TITLE: Light adaptation alters the source of inhibition to the mouse retinal OFF pathway

Running title: Light adaptation alters OFF bipolar cell inhibition

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Abstract

Sensory systems must avoid saturation to encode a wide range of stimulus intensities. One way the retina accomplishes this is by using both dim light-sensing rod and bright light-sensing cone photoreceptor circuits. OFF cone bipolar cells are a key point in this process, as they receive both excitatory input from cones and inhibitory input from AII amacrine cells via the rod pathway. However, in addition to AII amacrine cell input, other inhibitory inputs from cone pathways also modulate OFF cone bipolar cell light signals. It is unknown how these inhibitory inputs to OFF cone bipolar cells change when switching between rod and cone pathways or if all OFF cone bipolar cells receive rod pathway input. We found that one group of OFF cone bipolar cells (types 1, 2, and 4) receive rod-mediated inhibitory inputs that likely come from the rod - AII amacrine cell pathway, while another group of OFF cone bipolar cells (type 3) do not. In both cases, dark-adapted rod dominant light responses showed a significant contribution of glycinergic inhibition, which decreased with light adaptation and was, surprisingly, compensated by an increase in GABAergic inhibition. As GABAergic input has distinct timing and spatial spread from glycinergic input, a shift from glycinergic to GABAergic inhibition could significantly alter OFF cone bipolar cell signaling to downstream OFF ganglion cells. Larger GABAergic input could reflect an adjustment of OFF bipolar cell spatial inhibition which may be one mechanism that contributes to retinal spatial sensitivity in the light.

Keywords: bipolar cell, gamma-aminobutyric acid (GABA), glycine, amacrine cell
Introduction:

Sensory systems must avoid signal saturation to encode a wide dynamic range of incoming information, for example when stepping outside into the bright sunlight from a dimly lit room. This occurs in the retina partly by using two photoreceptors with different sensitivities – rod photoreceptors that sense dim light and cone photoreceptors that sense brighter light. Light information sent to bipolar cells (BCs) forms three primary pathways. ON and OFF cone BCs respond to the onset and offset of light signals from cones respectively, while rod BCs respond to the onset of light signals from rods. ON and OFF cone BCs synapse onto ON and OFF ganglion cells (GC) respectively, which are the output neurons of the retina. Rod BCs are unique however in that they do not contact GCs directly, instead they synapse onto the AII amacrine cell (AC) (McGuire et al. 1984; Strettoi et al. 1990).

Rod BCs and AII ACs are part of a specialized rod pathway which makes use of existing cone circuitry (Figure 1A). Light onset signals from rods are relayed from rod BCs to AII ACs, which have electrical connections with ON BCs (Chun et al. 1993; Deans et al. 2002; Strettoi et al. 1992; Trexler et al. 2001). These electrical connections can also be bi-directional, to relay cone light information to the AII AC, but are more robust in the AII AC → ON BC direction (Beaudoin et al. 2008; Manookin et al. 2008; Munch et al. 2009; Trexler et al. 2005; Trexler et al. 2001; Veruki and Hartveit 2002). Light offset signals from rods are also relayed through the AII AC, which has anatomical connections with OFF BCs and OFF GCs (Grunert and Wässle 1996; Haverkamp et al. 2003; Strettoi et al. 1994). As the AII AC releases glycine, it inhibits the OFF pathway at light onset and the removal of inhibition at light offset serves as an “Off” signal. OFF BCs may also receive rod input through direct connections or rod-cone coupling providing...
alternate routes for light offset signals (DeVries and Baylor 1995; Tsukamoto et al. 2001; Volgyi et al. 2004).

A recent paper (Arman and Sampath 2012) suggested that the AII AC-OFF GC connection determines the response to very dim light stimuli, signaling rod threshold responses. However, OFF BCs have a prominent localization of glycine receptors (Haverkamp et al. 2003; Sassoe-Pognetto et al. 1994) showing large spontaneous (Eggers and Lukasiewicz 2006b; Ivanova et al. 2006) and light-evoked (Eggers et al. 2007) glycine receptor-mediated currents compared to rod BCs. Although glycinergic inputs to OFF BCs may not be crucial for determining the scotopic threshold of the retina (Arman and Sampath 2012), there are clearly significant AII AC inputs to OFF BCs that are likely mediating the significant glycinergic input that OFF BCs receive at brighter rod and potentially cone light intensities, which have not previously been tested. Additionally OFF BCs are divided into at least 4 main subtypes (Ghosh et al. 2004) and previous anatomical work has suggested that at least one subtype of OFF BC does not receive input from the AII AC (Tsukamoto et al. 2001). The strength and importance of this connection therefore likely varies across OFF BC pathways, but this has not previously been determined physiologically.

OFF BC signaling to GCs is also shaped by inhibition from other ACs, which are roughly divided into narrow-field glycinergic and wide-field GABAergic groups (Menger et al. 1998; Pourcho and Goebel 1985; 1983; Vaney 1990). AC inhibitory inputs combine their signals onto BCs which has been shown to significantly modulate the output of BCs (Eggers and Lukasiewicz 2006a; 2010; 2006b; Eggers et al. 2007; Sagdullaev et al. 2006). Since rod and cone pathways
signal at different light intensities, applying a bright background light that causes rod saturation allows the two pathways to be investigated separately (Dacheux and Raviola 1986; Xin and Bloomfield 1999). It is unknown how glycinergic input to OFF BCs changes when switching between rod and cone pathways or if there are functional differences in glycinergic inputs to multiple OFF BC subtypes (Figure 1B) with rod vs. cone activation (Euler and Wässle 1995; Ghosh et al. 2004; Pignatelli and Strettoi 2004).

Here we investigated how inhibition to OFF BC subtypes changes with light adaptation. In order to elucidate how light adaptation is affecting the different OFF BC circuits, we examined the physiological connections that the rod pathway has with the different OFF BC subtypes. We found that one type of OFF BC is not contacted by the rod BC–AII AC while the other three types are. A decrease in AII AC activity, due to a decrease in rod pathway signaling with light adaptation, might result in a decrease in inhibitory input to OFF BCs. Surprisingly, we found that a compensatory switch from glycine to GABA-mediated OFF BC inhibition occurs with light adaptation that may alter OFF BC spatial inhibition and sensitivity.

Methods:

Mouse retinal slice preparation

Animal protocols were approved by the University of Arizona Institutional Animal Care and Use Committee (IACUC). As described previously (Eggers and Lukasiewicz 2006a; Eggers et al. 2013), male mice (C57BL/6J strain, Jackson Laboratories, Bar Harbor, ME, USA) 35-60 days of age were euthanized using carbon dioxide. Their eyes were enucleated and the cornea and lens removed. The eyecup was incubated for 20 min in cold extracellular solution (see Solution and
Drugs) with 800 units/mL of hyaluronidase to dissolve the remaining vitreous humor. The hyaluronidase solution was then replaced with ice cold, oxygenated extracellular solution and the retina was dissected out of the eyecup. Following removal, the retina was trimmed down into one large flat rectangle by removing the peripheral retina and leaving only the central retina surrounding the optic disc. A nitrocellulose membrane filter paper (0.45 μm pore size, Millipore, Ireland) was placed on the retina section which was transferred to a hand chopper. An average of six 250 μm slices were cut, rotated 90º, and mounted onto glass cover slips using vacuum grease. Cells used from these slices were never more than 700 μm away from the center of the retina and only cells near the center of each slice were used for recordings. In this way, much of the differential input due to the dorsal-ventral cone opsin gradient reported in mice was mitigated (Applebury et al. 2000; Haverkamp et al. 2005). The tissue was maintained in oxygenated extracellular solution at room temperature. All dissection procedures were performed under infrared illumination to preserve the light sensitivity of the preparations.

Solutions and drugs

The extracellular recording solution used for dissection and to examine light-evoked currents contained (in mM) 125 NaCl, 2.5 KCl, 1 MgCl2, 1.25 NaH2PO4, 2 CaCl2, 20 glucose, and 26 NaHCO3 and was bubbled with 95% O2-5% CO2. The intracellular solution contained (in mM) 120 CsOH, 120 gluconic acid, 1 MgCl2, 10 HEPES, 10 TEA-Cl, 10 phosphocreatine-Na2, 4 Mg-ATP, 0.5 Na-GTP, 10 EGTA and 50 μM Alexa Fluor 488 (Invitrogen, Carlsbad, California, USA) and was adjusted to pH 7.2 with CsOH. To isolate the inhibitory receptor (R) inputs, SR-95531 (SR, 20 μM) to block GABA_A Rs, (1,2,5,6-tetrahydropyridine-4yl) methyphosphinic acid (TPMPA, 50 μM) to block GABA_C Rs, and strychnine (1 μM) to block glycine Rs were used.
Strychnine was washed on first, followed by TPMPA and finally SR-95531 to prevent serial inhibitory effects (Eggers and Lukasiewicz 2006a; 2010). To block synapse-activated inhibition, CNQX (25 μM) was used to block AMPA and KainateRs and AP-5 (50 μM, Santa Cruz Biotechnology, Santa Cruz, CA) was used to block NMDARs. All drug solutions were washed on the slice for 5 min before recordings began using a gravity-driven superfusion system (Cell Microcontrols, Norfolk, VA) at a rate of ~1-2 mL/minute. Unless otherwise indicated, all chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA).

**Whole cell recordings**

Whole cell patch clamp recordings, sampled at 10 kHz, were made from BCs and ACs from retinal slices. Light-evoked inhibitory post synaptic currents (L-IPSCs) and spontaneous (s)IPSCs were recorded from retinal BCs voltage clamped to 0 mV, the reversal potential of nonselective cation channel currents. BC recordings were stable and no rundown of the light response was observed over the recording period. Light-evoked excitatory post synaptic currents (L-EPSCs) and sEPSCs were recorded from ACs voltage clamped to -60 mV, the reversal potential for chloride channel-mediated currents. Liquid junction potentials of 20 mV were corrected at the beginning of each recording. Electrodes were pulled from borosilicate glass (World Precision Instruments, Sarasota, FL) on a P97 Flaming/Brown puller (Sutter Instruments, Novato, California, USA) and had resistances of 5-7 MΩ. Mice were dark-adapted overnight, and all recording procedures were performed in the dark under infrared illumination to preserve the light sensitivity of the slices. Recordings were made in extracellular solution heated to 32°C, using thin stage and inline heaters (Cell Microcontrols, Norfolk, VA). Responses were filtered at 6 kHz with the four-pole Bessel filter on a Multi-clamp 700B patch-clamp amplifier (Molecular
Devices, Sunnyvale, California, USA) and digitized with a Digidata 1140 data acquisition system (Molecular Devices, Sunnyvale, California, USA).

Morphological identification of cell subtypes
Alexa 488 included in the recording pipette was used to label OFF BC and AC subtypes. They were classified as either type 1/2, 3, 4 OFF BCs or AII ACs based on their axonal morphologies and stratification within the inner plexiform layers and the position of their somas in the inner nuclear layer (Ghosh et al. 2004). Type 1 and 2 BCs were difficult to distinguish based on morphology, so their results were pooled. Additionally, although subtypes of the OFF type 3 BC have been described, (Mataruga et al. 2007; Wassle et al. 2009) their morphologies were quite similar so all OFF type 3 cells were pooled. The cells were imaged with Nikon Digital Sight camera with Elements software using a Nikon Intensilight C-HGFIE Fluorescent lamp (Nikon Instruments, Tokyo, Japan). Photoshop (Adobe, Seattle, WA) and Elements software were used to measure and trace the cells. Detailed analysis of axon terminal morphology was performed on a subset of all recorded OFF BCs (n=24) to determine anatomical differences between OFF BC types, and these criteria were subsequently used to identify OFF BCs. Cell tracings are provided for easy quantification as well as visualization since the fluorescent images of OFF BC terminal morphologies were often at multiple depths vertically in the slice preparation.

Light stimuli
Full-field light stimuli were evoked using a light-emitting diode (LED, Agilent HLMP-3950, $\lambda_{peak} = 525$ nm, Palo Alto, CA) projected through the camera port of the microscope, which elicited a strong response in both dark- and light-adapted conditions. We focused our recordings
on cells located within the regions of mixed green/UV cone opsin input (Applebury et al. 2000; Haverkamp et al. 2005) where there was little difference between BC or GC cell responses to green light (Breuninger et al. 2011; Wang et al. 2011). Thus, 525 nm light should stimulate both rods (peak sensitivity at 500 nm) and cones robustly. The stimulus intensity (max of \(9.5 \times 10^5\) photons/\(\mu m^2/sec\)), background rod-saturating light (950 photons/\(\mu m^2/sec\)) and duration (30 ms) were controlled with Clampex software by varying the current through the LED. The background intensity was chosen as it was shown to maximally activate rods (Wang and Kefalov 2009). A long light stimulus (500 ms) was used to determine the type of inhibition to all recorded BCs (ON or OFF) as well as the type of excitation of the ACs. A rod saturating background light was applied for 5 min to light-adapt the retina slice and was sustained throughout all light-adapted recordings.

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 and decay to 37% of the peak (D37) were measured in each condition. Due to the significant amount of spontaneous activity, it was difficult to measure a peak from OFF BC L-IPSCs. Therefore to estimate the peak and D37, average traces were decimated (100 fold) and each point was replaced with the average of those data points to limit variations due to spontaneous activity. To determine changes in total current, the Q was measured, which represents the magnitude of the response. Q was measured in Clampfit over the length of the response, typically 1-2 seconds, using the same time parameters in each condition for the same cell. All example response traces show responses to the max light flash intensity of \(9.5 \times 10^5\) photons/\(\mu m^2/sec\).
photons/µm²/sec. For intensity response curves, light-evoked responses were normalized to the maximal response in the dark-adapted condition. The normalized data were plotted against the log₁₀ of the stimulus intensity.

Spontaneous inhibitory (sIPSC) and excitatory (sEPSC) post-synaptic current data were analyzed using Clampfit software. A sIPSC or sEPSC template was calculated for each data file, using the average of about 10 prototypical events from the recording. The software used this template to automatically detect spontaneous events which were manually accepted or rejected based on strict criteria: events used to calculate the frequency were rejected if they appeared to be noise and events used to calculate the average peak amplitude were rejected if they appeared to be noise or consisted of two or more overlapping events. Frequency was calculated by dividing the number of events by the recording time. Peak amplitude and inter-event interval histogram distributions were normalized to the number of events. The signal-noise-ratio (SNR), which gives a measurement of the actual response signaled over the background noise, was calculated by dividing peak amplitude of the L-IPSC by the variance of the baseline.

Student’s t-test (2-tailed, paired) was used to compare response characteristics before and after drug application or light adaptation. Standard student’s t-test (2-tailed, unequal variance) was used to compare values between different cells. Differences were considered significant when \( p < 0.05 \) (\(*,†,‡\)) and \( p < 0.01 \) (\(**,††\)). All averaged data are reported as means ± SEM. The distributions of sIPSC and sEPSC amplitude and inter-event interval values were compared using the Kolmogorov-Smirnov test (K-S) with significance being \( p < 0.05 \).
Results:

**OFF BC subtypes receive different sources of inhibition in the dark**

Previous studies have shown that OFF BCs receive glycine, GABA$_A$, and GABA$_C$-mediated inhibition (Eggers et al. 2007; Euler and Wässle 1998; Ivanova et al. 2006), that likely includes both rod pathway connections from rod BC-AII AC inputs and cone pathway AC input driven by ON and OFF cone BCs (Figure 1A). However, these previous studies did not determine if OFF BCs receive rod BC-AII AC mediated inputs or if the light-evoked inhibitory inputs vary between OFF BC subtype. To resolve this, we identified 3 OFF BC subtypes by the stratification of their axon terminals within the inner plexiform layer (IPL) (Ghosh et al. 2004). We quantified the percentage of the IPL in which OFF BC axon terminals ramify (0% = outer nuclear layer/IPL border) and found that type 4 OFF BCs have terminals that ramify in 1±1-38±8% (n = 4) of the IPL, type 3 OFF BCs have terminals that cover 17±2-50±4% (n = 11) of the IPL and both type 1 and type 2 OFF BCs have terminals that cover 1±1-16±4% of the IPL (n = 9) (Figure 1B).

Because of their similar morphology, type 1 and type 2 OFF BCs were difficult to differentiate and their data were pooled together in these measurements.

It is not known if all of these OFF BC subtypes receive input from the rod-rod BC-AII AC circuit. A previous study suggested that one type of OFF BC (likely type 4) had significant anatomical contacts from AII ACs, while another OFF BC (likely type 3) did not (Tsukamoto et al. 2001). To test for physiological differences in potential AII AC inputs, we recorded L-IPSCs from dark-adapted retinas, where rod pathways are active, in response to long light stimuli to categorize the inhibition as ON, OFF or ON/OFF. If an OFF BC only receives inhibition at the
offset of light, then it could not be receiving rod BC-AII AC mediated inhibition that is activated at light onset. However, if an OFF BC received ON inhibition, then it may be due in part to the rod pathway and coming from the AII AC. We found that the responses of the OFF BC subtypes to long light stimuli differ. OFF BC types 1, 2, and 4 (OFF1,2,4) have an ON inhibitory response while OFF BC type 3 (OFF3) has an OFF inhibitory response (Figure 1C). This suggests that OFF3 BCs do not receive rod pathway input from AII ACs. To quantify this, we measured the charge transfer (Q) 500 ms before, during, and after the long light stimulus. OFF1,2,4 BCs had a significantly higher Q only during the light stimulus (normalized to the baseline Q, Figure 1D). OFF3 BCs had a significantly higher normalized Q only after light offset (Figure 1D). OFF1,2,4 responses during light offset and OFF3 responses during light onset were not different than the baseline (p > 0.05). We found no significant difference between the light responses of OFF BC types 1 and 2 and OFF BC type 4, so these data have been pooled as OFF BC types that all receive potential input from AII ACs.

To confirm our results on connections between AII ACs and OFF BCs, we used the fact that AII ACs are activated both by rod pathway activation and by ON cone BC activation through gap junction connections. Since the ON BC AII activation requires only metabotropic glutamateRs, we recorded OFF BC L-IPSCs in light-adapted conditions with CNQX and AP-5 to block AMPA/Kainate and NMDA receptors. Blocking these receptors blocks both OFF BC activation as well as all chemical synapse-activated inhibition so that only ON BC pathways are active. We found that OFF3 BCs are not contacted by the AII ACs as their inhibition is abolished in light-adapted conditions with CNQX and AP-5 (Fig 1E,F, n = 5). In contrast, OFF1,2,4 BCs had significant inhibition remaining after blocking all ionotopic glutamateRs (Fig 1E,F, 42±19% of
total inhibition at the maximum light intensity), suggesting significant contribution from ON BC-activated AII ACs (n = 4), similar to the input from AII ACs to OFF GCs shown previously (Manookin et al. 2008; Munch et al. 2009; Murphy and Rieke 2008). This inhibition was blocked by strychnine (n = 2, data not shown), showing that it was coming from glycinergic ACs, presumably the AII. From these results we can confirm that only OFF1,2,4 BCs are contacted by an ON pathway mediated by the rod-AII AC. The remaining inhibition in OFF1,2,4 BCs also suggests that cones are being robustly stimulated with a 525 nm LED in a light-adapted condition.

As seen in the examples in Figure 1C and 1D, the sIPSCs from OFF 1,2,4 and OFF3 BCs also appear to have different amplitudes and frequency, with OFF 1,2,4 BCs receiving much more spontaneous input. If inhibitory input to the OFF1,2,4 and OFF3 BCs is coming from different sources we would expect that the spontaneous activity might also be different, as spontaneous activity varies between neurons. We measured sIPSCs in the absence of light stimuli from dark-adapted retinas. The sIPSC peak amplitude was significantly lower in OFF3 BCs (16±1 pA, n = 11) than in OFF1,2,4 BCs (38±7 pA, n = 13) (p < 0.01). Additionally, the sIPSC frequency was also significantly lower in OFF3 BCs (7±3 Hz, n = 12) than OFF1,2,4 BCs (25±6 Hz, n = 15) (p < 0.05). The lower amplitude and frequency of sIPSCs suggests that OFF3 and OFF1,2,4 BCs are receiving inputs from distinct sources.

Dark-adapted L-IPSCs are mostly glycinergic in OFF1,2,4 but not OFF3 BCs

The differences in OFF1,2,4 and OFF3 BC L-IPSCs also suggest that they are receiving distinct inhibitory inputs from different sources. The majority of the dark-adapted inhibition to OFF 1,2,4
BCs likely comes from the rod BC-AII AC pathway, leading to the large glycinergic light-evoked currents previously observed by Eggers et al. (2007), since the rod pathway should be the most activated in the dark-adapted state. However, our results from Figure 1 suggest that the OFF3 BCs are not receiving AII input and thus may not have large glycinergic inputs. To test this, we recorded L-IPSCs and sIPSCs from dark-adapted OFF BCs in control conditions and with application of strychnine, a glycineR antagonist, to separate glycinergic and GABAergic contributions (Eggers et al. 2007). There was a significant decrease in OFF1,2,4 BC L-IPSCs ($n = 6$) with application of strychnine (Figure 2 A,C) at all light intensities used ($p < 0.01$). In contrast, OFF3 BCs ($n = 4$) only had significant decreases in L-IPSCs at the brighter light intensities (Figure 2 B,D), while at the two dimmest light intensities, glycineR blockade produced no significant change. In dark-adapted conditions, OFF1,2,4 L-IPSCs were dominated by glycine (80±5% of the total inhibition at the maximum light intensity) in contrast to OFF3 BC responses (51±4%, $p < 0.05$). These results suggest that in the dark, the main inhibition to OFF1,2,4 BCs is glycinergic mainly mediated by the rod BC-AII AC pathway while only half of the inhibition to OFF3 BCs was due to glycine input from OFF cone activated ACs. Since glycinergic and GABAergic inhibition have distinct time courses (Eggers and Lukasiewicz 2006b; Eggers et al. 2007), this could contribute to further differences between signaling of OFF BC subtypes.

**Dark-adapted sIPSCs are primarily glycinergic in both OFF1,2,4 and OFF3 BCs**

As may be seen in Figures 1 and 2, OFF BCs have a significant amount of background spontaneous activity, which reflect their inputs and may affect signal transmission of light-evoked responses. Although the light-evoked inputs to the classes of OFF BCs differ, sIPSCs for
both OFF1,2,4 and OFF3 BCs in the dark were mostly due to glycineR activation. Strychnine abolished sIPSCs in half of the OFF1,2,4 and a quarter of the OFF3 BCs. Of the spontaneous events that remained, there was a significant reduction in the sIPSC peak amplitude for OFF1,2,4 (K-S \( p < 0.01, n = 3 \)) and OFF3 BCs (K-S \( p < 0.01, n = 3 \)) (Figure 3 A,B,C,D) but no change in the decay \( \tau \) of the sIPSCs in either group (OFF1,2,4, \( n = 3 \), \( p = 0.88 \) or OFF3 BCs, \( n = 3 \), \( p = 0.79 \), data not shown). Although small differences in the decay of GABA\( _A \) and glycineR mediated sIPSCs have previously been shown (Eggers et al. 2007), the lack of a significant change in the kinetics of the sIPSCs that remain after strychnine, presumably due to GABA\( _A \)R input (Eggers et al. 2007), could be due to the very small sample of sIPSCs that remained in only a handful of cells. There was also a significant decrease in sIPSC frequency with strychnine application for both OFF1,2,4 (\( n = 6 \)) and OFF3 (\( n = 4 \)) BCs (\( p < 0.01 \)). These results suggest that in the dark, most of the tonic spontaneous inhibition to OFF BCs is due to glycineR mediated currents likely coming from both the AII AC and other ON ACs for OFF1,2,4 BCs and only OFF glycineR ACs for OFF3 BCs.

Light adaptation alters the timing and charge transfer of AII AC L-EPSCs

We have found that dark-adapted inhibition to OFF1,2,4 and OFF3 BCs varies, likely due to AII AC versus non-AII AC inputs. However, since OFF BCs function in both rod and cone dominant conditions, switching from rod to cone pathways which could modulate inhibition to the OFF BC subtypes differently. Light adaptation has been used to separate the rod and cone pathways by applying a maximally rod activating background light to the retina (Xin and Bloomfield 1999). If the rod pathway is saturated via light adaptation, then the amount of excitation the rod BC gives to the AII AC should decrease. However, AII ACs can also be directly activated in cone-
dominant conditions by ON BCs through electrical connections (Strettoi et al. 1994; Trexler et al. 2001; Volgyi et al. 2004). To confirm that activation of AII ACs declines when switching from rod to cone activation with our light stimulus, we recorded light-evoked excitatory post synaptic currents (L-EPSCs) from AII ACs in dark- and light-adapted (maximal rod pathway activation of 950 photons/μm²) (Wang and Kefalov 2009) conditions.

AII ACs were identified prior to recording by their characteristic soma shape and large proximal dendrite and labeled with a fluorescent dye, Alexa 488, during the recording to quantify their morphology after the recording. We found that AII AC processes covered between 10±2 and 97±4% of the IPL (n = 6) (Figure 4B). With light adaptation, there was a significant decrease in the Q of the AII L-EPSCs at each light intensity (n = 9, Figure 4A,C). In addition, there was a significant decrease in the normalized peak amplitude (to 43±7%) and D37 (to 69±8%) of the L-EPSC (Figure 4D). The remaining excitatory input is most likely due to cone activation via electrical connections of the AII AC and ON BCs which is smaller than the high gain rod BC-AII AC synapse (Pang et al. 2004; Volgyi et al. 2004; Xin and Bloomfield 1999). Although previous studies show that AII ACs do function during light-adapted cone dominant conditions by receiving rod input via rod-cone coupling and ON BC-AII AC electrical coupling (Demb and Singer 2012; Manookin et al. 2008; Munch et al. 2009), our results, in combination with Figure 1E,F and other studies, show that this drive is less robust than AII AC activation in the dark. AII AC sEPSCs were also recorded under both dark- and light-adapted conditions. With light adaptation, the sEPSC peak amplitude significantly decreased from 22±3 pA to 14.5±2 pA (n = 8) (Figure 4E,F,G). In addition, the normalized sEPSC frequency significantly decreased to 45±10% of control with light adaptation (n = 9) and the inter-event interval significantly
increased (487% of dark-adapted inter-event interval average) (Figure 4H). These data suggest that AII AC inhibition to OFF BCs decreases with our light adapting stimulus.

The magnitudes of OFF BC L-IPSCs are unaffected by switching from rod to cone circuits

We have shown that in the dark, OFF1,2,4 BCs receive mostly glycinergic input, likely from the AII ACs, while only about half of OFF3 BC L-IPSCs consist of glycineR-mediated currents from other AC types. However, it is unknown how inhibition to either OFF1,2,4 or OFF3 BCs will change when the cone pathways are dominant. To determine if there are changes in inhibition to OFF BCs with light adaptation, L-IPSCs were recorded from OFF1,2,4 and OFF3 BCs under a rod-adapting background light. If OFF1,2,4 BCs are receiving primarily rod-mediated inhibition we would expect there to be a significant reduction in L-IPSCs after light adaptation, while we might not expect OFF3 BCs L-IPSCs to significantly change since they are receiving cone pathway inhibition. Surprisingly, under these conditions, there was no significant difference in the Q in either OFF1,2,4 (n = 13, p > 0.05) or OFF3 (n = 12, p > 0.05) BCs with light adaptation at any stimulus intensity (Figure 5A,B,C). The absence of any change in the Q of the L-IPSC in OFF1,2,4 BCs suggests cone-pathway mediated inhibition is compensating for the reduction in rod-pathway mediated inhibition from the AII AC.

A change in the source of inhibition to OFF1,2,4 BCs from AII ACs to cone pathway ACs was also suggested by the significant decrease in the normalized peak amplitude (63±9%, p < 0.01) and a significant increase in the normalized D37 (193±30%, p < 0.05) (Figure 5D). In dark-adapted conditions there was no significant difference in the D37 between the OFF1,2,4 BC L-IPSCs and the AII AC L-EPSCs (AII D37 of 147±40 ms, OFF1,2,4 BC D37 of 142±45 ms, p >
0.05), while in the light they significantly differed (AII D37 of 104±29 ms, OFF1,2,4 BC D37 of 195±34 ms, p = 0.05), further suggesting different sources of input. There was no difference in the time-to-peak between dark- and light-adapted conditions for either OFF BC group (p > 0.05, data not shown). In contrast, the lack of change in the Q or timing parameters (D37 107±27, p=0.8) of OFF3 BCs agrees with our previous conclusion that their inhibition comes from cone pathways.

Additionally, there were differences in L-IPSC timing parameters between OFF1,2,4 and OFF3 BCs in the dark and light. There was a significant difference in the time-to-peak of L-IPSCs between OFF1,2,4 and OFF3 BCs in the dark (OFF1,2,4 average of 125±24 ms, OFF3 average of 213±23 ms, p < 0.05) and in the light (OFF1,2,4 average of 124±33 ms, OFF3 average of 222±21 ms, p < 0.05). It makes sense for OFF3 BCs to have longer response delays as they begin to receive inhibitory input only when the light turns off. Lastly, the L-IPSC D37 of OFF1,2,4 BCs was significantly longer than OFF3 BCs only in the light (OFF1,2,4 average of 195±38 ms, OFF3 average of 90±16 ms, p < 0.05). The timing differences of L-IPSCs between the two groups of BCs highlight their different circuitry connections and pathways.

*Light adaptation differentially modulates OFF1,2,4 and OFF3 BC sIPSCs*

sIPSCs in OFF BCs form the background inhibition they are receiving from all AC inputs with no external stimulus involved. Thus, any modulation of the sIPSCs would change the baseline level of inhibition the cells are receiving and in turn, alter their glutamate release onto GCs. In contrast to the L-IPSCs, the sIPSCs of OFF1,2,4 and OFF3 BCs were differentially changed with a switch to cone pathways under light-adapted conditions. The sIPSC peak amplitude of
OFF1,2,4 BCs decreased significantly (38±9 to 21±4 pA, K-S p < 0.01, Figure 6A,C,E). This is consistent with the idea that a majority of these glycinergic sIPSCs are coming from the rod BC-AII AC mediated pathway, as we show in Figure 4 that spontaneous AII AC activity decreases significantly with light. There was no change in the sIPSC peak amplitude in OFF3 BCs (13.58±1.54 to 15±3 pA, K-S p > 0.05, Figure 6B,D,F) further suggesting that the sIPSCs of OFF3 BCs are coming from cone-dependent OFF pathways. The decay τ of the sIPSCs did not significantly change with light adaptation in OFF1,2,4 (n = 8, p = 0.68) or OFF3 BCs (n = 7, p = 0.93) (data not shown). However, the sIPSC frequency normalized to the dark-adapted condition significantly decreased in the light in both OFF1,2,4 (to 69±11%, n = 10, p < 0.05) and OFF3 BCs (to 51±10%, n = 9, p < 0.01). As a result, the inter-event intervals of the sIPSCs also significantly increased in both OFF BC groups (119% of dark-adapted inter-event interval average in OFF1,2,4 BCs and 166% of dark-adapted inter-event interval average in OFF3 BCs) (Figure 6G,H). Taken together, these results suggest that with light adaptation, OFF1,2,4 BCs are losing a main source of spontaneous inhibition (rod BC-AII AC pathway) due to a decrease in both peak amplitude and frequency whereas OFF3 BCs may just be receiving fewer overall sIPSCs from the same ACs.

Changes in the signal-to-noise ratio suggest distinct sources of input

Changes in the background activity of OFF BC inhibition with light adaptation suggested that the strength of signal over background noise might also be changing. This can be estimated by calculating the signal-to-noise ratio (SNR) of a response. The SNR was calculated by dividing the peak amplitude of the L-IPSC by the variance of 100 ms of baseline, which accurately represented baseline variation for the given cell. Given that OFF1,2,4 BCs have significantly
more inhibitory spontaneous activity than OFF3 BCs, we predicted that the SNR for their responses might be different as well, which would affect the efficiency of OFF BC signal transmission to OFF GCs. For this reason, we calculated the SNR of OFF1,2,4 (n = 13) and OFF3 BCs (n = 11) in both dark- and light-adapted conditions to determine if there was a change in the relationship between the L-IPSC and the baseline sIPSCs. The SNR significantly increased for both OFF1,2,4 and OFF3 BCs with light adaptation (p < 0.05) suggesting that the decrease in the sIPSCs helps to magnify the L-IPSC (Figure 7 A,B). In the light, when the cone pathways are active, the light-evoked inhibitory input to OFF BCs is larger relative to the dark-adapted state as a result of the decrease in background noise. However, the SNR was significantly larger in OFF3 BCs in both dark- and light-adapted conditions (Figure 7C). This is most likely a result of the significantly fewer sIPSCs in OFF3 BCs (Fig 1). Our results further support that OFF1,2,4 and OFF3 BCs are receiving inhibitory input from distinct sources, and suggest that in general, cone-mediated inputs have a higher signal-to-noise ratio than rod-mediated inputs. A previous study (Arman and Sampath 2012) showed that glycinergic inputs do not significantly modulate the SNR (calculated using the unequal variance model of $d'$ or $d_a$), of very dim light voltage responses in OFF BCs. Our results however suggest that at brighter rod and cone light intensities this may be an important mechanism for determining the gain of inhibition to OFF BCs, possibly allowing for larger light-evoked modulation of the OFF BC excitatory signal.

Dark-adapted AII ACs receive a large amount of spontaneous activity (Figure 4), which sets the background for light-evoked signals. Thus, any changes in the sEPSCs could change the light signal to postsynaptic OFF BCs. As a result of the decrease in the peak amplitude and frequency of spontaneous activity, the SNR of the AII AC did not change (n = 9, p = 0.18, Figure 7D). The
increase in SNR of OFF BCs with light adaptation, contrasted to the unchanged SNR of the AII AC suggests that OFF1,2,4 BCs that receive primarily AII AC input in the dark must be getting input from other sources in the light. The smaller AII AC signal, presumably due to both less quantal release from RBCs and rod saturation, could not be providing the drive for the increase of the OFF BC signal relative to spontaneous noise. Taken together, these findings indicate that light adaptation increases the SNR, allowing for more sensitive higher visual responses in the light-adapted condition.

Increased GABAergic input provides compensatory light-adapted inhibition to OFF BCs

In dark-adapted conditions, the L-IPSCs and sIPSCs in OFF1,2,4 BCs were due mostly to glycinergic input, presumably from AII ACs. However, because there was no change in the L-IPSCs of OFF1,2,4 BCs with light adaptation, while the AII AC activation significantly decreases, it is necessary to determine what input is mediating the compensatory inhibition. To investigate the inhibition OFF BCs are receiving in the light, strychnine was applied to the preparation to block glycineRs followed by TPMPA and finally SR-95531 to block GABAC and GABAARs respectively. In OFF1,2,4 BCs, strychnine significantly decreased the L-IPSCs at all light intensities (n = 11) and application of SR-95531 and TPMPA abolished all light-evoked responses (n = 5) (Figure 8 A,C). There was no significant reduction in L-IPSCs after TPMPA application (data not shown, 37±7% after strychnine to 50±25% after TPMPA of the total initial Q, p > 0.05) but the additional application of SR-95531 abolished the light responses in all cells tested (n = 4). This suggests that light-adapted OFF BCs, like dark-adapted OFF BCs (Eggers et al. 2007), receive very little GABACR mediated input. The OFF1,2,4 BC L-IPSCs switched to significantly more GABA input mediated by GABAARs (43±10% glycine, 57±10% GABA, p <
0.05) when going from dark- to light-adapted conditions (Figure 8E). The decrease in glycinergic input to OFF1,2,4 BCs (37±11%) with light adaptation correlates with the decrease in excitatory input to AII ACs (54±10%) in the light, suggesting the majority of the glycinergic input comes from this connection (Welch’s t-test, p = 0.08).

Although OFF3 BCs receive no input from AII ACs and have much less glycinergic input in the dark than the OFF1,2,4 BCs (Figure 8E, p < 0.05), the rod pathway input eventually is distributed across at least some cone BC pathways via electrical synapses. This could mean that the activation of the OFF cone BCs and OFF ACs that send input to the OFF3 BCs could vary between rod and cone dominant conditions. In OFF3 BCs, strychnine had no significant effects on the light-adapted L-IPSCs (n = 8) and application of SR95531 and TPMPA abolished all light-evoked responses (n = 5) (Figure 8B,D). Similar to OFF1,2,4 BCs, TPMPA application had no effect (data not shown, 93±14% after strychnine to 79±19% after TPMPA of the total initial Q, p > 0.05) but the additional application of SR-95531 virtually eliminated the light responses in all cells tested (n = 4, 12±4% after SR-95531, p < 0.05). The OFF3 BC L-IPSCs switched to solely GABA<sub>A</sub>R mediated input (p < 0.05) in the light. There was a significantly smaller percentage of light-evoked glycineR input to OFF3 than OFF1,2,4 BCs in both dark- and light-adapted conditions (Figure 8E, p < 0.05), likely due to remaining AII AC inputs to OFF 1,2,4 BCs in the light. These results suggest cone pathway activation significantly alters the proportions of inhibition to switch in favor of GABAergic input to OFF1,2,4 and OFF3 BCs, effectively altering the type and source of inhibition that the OFF BCs receive.
Unlike the L-IPSCs, the sIPSCs in light-adapted conditions were mostly mediated by glycinergic input for both OFF1,2,4 and OFF3 BCs (data not shown). For most cells, application of strychnine eliminated all sIPSCs, causing a significant decrease in frequency in OFF1,2,4 (to 20±16% of total frequency, n = 8) and OFF3 (to 21±16% of total frequency, n = 6) BCs (p < 0.01). For the cells where some sIPSCs remained, there was no significant difference in the sIPSC peak amplitude of OFF1,2,4 (n = 3, p = 0.25) and OFF3 (n = 2, p = 0.54) BCs. The decay τ of the sIPSCs did not significantly change in the light when glycineRs were blocked in OFF1,2,4 (n = 3, p = 0.14) or OFF3 (n = 3, p = 0.14) BCs. Despite the switch of the L-IPSCs to larger GABAergic input, the sIPSCs of both OFF1,2,4 and OFF3 BCs in the light are still mediated by glycine release, suggesting that glycinergic ACs high basal rate of neurotransmitter release plays a role of setting background inhibition to OFF BCs.

Discussion:
In natural visual situations, light levels may activate both rods and cones simultaneously and changes in background light levels may emphasize rod or cone activation. Several studies have analyzed retinal signaling across different background light levels and have shown that gain and noise in the rod pathway changes in turn (Dunn et al. 2006; Dunn and Rieke 2008). Here we show that the ambient light state modulates the source and noise level of OFF BC inhibition when switching between rod and cone pathways. When a rod-saturating background was applied, glycinergic input to OFF BCs was reduced, but this reduction was compensated by an increase in GABAergic input. In the dark, OFF1,2,4 BCs are receiving substantial glycinergic input from the rod-AII AC pathway while in the light they receive equal amounts of glycine and GABAergic input from ON cone pathways (Figure 9A,C). In contrast, OFF 3 BCs, which do not
receive input from the rod pathway AII AC, receive much more GABAergic input in both the
dark- and light-adapted conditions from OFF cone pathways (Figure 9B,D). Although the total
amount of inhibition to OFF BCs did not change, the SNR increased with light adaptation due to
a decrease in spontaneous baseline inhibition to OFF BCs and was different between the rod and
cone activated pathways. Collectively, these suggest a specialization among OFF BC pathways
for light information from the rods and cones.

AII ACs target specific OFF BC pathways

The AII AC-OFF BC synapses are prominent morphologically (McGuire et al. 1984; Strettoi et
al. 1990), but had not been directly investigated physiologically, A previous report concluded
that AII AC-OFF GC connections, but not AII AC-OFF BC connections, signal the threshold of
rod responses (Arman and Sampath 2012) and did not find large glycinergic inputs in response to
the dim light intensities tested or changes in the SNR when blocking glycinergic inputs.
However, our data show that when using brighter rod light stimuli, OFF BCs receive robust AII
AC input, that is targeted to 3 subtypes (OFF 1,2,4) of OFF BCs (Figure 1). This supports a
previous morphological study that showed OFF4 BCs, but not OFF3 BCs, received AII AC
inputs (Tsukamoto et al. 2001). The potential differences in sensitivity between OFF BCs and
OFF GCs to AII input may be due to differential synapse structure between the AII and OFF
BCs and OFF GCs.

Increased GABAergic inhibition with light adaptation may modulate the spatial sensitivity of the
OFF pathway
We show that decreases in glycinergic OFF BC inhibition are compensated by increases in GABAergic input under light-adapted conditions. Glycinergic ACs such as the AII AC are narrow-field cells and thus may not mediate wide spatial inhibition, whereas GABAergic AC processes span long lengths of the retina (Menger et al. 1998; Pourcho and Goebel 1985; 1983; Vaney 1990). Although, in the dark AII ACs are connected via gap junctions that increase their spatial range to about 200-400 µm in very dim light, they are uncoupled with light adaptation and their spatial spread is much lower than potentially coupled wide-field GABAergic ACs (1000-4000 µm) (Bloomfield et al. 1997; Xin and Bloomfield 1999; 1997). Thus the switch from glycinergic to GABAergic inhibition has the potential to widen the spatial inhibitory surrounds of OFF BCs which may change their spatial sensitivity.

Many previous studies have shown that GC spatial sensitivity increases when moving from dim to bright light conditions (Barlow et al. 1957; Kuffler 1953; Maffei et al. 1971; Troy et al. 1999), which has been suggested to be due to changes in inner retinal circuitry (Dedek et al. 2008). Our findings suggest that changing inhibition to BCs may be one signaling mechanism to downstream GCs to enable more differentiation between spatially distinct light signals. It may be more important to have narrow-field inhibition in the dark where there is no need for high spatial sensitivity, as shown by the large convergence of rod signals (Vaney 1991). In light-adapted conditions, cone vision mediates the highest spatial acuity in order to differentiate novel light signals from the background luminance. This is formed from GABAergic input modulating center-surround organization which is an important feature for GC spatial tuning (Cook and McReynolds 1998; Flores-Herr et al. 2001; O'Brien et al. 2003; Volgyi et al. 2002). Increasing
GABAergic inhibition to OFF BCs could be one way of adjusting the inhibitory surrounds of BCs to affect the spatial center signals that GCs receive.

One potential mechanism for modulation of OFF BC inhibition is the increase in dopamine levels in the light (Doyle et al. 2002). Dopamine was found to potentiate GABA$_A$R currents possibly through phosphorylation by the cAMP-PKA signaling cascade (Feigenspan and Bormann 1994). This mechanism could cause circuitry changes such that cone activated GABAergic ACs are potentiated in the light while glycinergic AC outputs are decreased. A logical progression of this idea is that the increase in GABAergic inhibition could be partly inhibiting AII AC glycinergic release in addition to inhibiting OFF BCs. However, several studies have shown that AII ACs in the light receive primarily glycine-mediated inhibition and not GABAergic inhibition (Gill et al. 2006; Weiss et al. 2008). Thus it is likely that inhibition of the AII AC is not the direct cause of the inhibitory switch and dopamine may be acting through other means. Additionally, the increase in the inhibitory SNR could modulate the relative output of OFF BCs to filter out unneeded background light information. Further examination is necessary to determine what role the increase in the SNR plays in OFF BC signaling and whether there is a change in the spatial signaling to these cells. This would be an interesting future expansion of the present study.

OFF BC subtypes are receiving and sending different types of signals

In this study we have investigated the potential role of the primary rod pathway which transfers dim light information through the rod BC-AII AC circuit to OFF BCs (Chun et al. 1993; Deans et al. 2002; McGuire et al. 1984; Sassoe-Poggetto et al. 1994; Strettoi et al. 1990; Trexler et al.
However, there are two other rod pathways: the secondary pathway, activated by moderate light which signals OFF BCs through electrical connections between rods and cones (DeVries and Baylor 1995; Volgyi et al. 2004) and the tertiary pathway where brighter light causes direct rod activation of OFF BCs (Tsukamoto et al. 2001; Volgyi et al. 2004). Since there are multiple OFF BC pathways that convey distinct portions of the light signal, it is possible that the inputs from these three rod pathways will vary among OFF BC types. Our conclusions in this study support previous anatomical work showing that OFF3 BCs do not have AII AC connections (Tsukamoto et al. 2001). Several studies have shown that OFF3-like BCs contact both rods and cones and may be the BC that mediates the alternative rod pathway from rods directly to BCs (Mataruga et al. 2007; Tsukamoto et al. 2001). Furthermore it has been recently shown that OFF BCs (types 1 and 2) may receive rod information via rod-cone coupling in scotopic conditions (Pang et al. 2012) whose input may also activate cone inhibitory circuits which provide inhibition to OFF BCs. This pathway may be responsible for additional inhibition to OFF BCs but requires a different set of investigations to elucidate specific connections and its role in the inhibitory switch with light adaptation. We have shown here that OFF1,2,4 subtypes may receive input from the primary rod pathway (rod BC-AII AC-OFF BC) while OFF3 BCs instead may be getting brighter light information from the secondary and tertiary rod pathways. Our data support the idea that portions of rod pathway signals are specialized to different OFF BC pathways, although more work is required to determine how the dynamic range and timing of rod pathway input to OFF BCs varies.

In summary, we found that L-IPSCs in OFF BCs change from primarily glycine-mediated to GABA-mediated between dark- and light-adapted conditions. This occurs in a pathway specific
manner that may serve to help retinal fine-tuning of spatial sensitivity during daytime vision so
that the visual scene can be accurately signaled. This type of switch allows the retina to make
efficient use of available neurons and to use them in multiple signaling modes, a mechanism that
may be common across many brain systems.

Acknowledgements:

We would like to thank Dan Stutman for cell tracing and measurements, Drs. Johnnie Moore-
Dotson and Regina Nobles for helpful comments on this manuscript, and Adam Bernstein for
technical assistance.

Grants:

- NIH grant EY018131 (EDE)
- University of Arizona NIH Graduate Training in Systems and Integrative Physiology
  grant 5T32GM008400 (REM)
- Science Foundation Arizona (REM)

Disclosures:

Neither author, Reece E Mazade or Erika D Eggers, have any potential conflict of interest with
the present study.
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**Figure 1.** Inhibition to OFF BCs in the dark-adapted retina.

A, schematic of the rod pathway circuitry. Rod photoreceptors (R) activated by dim light release glutamate onto rod bipolar cells (RB) which release glutamate onto AII amacrine cells (AC). AII ACs release glycine onto OFF cone bipolar cells (OFF BC) while transmitting the depolarizing signal to ON cone bipolar cells (ON BC) via gap junctions. Cone photoreceptors (C) are activated by brighter light and release glutamate onto OFF and ON BCs. Activation of these bipolar cells in turn releases glutamate onto other GABAergic (GABA) and glycinergic (Gly) amacrine cells which also have inputs onto OFF BCs. Additionally, activation of ON BCs can depolarize AIIIs through electrical gap junctions. INL = inner nuclear layer, IPL = inner plexiform layer.

B, example cell morphology traces of #4, #3, and #1/2 OFF BCs. #4 OFF BCs have terminals that ramify in 1-38% (n = 4) of the IPL, #3 OFF BCs have terminals that ramify in 17-50% (n = 11) of the IPL and #1/2 OFF BCs have terminals that ramify in 1-16% of the IPL (n = 9). #1 and #2 OFF BCs are difficult to morphologically differentiate and so have been pooled.

C, example L-IPSCs recorded from an OFF4 (representing the OFF 1,2,4 group that we have pooled together) and an OFF3 BC to a 500 ms stimulus (dark gray bar). OFF1,2,4 BCs show an inhibitory response with the onset of light while OFF3 BCs show an inhibitory response with the offset of light. The recording paradigm is shown to the left.

D, OFF1,2,4 (n = 12) cells have a significantly higher charge transfer during light onset while OFF3 (n = 10) BCs have significantly higher charge transfer during light offset. The dashed gray line represents the normalized baseline responses. OFF1,2,4 during light offset and OFF3 during light onset were not different than the baseline (p > 0.05).
E, example L-IPSCs recorded from an OFF4 and OFF3 BC to a 30 ms stimulus (dark grey bar) in the light and with the application of CNQX and AP5. L-IPSCs remained in OFF1,2,4 BCs but were abolished in OFF3 BCs with CNQX and AP5.

F, intensity response curves of charge transfer normalized to the max light-adapted L-IPSC in OFF1,2,4 (n = 4) and OFF3 (n = 5) light-adapted BCs. CNQX and AP5 significantly reduced OFF1,2,4 L-IPSCs (*) at the brighter light intensities but abolished OFF3 BC L-IPSCs at all light intensities. (* = p < 0.05, ** = p < 0.01)

Figure 2. Dark-adapted L-IPSCs are mostly mediated by glycinergic input in OFF1,2,4 but not OFF 3 BCs.

A,B, example L-IPSCs recorded from an OFF1/2 and OFF3 BC, respectively, in response to a 30 ms flash of light (dark gray bar below L-IPSC). The dark-adapted L-IPSC (black trace) was greatly reduced by application of strychnine (gray trace). The recording paradigm is shown to the left with glycine receptors blocked (Ø).

C,D, intensity response curves of normalized charge transfer of OFF1,2,4 (n = 6) and OFF3 BCs (n = 4), respectively, to the max dark-adapted L-IPSCs. Strychnine significantly reduced OFF1,2,4 L-IPSCs at all light intensities while only the three highest light intensities were reduced in OFF3 BCs. Glycinergic inhibition in OFF3 BCs appears to be a smaller component than in OFF1,2,4 BCs (p<0.05). (* = p < 0.05, ** = p < 0.01)
**Figure 3.** Dark-adapted sIPSCs are mediated mainly by glycinergic input in both OFF1,2,4 and OFF3 cells. 

*Panel A,B*, example traces showing the sIPSCs from an OFF4 and OFF3 BC, respectively, in dark adapted conditions (black trace) and with application of strychnine (gray trace). The recording paradigm is shown to the left with glycine receptors blocked (Ø).

*C,D*, sIPSC peak amplitude histogram distributions (normalized to number of events) of the OFF1,2,4 and OFF3 BC from panel *A* and *B* respectively. Application of strychnine significantly reduced the sIPSC peak amplitude for OFF1,2,4 BCs (K-S p < 0.01) and OFF3 BCs (K-S p < 0.01). Arrows show the average amplitude of the sIPSC. Insets are averages of dark-adapted (black line) and strychnine application (gray line) sIPSCs. Scale bars are the same for both insets. (** = p < 0.01)**

**Figure 4.** Light adaptation alters the timing and the charge transfer of the AII L-EPSC and the magnitude and frequency of sEPSCs.

*Panel A*, L-EPSCs from an AII AC in response to a 30 ms flash of light (dark gray bar above the L-EPSC). There was a significant reduction of the dark-adapted L-EPSC (black trace) with light adaptation (gray trace). The recording paradigm is shown to the left.

*Panel B*, example cell morphology traces of an AII AC. AII ACs have terminals that cover 10 - 97% (n = 6) of the IPL. They have lobular terminals in the OFF BC sublamina of the IPL, responsible for glycine release, and longer thinner processes in the ON sub lamina which make gap junction connections to ON BCs.
C, intensity response curves of the charge transfer normalized to the max dark-adapted L-EPSC in AII dark- and light-adapted conditions (n = 9). There was a significant difference in the charge transfer with light adaptation at all intensities measured.

D, normalized change in L-EPSC response parameters of AII (n = 9) ACs with light adaptation. In the light, the peak amplitude and decay time (D37) significantly decreased.

E, example traces showing the sEPSCs of AII ACs in dark-adapted (black trace) and light-adapted conditions (gray trace). The recording paradigm is shown to the left.

F, sEPSC peak amplitude histogram distribution (normalized to number of events) of the AII AC seen in panel E. Light adaptation significantly reduced the sEPSC peak amplitude for the AII AC (K-S P < 0.01). Arrows show the average amplitude of the sEPSCs. The inset is an average of dark-adapted (black line) and light-adapted (gray line) sEPSCs.

G, the peak amplitude of sEPSCs was significantly decreased in AII ACs following light-adaptation (n = 8).

H, sEPSC inter-event interval histogram distribution (normalized to number of events) of the AII AC seen in panel E. Light adaptation significantly increased the sEPSC inter-event interval for the AII AC (K-S P < 0.01). Arrows show the average interval between the sEPSCs. (* = p < 0.05, ** = p < 0.01)

Figure 5. Light adaptation alters the timing of the L-IPSC in only OFF1,2,4 BCs while the magnitude of L-IPSCs remain unaffected for each BC group.

A,B, example L-IPSCs from an OFF1/2 and OFF3 BC, respectively, in response to a 30 ms flash of light (dark gray bar below L-IPSC trace). There was no significant reduction of the dark-
adapted L-IPSC (black trace) with light adaptation (gray trace). The recording paradigm is shown to the left.

C, intensity response curves of charge transfer normalized to the max dark-adapted L-IPSC in OFF1,2,4 (n = 13) and OFF3 (n = 12) dark- and light-adapted L-IPSCs. There was no significant difference in the charge transfer between OFF1,2,4 and OFF3 BC with light adaptation.

D, normalized change in L-IPSC response parameters of OFF1,2,4 (n = 13) and OFF3 (n = 10) BCs to max dark-adapted response with light adaptation. The peak amplitude was significantly reduced while the decay time was significantly increased in OFF1,2,4 BCs. In OFF3 BCs, neither the peak amplitude nor the decay time significantly changed. (* = p < 0.05, ** = p < 0.01)

Figure 6. Light adaptation decreased sIPSC peak amplitude in OFF1,2,4 BCs but not OFF3 BCs.

A,B, example traces showing the sIPSCs from an OFF1/2 and OFF3 BC, respectively, in dark-adapted (black trace) and light-adapted conditions (gray trace). The recording paradigm is shown to the left. The sIPSC peak amplitude histogram distributions (normalized to number of events) of the OFF1,2,4 and OFF3 BCs are shown in panels C and D respectively. Light adaptation significantly reduced the sIPSC amplitude for OFF1,2,4 BCs (K-S p < 0.01) but not OFF3 BCs (K-S p > 0.05). Arrows show the average peak amplitude of the sIPSCs. Insets are averages of dark-adapted (black line) and light-adapted (gray line) sIPSCs. Scale bars are the same for both insets.

E,F, the peak amplitude of sIPSCs was significantly decreased in OFF1,2,4 BCs (E, n = 10) following light adaptation and did not change in OFF3 BCs (F, n = 8).
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$G,H$, sEPSC inter-event interval histogram distributions (normalized to number of events) of the OFF1/2 and OFF3 BCs seen in panel $A$ and $B$, respectively. Light adaptation significantly increased the sIPSC inter-event interval for both cell groups (K-S $P < 0.01$). Arrows show the average interval between the sIPSCs. (* = $p < 0.05$, ** = $p < 0.01$)

**Figure 7.** The signal-to-noise ratio (SNR) increased in both OFF BC groups with light adaptation, but did not change in AII ACs.

$A,B$, SNRs in OFF1,2,4 BCs ($n = 13$) and OFF3 cells ($n = 11$), respectively. There was a significant increase in the SNR in the OFF1,2,4 as well as OFF3 BCs with light adaptation.

$C$, the SNR comparing OFF1,2,4 ($n = 13$) and OFF3 ($n = 11$) BCs in dark- and light-adapted conditions. In both instances, there was a significantly higher SNR in OFF3 BCs. The SNR ratio was calculated by dividing the peak amplitude of the L-IPSC by the variance of 100 ms of the baseline current. (* = $p < 0.05$)

$D$, the SNR of AII AC light responses in dark- and light-adapted conditions. There was no significant difference between the conditions ($n = 9$). (* = $p < 0.05$, ** = $p < 0.01$)

**Figure 8.** With light adaptation, both OFF BC groups received compensatory GABAergic inhibition.

$A,B$, example light-adapted L-IPSCs from an OFF1/2 and OFF3 BC, respectively, in response to a 30 ms flash of light (dark gray bar below the L-IPSC). The light-adapted L-IPSC (black trace) was unchanged with isolation of GABAergic input (light gray strychnine trace) in OFF3 BCs only. OFF1,2,4 light-adapted L-IPSCs decreased when GABAergic input was isolated. All L-
IPSCs were absent when both glycine and GABA receptors were blocked. The recording paradigm is shown to the left with GABA and glycine receptors blocked (Ø).

C,D, intensity response curves of charge transfer normalized to the max light-adapted L-IPSC in OFF1,2,4 (n = 11) and OFF3 (n = 8) BCs, respectively. Addition of strychnine had no effect on the L-IPSCs of OFF3 BCs but significantly decreased the L-IPSCs of OFF1,2,4 BCs. An * indicates a significant difference between the light-adapted control and strychnine application while a † indicates a significant difference between the strychnine treatment and the GABAR blockade (SR95531 + TPMPA).

E, the relative proportions of glycinergic and GABAergic input to OFF BCs under dark- and light-adapted conditions for OFF1,2,4 (n = 5, n = 11 respectively) and OFF3 (n = 5, n = 8, respectively) subtypes. With light adaptation, OFF1,2,4 BCs had a significant decrease in glycinergic input and OFF3 BCs had virtually no glycine response. OFF1,2,4 BCs had significantly more glycinergic input than OFF3 BCs in both light conditions. An * indicates a significant difference between the dark- and light-adapted conditions within an OFF BC group where as a # indicates a significant difference between OFF1,2,4 and OFF3 BCs in each light condition. (*, †, # = p < 0.05, **, †† = p < 0.01)

Figure 9. Summary schematics showing inhibitory input to OFF1,2,4 and OFF3 BCs under dark- and light-adapted conditions. A, under dark-adapted conditions, OFF1,2,4 BCs receive large light-evoked glycinergic inhibition from the rod-AII AC pathway in addition to other glycinergic and GABAergic inputs. This is done through crossover inhibition via excitation of the AII AC with dim light and serves as an ON inhibitory signal to the OFF pathway.
B, under dark-adapted conditions, OFF3 BCs receive large light-evoked glycinergic and GABAergic inhibition from OFF cone pathways activated by brighter light intensities.

C, under light-adapted conditions OFF1,2,4 BCs receive compensatory GABAergic and glycinergic inhibition from cone activated ACs. The AII AC may receive depolarizing signals through gap junction connections with ON BCs, and partly mediate inhibitory currents in the OFF1,2,4 BCs (Fig. 1E,F). However, there is an overall higher proportion of GABAergic input in the light.

D, in light-adapted conditions, OFF3 BCs receive compensatory inhibition solely mediated by GABAergic input from OFF cone activated ACs.
A. 30 ms OFF1,2,4 L-IPSC

B. OFF3 L-IPSC

C. Normalized Charge Transfer ($Q_r$) vs. Luminance (photons/µm²/sec)

D. Normalized Charge Transfer ($Q_r$) vs. Luminance (photons/µm²/sec)

[Graphs showing data for OFF1,2,4 and OFF3 L-IPSC with responses to different luminance levels and normalized charge transfer values.]

** and * indicate statistical significance.
Figure A: Dark-adapted OFF1,2,4 sIPSCs

Figure B: Dark-adapted OFF3 sIPSCs

Figure C: Normalized frequency vs. sIPSC peak amplitude (pA) for dark-adapted and Strychnine conditions.

Figure D: Normalized frequency vs. sIPSC peak amplitude (pA) for dark-adapted and Strychnine conditions.
**A.** Diagram showing the response of AII cells to RB stimulation, with 30 ms time resolution. The black curve represents the response under dark-adapted conditions, while the gray curve represents the response under light-adapted conditions.

**B.** Graph showing the percentage of IPL covered as a function of inter-event interval (ms) for both dark-adapted (black) and light-adapted (gray) conditions. The graph includes a vertical dotted line at 10 ms, indicating a notable change in normalized frequency.

**C.** Graph illustrating the normalized change in charge transfer (Q) as a function of luminance (photons/µm²/sec). The x-axis represents luminance values ranging from $9.5 \times 10^3$ to $9.5 \times 10^5$, and the y-axis shows normalized change values ranging from 0 to 1.4. The graph contains error bars for both dark-adapted and light-adapted conditions.

**D.** Bar graph comparing peak amplitude and D37 values between dark-adapted and light-adapted conditions. The black bars represent peak amplitudes, and the gray bars represent D37 values. Asterisks denote statistically significant differences.

**E.** Diagram showing AII cell responses to stimulation with 200 ms time resolution. The dark-adapted condition is represented by the black curve, and the light-adapted condition is represented by the gray curve.

**F.** Graph showing the normalized frequency of sEPSC peak amplitudes in pA. The x-axis represents sEPSC peak amplitude values ranging from 0 to 125 pA, and the y-axis represents normalized frequency values ranging from 0 to 0.07. The graph includes a dotted line at 25 pA with a 1 ms time resolution.

**G.** Graph illustrating the normalized frequency of sEPSC peak amplitude values ranging from 10 to 50 pA for both dark-adapted and light-adapted conditions. Asterisks denote significant changes.

**H.** Graph comparing normalized frequency values between dark-adapted and light-adapted conditions as a function of inter-event interval (ms). The x-axis represents inter-event interval values ranging from 0 to 100 ms, and the y-axis represents normalized frequency values ranging from 0 to 0.6. Asterisks denote significant differences.
A. OFF1,2,4 L-IPSC

B. OFF3 L-IPSC

C. Normalized Change Transfer (Q)

D. Peak Amplitude D37
A. OFF1,2,4 L-IPSC

B. OFF3 L-IPSC

C.

D.

E.

** Normalized Charge Transfer (Q)**

** Luminance (photons/µm²/sec)**

** Percent of L-IPSC**
A. Dark Adapted (Rods Active)

B. Dark Adapted (Rods Active)

C. Light Adapted (Cones Active)

D. Light Adapted (Cones Active)