al. 2006). The decrease in inhibition was not due to a change in the tonic inhibition to rod BCs, as during light adaptation there is no significant change in the baseline current (dark baseline – light baseline = 2.3 ± 2.0 pA, p=0.22).

To assess the general activity of ACs giving input to rod BCs we also recorded sIPSCs in dark-adapted and light-adapted retinas. In the rod BC, sIPSCs in control conditions are mediated by glycineRs and GABA\textsubscript{A}Rs (Eggers and Lukasiewicz 2006a; b). We found that sIPSCs in rod BCs were significantly decreased by light adaptation (Figure 3A). This was caused by both a decrease in the frequency of sIPSCs (Figure 3B, n=7, p<0.005) and a shift of the peak amplitude distribution to smaller peak amplitudes (Figure 3C). We analyzed changes in the peak amplitude of sIPSCs for all rod BCs where more than 5 sIPSCs remained after light adaptation. The average peak amplitude of all rod BC sIPSCs was significantly decreased (Figure 3D, n=5, p<0.05). This suggests that ACs that inhibit rod BCs are less spontaneously active when a rod-saturating background is applied, although their activity is not completely suppressed since spontaneous inputs remained in many rod BCs.

Rod outputs are significantly decreased with light adaptation

To ensure that the light intensities we used are significantly decreasing rod signals, we recorded light-evoked excitatory post-synaptic potentials (L-EPSCs) from A17 ACs in dark and light adapted conditions. A17 ACs receive all excitatory input from rod BCs, and feedback GABAergic inhibition onto rod BC terminals. Thus L-EPSCs of A17 ACs are also a measure of the direct rod-activated feedback inhibition as well. We found that the Q (Figure 4B, n=4, p<0.01) and peak amplitude (p<0.05) of A17 AC L-EPSCs were significantly decreased with
light adaptation at all intensities used. What little current remained was faster (D37 = 47 ± 3% of control, p<0.01), suggesting it might be coming from rod to cone coupling instead of direct rod inputs, as cone signaling is faster than rod signaling (Ashmore and Copenhagen 1980; Copenhagen et al. 1983; Schnapf and Copenhagen 1982). On average the Q of the A17 L-EPSCs in response to the maximum intensity was decreased to 26 ± 5% of control by light adaptation. In contrast the rod BC L-IPSC was decreased to 10 ± 4% of control by light adaptation, a significantly larger decrease than that of the A17 L-EPSC (p<0.05). This suggests that light adaptation is changing more facets of rod BC inhibition than just a decrease in rod signaling.

Switching from rod to cone pathways does not affect OFF cone BC L-IPSCs

We previously have recorded L-ISPCs from all BC types in dark-adapted retinas (Eggers et al. 2007), but did not investigate L-IPSCs in light-adapted retinas. To determine if the decrease in L-IPSCs that we observe in rod BCs is specific to the rod pathway, we measured L-IPSCs from OFF cone BCs in dark and light adapted retinas. OFF cone BCs receive significant inhibition from rod BC activated AII ACs (Grunert and Wässle 1996; Haverkamp et al. 2003; Strettoi et al. 1994), but also from other ACs (Eggers et al. 2007; Ivanova et al. 2006). Although the AII connections may not be the most important determinant of the very dim rod threshold observed in the OFF pathway (Arman and Sampath 2012), the connection between AII ACs and OFF cone BCs is very strong (Grunert and Wässle 1996; Haverkamp et al. 2003; Strettoi et al. 1994) and likely activated at brighter light intensities (Pang et al. 2012) through the gap junctional connection between ON cone BCs and AII ACs that remains active in bright light. They are therefore a good control for switching between rod and cone inhibition. We found that L-IPSCs of OFF cone BCs were not changed by our rod-adapting background at any of the intensities we
tested (Figure 5, p=0.9, n=8). There were also no significant changes in the timing of L-IPSCs (D37 p=0.2; time to peak p=0.3). This suggests that, unlike rod BCs, OFF cone BCs are receiving additional inhibition from other sources when their rod-mediated inhibition from AII ACs is decreased.

Serial connections between ACs limit cone-mediated inhibition to rod BCs

We have previously shown that GABA_A-R-mediated connections between ACs can modulate inhibition to all BC types (Eggers and Lukasiewicz 2006a; 2010) in dark adapted retinas. Blocking these connections with an antagonist to GABA_A-Rs causes an increase in GABAergic inhibition to the rod BC. To determine if these serial connections are affected by light adaptation, we blocked GABA_A-Rs with SR-95531 and recorded L-ISPCs in response to multiple intensities in dark-adapted and light-adapted retinas (Figure 6A). We expected that if the amount of inhibition between ACs is unaffected by a background light, rod BC L-IPSCs would still be suppressed in the light adapted conditions when these serial connections were blocked. However, when these connections were blocked we did not see a decrease in rod BC L-IPSCs with light adaptation. Instead there was no significant difference between the Q of dark-adapted and light-adapted L-IPSCs when GABA_A-Rs were blocked (Figure 6B). This led to a light-adapted L-IPSC Q when GABA_A-Rs were blocked that was significantly greater than light-adapted L-IPSCs when GABA_A-Rs are active (control Figure 6B). Although the suppression of the A17 input onto GABA_C-Rs is presumably still present after GABA_A-Rs are blocked, blocking serial connections causes a significant increase to rod BC inhibition in the dark (Eggers and Lukasiewicz 2006a; 2010), so that this decrease is overcome by the increased GABA release (SR was 178 ± 23% of control at the highest light intensity in the dark-adapted retina). The L-IPSC at the maximum
light intensity also became faster in light-adapted conditions. When GABA_A Rs were blocked, the
D37 of rod BC L-IPSCs was decreased from 200.2 ± 45.9 ms in the dark to 114.4 ± 14.0 ms in
light-adapted conditions, an average of 67.2 ± 11.6% of dark-adapted D37. This suggests that
cone pathways were being activated that were normally suppressed by serial connections
between ACs in the light-adapted conditions.

We also recorded the response of OFF cone BC L-IPSCs to light adaptation after blocking
GABA_A Rs and also saw no differences between the Q of dark-adapted and light-adapted
conditions (Figure 6C,D). In both control conditions and when GABA_A receptors were blocked
OFF cone BCs showed no significant decrease in L-IPSCs with light adaptation. When
GABA_A Rs were blocked, the D37 of OFF BC L-IPSCs was also decreased from 319.0 ± 42.4 ms
to 178.3 ± 48.5 ms, an average of 52.7 ± 10.1% of the dark-adapted D37. This suggests that for
rod BCs, but not for OFF BCs, activation of inhibitory connections between ACs after light
adaptation is serving to limit rod BC inhibition.

Rod BC sIPSCs when GABA_A Rs were blocked were reduced by light-adaptation

When GABA_A Rs are blocked throughout the retina, GABAergic ACs are disinhibited, which
increases GABAergic input mediated by GABA_C Rs to rod BCs (Eggers and Lukasiewicz
2006a). However, in the absence of compounds that increase AC depolarization, such as kainate
(Eggers and Lukasiewicz 2006b), rod BC sIPSCs are composed only of inputs onto GABA_A Rs
and glycineRs. Since GABA_A Rs are blocked in the presence of SR-95531, we can monitor how
glycinergic inhibition to rod BC sIPSCs changes with light adaptation. We found that glycineR
sIPSCs were significantly reduced by light adaptation (Figure 7A). This resulted in a significant
decrease in the frequency of sIPSCs (Figure 7B, n=6, p<0.01). Additionally, we observed a small shift of the peak amplitude distribution to smaller peak amplitudes (Figure 7C). We analyzed changes in the peak amplitude of sIPSCs for all rod BCs where more than 5 sIPSCs remained after light adaptation. The average peak amplitude was significantly decreased (Figure 7D, n=4, p<0.05). This suggests that glycinergic ACs that send inhibition to rod BCs are still less active when a rod-saturating background is applied, even when GABAARs are blocked so that GABAergic ACs are disinhibited. This makes sense, as we have previously shown that disinhibiting the GABAergic ACs affects only GABAergic, and not glycinergic, inputs to rod BCs (Eggers and Lukasiewicz 2006a). This decrease in glycinergic input is likely present in the results in Figure 6, but it is not observed because the GABAergic mediated input dominated the rod BC L-IPSCs. In a separate experiment we also compared GABAAR sIPSCs (in the presence of strychnine) and found that the sIPSC frequency was significantly decreased to 0.18 ± 0.05 (p<0.05, n=3) of the GABAergic frequency in the dark. Therefore the decrease of sIPSCs with light adaptation seen in Figure 3 results from a decrease in both GABAergic and glycineR sIPSCs.

**Discussion**

We have shown that, surprisingly, inhibition to rod BCs is significantly regulated by a change in background light level. Light-evoked and spontaneous inhibition to rod BCs is significantly reduced with a rod-adapting background, while light-evoked OFF BC inhibition is unchanged. This occurs by two mechanisms: a reduction in the A17 AC rod-pathway mediated inhibition, as well as increased activity between GABAergic ACs with light adaptation that limits rod BC inhibition (Figure 8). This suggests that rod BC inhibition is limited in circumstances where it is
not needed, when the rods are significantly less active after light adaptation. This might allow the small signals from cone-rod coupling to pass through the rod signaling system in light adapted conditions. In contrast OFF cone BCs still receive strong inhibition after light adaptation, as they are active in both the rod and cone circuits.

Reduction of rod BC inhibition with light adaptation

In this study we found that light-evoked inhibition to rod BCs is almost non-existent after light adaptation. We have previously shown that rod BCs receive significant inhibition in dark-adapted conditions from GABAergic and glycinergic ACs (Eggers and Lukasiewicz 2006a; b). A significant portion of this inhibition comes from reciprocal connections with A17 ACs, with contributions from other ACs not activated by the rod pathways (Chavez and Diamond 2008; Chavez et al. 2010). Although rod signals cross over into the cone pathways, through activation of AII ACs and ON cone BCs by rod BCs, this is a secondary connection. Thus it makes sense that the contribution of direct rod pathway activated inhibition from A17 ACs would be greater than that coming from the cone pathways and rod BC inhibition from this source would show a significant decrease.

Yet, the observation that rod BC inhibition is almost gone in light-adapted conditions is somewhat puzzling because rod BCs receive inputs from non-rod pathway activated ACs, most notably onto glycine receptors that cannot be activated by the GABAergic A17 AC (Eggers and Lukasiewicz 2006a; b). This implies there must be some active suppression of rod BC inhibition with light adaptation in addition to a decrease due to rod saturation. Additionally, in dark-adapted retinas at brighter light intensities, where rods are presumably already saturated, rod BCs
can also receive additional “inhibitory” input from the activation of the Cl\(^-\) channel that is
coupled to the EAAT glutamate transporter (Ichinose and Lukasiewicz 2012; Veruki et al. 2006).
However, in light-adapted conditions rods are mostly saturated and not significantly responsive
to light, so rod BCs should not be depolarized to a significant extent and this EAAT current is
not likely to be active. Any inhibition received by a rod BC might be considered as excess, since
the rod BCs activation is already significantly reduced by rod adaptation (Figure 4), and only
small cone-rod coupling signals remain.

Our results suggest that light-adapted rod BC inhibition is decreased, at least in part, by an
increase in the activation of serial GABA\(_A\)-mediated synapses between ACs. We have
previously shown that serial synapses between ACs are preferentially activated by large light
stimuli (Eggers and Lukasiewicz 2010). In this study we only used full-field light stimuli, so we
did not test for any differences in the spatial extent of the stimulus. However, the dependence on
stimulus size of serial synapse activation suggests that rod BC inhibition could be preferentially
activated in light-adapted retinas by a small light stimulus. If this were the case, it could allow a
small inhibitory signal to dampen any responses in the rod BCs coming from rod-cone coupling
(DeVries and Baylor 1995; Wu and Yang 1988), without requiring a large inhibitory input from
wide-field ACs. This is an interesting topic for exploration in a future study.

Another mechanism that could potentially be reflected in our results showing decreases in light-
evoked inhibition to rod BCs is dopamine modulation of inhibitory receptors. Light adaptation of
the retina leads to increases in levels of dopamine release (Bloomfield and Dacheux 2001; Doyle
et al. 2002). Previous studies have shown that GABA\(_C\)-R-mediated currents are reduced by
dopamine (Dong and Werblin 1994; Wellis and Werblin 1995). Since GABA<sub>C</sub>Rs are the primary carriers of current in the rod BCs (Eggers and Lukasiewicz 2006a; Euler and Wässle 1998; Shields et al. 2000), a reduction in GABA<sub>C</sub>-R currents could also cause a significant decrease in rod BC inhibition with light adaptation. Additionally, another study has shown that GABA<sub>A</sub>Rs can be potentiated by dopamine (Feigenspan and Bormann 1994), which would also serve to decrease inhibition to rod BCs through the enhancement of communication through serial synapses that ordinarily suppress rod BC inhibition (Eggers and Lukasiewicz 2006a; 2010).

We also found that both GABA<sub>A</sub> and glycine receptor-mediated spontaneous inhibitory inputs to rod BCs were significantly decreased, but not eliminated by light adaptation. Spontaneous inputs to rod BCs come from both the spontaneous fusion of vesicles of neurotransmitter in the presynaptic ACs that is independent of Ca<sup>2+</sup> and the Ca<sup>2+</sup> triggered fusion of vesicles that reflect the general state of activity in the presynaptic AC in the absence of a light stimulus. As the rate of non Ca<sup>2+</sup> triggered fusion of vesicles likely does not change with light adaptation state, the decrease in spontaneous release with light adaptation shows that the general depolarization level of ACs releasing GABA and glycine onto rod BCs is decreased with light adaptation. Although the small changes in glycine receptor sIPSC amplitude could also reflect a postsynaptic change in receptor properties, these changes could also reflect reduced simultaneous fusion of two vesicles of neurotransmitter in the ACs, an additional presynaptic mechanism. A decrease in spontaneous inhibition, in addition to revealing a decrease in the general activity of presynaptic ACs, can also decrease the synaptic “noise” of a cell which would increase the signal to noise ratio. While the light-adapting intensity used here eliminated rod BC L-IPSCs, at more moderately adapting intensities this could be an important mechanism.
Together all of the mechanisms we have discussed here – a decrease in rod pathway mediated inhibition, an increase in the activation of serial connections between ACs and a potential decrease in GABA\textsubscript{C}R response due to dopamine - could contribute to a selective decrease in rod BC inhibition in the light-adapted retina, when rods and rod BCs are not active. This could be an additional adaptive mechanism that the retina uses to prioritize cone pathway signaling.
Acknowledgements: We thank members of the Eggers laboratory for helpful discussion and comments on this manuscript and Adam Bernstein for technical assistance.

Grants: This work was supported by NIH grant EY018131 (EDE) and T32HL007249 (REM).
References


Figure Legends

Figure 1. Potential pathways of rod bipolar cell (BC) inhibition. 1. In dark-adapted retinas where rod (R) pathways are active, rod BCs (RB) activate A17 ACs (+, glutamate) and receive significant GABAergic feedback inhibition (-, GABA) from those A17 ACs (dark grey pathway). 2. They also receive a small amount of glycinergic inhibition (not shown here) and GABAergic inhibition (-) that comes from cone (C) activated pathways, activated (+) by ON cone BCs (ON). 3. Input from GABAergic ACs onto rod BCs is modulated by GABAA receptor-mediated serial connections between GABAergic ACs.

Figure 2. Rod BC L-IPSCs are significantly decreased by adaptation with a rod saturating light. A. Example L-IPSCs in response to the maximum light intensity (30 ms light stimulus – dark grey bar) used from rod BCs in a dark-adapted retina and with a rod-saturating background (dark grey bar shows timing of light stimulus). B. The charge transfer (Q) of L-IPSCs in response to many intensities of light was normalized to the response at the maximum light intensity in dark-adapted conditions. L-IPSCs in light adapted conditions were significantly decreased at all intensities used (*, p<0.01, n=6).

Figure 3. Rod BC sIPSCs are significantly decreased by adaptation with a rod saturating light. A. sIPSCs recorded from a rod BC in a dark-adapted retina and with a rod-saturating background (two traces from each condition are shown – one in black and one in dark grey). B. For all rod BCs, the frequencies of sIPSCs decreased from 0.33 ± 0.13 Hz to 0.11 ± 0.06 Hz (n=7, p<0.05). Large triangles denote average frequencies. C. Normalized histogram and cumulative probability histogram of peak amplitudes from the example in A. Light-adaptation
significantly decreased the peak amplitude (K-S p<0.01). Large circles denote average peak amplitudes ± SEM. D. For all rod BCs, the peak amplitudes of sIPSCs decreased from 17.1 ± 4.1 pA to 11.3 ± 2.5 pA (n=5, p<0.05). Large triangles denote average amplitudes.

Figure 4. A17 AC L-EPSCs are significantly decreased by adaptation with a rod saturating light. A. Example L-EPSCs from an A17 AC in a dark-adapted retina and with a rod-saturating background. B. The Q of L-EPSCs in response to many intensities of light was normalized to the response to the maximum light intensity in dark-adapted conditions. L-EPSCs in light adapted conditions were significantly decreased at all intensities used (** p<0.01, n=4).

Figure 5. OFF cone BC L-IPSCs are not changed by adaptation with a rod saturating light. A. Example L-IPSCs from an OFF cone BC in a dark-adapted retina and with a rod-saturating background. B. The Q of L-IPSCs in response to many intensities of light was normalized to the response at the maximum light intensity in dark-adapted conditions. There was no significant difference between the L-IPSCs in dark- and light-adapted OFF cone BCs (p=0.9, n=8).

Figure 6. When serial connections between ACs are blocked with a GABA_A antagonist rod BC L-IPSCs were not decreased by light adaptation. A. Example L-IPSCs recorded from a rod BC in a dark-adapted retina in the presence of GABA_A antagonist SR-95531 (20 μM) and with a rod-saturating background. B. The Q of rod BC L-IPSCs in response to many intensities of light was normalized to the response to the maximum light intensity in dark-adapted conditions in the presence of SR-95531. Control data from Figure 2 is added for comparison (dotted lines).
Rod BC L-IPSCs in light adapted, SR-95531 conditions were not significantly decreased, and were significantly greater than L-IPSCs in control conditions (*, p<0.05, n=5) at all intensities used. C. For comparison purposes we also show L-IPSCs from an OFF cone BC, in SR-95531, in dark and light adapted conditions (n=5). D. In contrast to rod BCs, OFF cone BCs did not change when light-adapted in either control or SR-95531.

Figure 7. Rod BC sIPSCs in the presence of SR-95531 are significantly decreased by adaptation with a rod saturating light. A. sIPSCs were recorded from a rod BC in a dark-adapted retina in the presence of SR-95531 and a rod-saturating background was applied (two traces from each condition are shown – one in black and one in dark grey). B. The frequency of rod BC sIPSCs (SR) decreased from 0.79 ± 0.41 Hz to 0.14 ± 0.08 Hz (n=6, p<0.01). Large triangles denote average frequencies. C. Normalized histogram and cumulative probability histogram of peak amplitudes from the example in A. Light-adaptation significantly decreased the peak amplitude (K-S p<0.01). Large circles denote average peak amplitudes ± SEM. D. The peak amplitude of rod BC sIPSCs (SR) decreased from 24.0 ± 3.2 pA to 17.2 ± 2.9 pA (n=4, p<0.05). Large triangles denote average amplitudes.

Figure 8. Distinct rod and cone pathways of rod BC inhibition. A. In dark-adapted retinas where rod pathways are active, rod BCs receive significant inhibition, likely from A17 ACs (white) and little inhibition from cone activated pathways (dark grey). B. When rod pathways (dark grey) were saturated with an adapting background, inhibition from the cone pathway (white) was suppressed by inhibitory connections between GABAergic ACs, resulting in little inhibition to rod BCs.
A. Rod BC, Control

- Dark Adapted
- Light Adapted

- 5 pA
- 200 ms

B. Light intensity (log units)

- Q (norm. to dark)

- Luminance (photons/μm²/sec)

- Dark-adapted
- Light-adapted

- * * *
A.

Dark-adapted

Light-adapted

B.

Frequency (Hz)

Dark-adapted  Light-adapted

C.

Cumulative Probability

Dark-adapted  Light-adapted

D.

Peak amplitude (pA)

Dark-adapted  Light-adapted

* Significant difference
A.

A17 AC L-EPSC

- Dark-adapted
- Light-adapted

200 ms

10 pA

B.

Q (norm. to dark)

Dark-adapted

Light-adapted

Luminance (photons/μm²/sec)

10¹ 10² 10³ 10⁴ 10⁵ 10⁶

0.0 0.5 1.0

** **

10² 10³ 10⁴ 10⁵ 10⁶

0.0 0.5 1.0

** **

10³ 10⁴ 10⁵ 10⁶

0.0 0.5 1.0

** **
A. OFF cone BC, Control

Dark-adapted  
Light-adapted

10 pA
200 ms

B. Dark-adapted  
Light-adapted

Q (norm. to dark)

Luminance (photons/μm²/sec)

0.0
0.4
0.8
1.2

10^2 10^3 10^4 10^5 10^6 10^7
A.

Dark-adapted (SR)

Light-adapted (SR)

B.

Frequency (Hz)

Dark-adapted
Light-adapted

C.

Cumulative Probability

D.

Peak amplitude (pA)

Dark-adapted (SR)
Light-adapted (SR)
A. Dark Adapted (Rods Dominate)

B. Light Adapted (Cones Dominate)