Cross inhibition from ON to OFF pathway improves the efficiency of contrast encoding in the mammalian retina

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Liang Z, Freed MA. Cross inhibition from ON to OFF pathway improves the efficiency of contrast encoding in the mammalian retina. J Neurophysiol 108: 2679–2688, 2012. First published August 29, 2012; doi:10.1152/jn.00589.2012.—The retina is divided into parallel and mostly independent ON and OFF pathways, but the ON pathway “cross” inhibits the OFF pathway. Cross inhibition was thought to improve signal processing by the OFF pathway, but its effect on contrast encoding had not been tested experimentally. To quantify the effect of cross inhibition on the encoding of contrast, we presented a dark flash to an in vitro preparation of the mammalian retina. We then recorded excitatory currents, inhibitory currents, membrane voltages, and spikes from OFF α-ganglion cells. The recordings were subjected to an ideal observer analysis that used Bayesian methods to determine how accurately the recordings detected the dark flash. We found that cross inhibition increases the detection accuracy of currents and membrane voltages. Yet these improvements in encoding do not fully reach the spike train, because cross inhibition also hyperpolarizes the OFF α-cell below spike threshold, preventing small signals in the membrane voltages at low contrast from reaching the spike train. The ultimate effect of cross inhibition is to increase the accuracy with which the spike train detects moderate contrast, but reduce the accuracy with which it detects low contrast. In apparent compensation for the loss of accuracy at low contrast, cross inhibition, by hyperpolarizing the OFF α-cell, reduces the number of spikes required to detect the dark flash and thereby increases encoding efficiency.

visual stimulus evokes a different response each time it is presented, sometimes producing a response identical to the response when the stimulus is absent (de Ruyter van Steveninck et al. 1997). As a result, a single neural response cannot predict whether a visual stimulus was presented with absolute certainty. Therefore, visual processing must take a probabilistic approach to detection that weighs the likelihood that a given neural response resulted from a given stimulus. Ideal observer analysis is a Bayesian method that formalizes this probabilistic approach. It is not assumed that the visual system analyzes neural responses in the same way as the ideal observer does. Instead, ideal observer analysis provides a measure of information that is available for a specific visual task. Thus the ideal observer does not provide a global measure of encoding like Shannon information, but a specific measure that can be related to visual behavior (Dhingra and Smith 2004; Geisler et al. 1991; Murphy and Rieke 2011; Xu et al. 2005).

We selected a particular type of OFF ganglion cell, the OFF α-cell, because its cross inhibitory circuit is well characterized (Manookin et al. 2008; Liang and Freed 2010). We presented the OFF α-cell with a specific visual task, which was to detect a dark flash. We recorded different instantiations of information as it flows from the α-cell’s synaptic input to its spike output and thus included postsynaptic currents, membrane voltages, and spikes. We submitted these instantiations to an ideal observer analysis that detected whether the dark flash had occurred or not, and, by blocking cross inhibition, we were able to assess whether cross inhibition normally increased the accuracy of detection. We found that the ON pathway improved the accuracy of the OFF α-cell’s excitatory currents and membrane potentials at low and moderate contrasts, but had a differential effect on the spike train, improving accuracy at moderate contrasts, but reducing accuracy at low contrast. In apparent compensation for this loss of accuracy at low contrast, cross inhibition reduces the number of spikes required to detect contrast, thus increasing the overall efficiency of contrast encoding.

THE RETINA IS DIVIDED INTO parallel and mostly independent ON and OFF pathways, but the ON pathway “cross” inhibits the OFF pathway. In the light-adapted retina, two effects of cross inhibition on signals in the OFF pathway have been found. First, cross inhibition rectifies excitatory currents in OFF ganglion cells, presumably by modifying the output of OFF bipolar cells, with the result that the excitatory currents respond to negative contrasts and much less to positive contrasts (Liang and Freed 2010; Manookin et al. 2008; Molnar et al. 2009; Renteria et al. 2006). Second, cross inhibition supplies to OFF ganglion cells inhibitory currents that are less rectified than the excitatory currents and respond more equally to negative and positive contrasts (Manookin et al. 2008). Yet it remained unclear whether cross inhibition improves the contrast sensitivity of the OFF pathway because sensitivity depends on both signal and noise, and the effects of cross inhibition on noise in the OFF pathway had not been measured.

We used an “ideal observer” analysis to quantify how accurately the OFF pathway detects contrast. A rationale for applying this method starts with the observation that the same
adjusted to \(\sim 300\) mOsm with glucose, and contained the following (in mM): 120 NaCl, 3.1 KCl, 0.5 KH\(_2\)PO\(_4\), 23 NaHCO\(_3\), 1.2 MgSO\(_4\), 1.15 CaCl\(_2\), plus amino acids and vitamins (pH 7.4).

**Recording.** A glass patch pipette (6–10 M\(\Omega\)) was formed with a Sutter P-87 puller (Sutter, Novato, CA). For extracellular recording of spikes by the loose patch method, the electrode contained Ames’ medium and formed a \(-1\)G\(\Omega\) seal. To record spikes in the whole cell mode, the pipette solution contained the following (in mM): 110 KOH, 110 gluconate, 10 NaCl, 1 EGTA 2.5 Na, 10 HEPES, 6 Lucifer yellow, and formed a \(-1\)G\(\Omega\) seal. To record currents or \(V_m\) in whole cell mode, spikes were blocked with a pipette solution containing the following (in mM): 110 CsOH, 120 gluconate, 10 NaCl, 1 EGTA 2.5 Na, 10 HEPES, 10 lidocaine 4-ethyl chloride (QX-314 Cl), 6 Lucifer Yellow. Pipette solutions were adjusted to 310 mOsm with glucose and to pH 7.2 with gluconate. Given these pipette solutions, the calculated reversal potential for gluconate channels (\(E_{\text{glu}}\) with equal permeability to Cs\(^+\), Na\(^+\), and K\(^+\)) was \(-4\) mV. The calculated reversal potential for GABA and glycine receptors (\(E_{\text{glyc}}\)) was approximately \(-67\) mV. Calculated values for \(E_{\text{glu}}\) and \(E_{\text{glyc}}\) have been confirmed by puffing agonists for glutamate and glycine receptors, respectively, on guinea pig OFF \(\alpha\)-cell dendrites under voltage clamp (Beaudoin et al. 2008).

Recordings were acquired with a Multiclamp 700B patch-clamp amplifier and digitized online at 4 kHz for current-clamp recordings and 2 kHz for voltage-clamp recordings using pClamp 10 (8-pole Bessel filter, \(f_c < 0.5\) digitization rate, Axon Instruments, Axon.com). The holding voltage \(V_{\text{hold}}\) was corrected for a calculated liquid junction potential of \(\sim 14\) mV. For excitatory currents, \(V_{\text{hold}}\) was not corrected for a voltage drop \(V_d\) due to the holding current \(I_{\text{hold}}\) across access resistance \(R_a\) because this voltage was small (\(R_a = 193.2 \pm 21\) M\(\Omega\), \(I_{\text{hold}} = -53.8 \pm 7.5\) pA, \(V_d = -1.1 \pm 0.2\) mV). For inhibitory currents, however, \(I_{\text{hold}}\) and \(V_{\text{hold}}\) were larger, and so we used the amplifier to compensate for 50–60% of the \(V_d\) (greater compensations produced oscillations that shortened recordings). To correct for the uncompensated \(V_d\), \(V_{\text{hold}}\) was calculated by the following formula:

\[
V_{\text{hold}} = V_{\text{uncorrected}} - I_{\text{hold}}R_a(1 – \% \text{ compensation}) \quad (1)
\]

where \(V_{\text{uncorrected}}\) is the uncorrected holding voltage. All drugs were added to the Ames’ medium. L-AP4 (L-2-amino-4-phosphonobutyric acid, TOCRIS, 25 \(\mu\)M) was used to block metatrophic glutamate receptors. Strychnine (TOCRIS, 2 \(\mu\)M) was used to block glycine receptors. MFA (meclofenamic acid; SIGMA 100 \(\mu\)M) was used to block gap junctions.

**Visual stimulus.** The stimulus was provided by a 556-nm light-emitting diode that projected diffusely over the entire \(-1\)cm\(^2\) field of retina. The circuitry driving the diode enabled a stimulus time constant of 140 \(\mu\)s. The flash stimulus was sampled at 1,000 Hz. The white-noise stimulus was randomly sampled at 1,000 Hz by low-pass filtering. The baseline intensity of the stimulus was \(3 \times 10^5\) photons \(\mu\)m\(^2\)s\(^{-1}\), resulting in a photoisomerization rate of \(4.6 \times 10^5\) R\(^*\)s\(^{-1}\) for a rod and \(3.3 \times 10^4\) R\(^*\)s\(^{-1}\) for an M cone (\(\lambda_{\text{max}} = 500\) nm, \(\lambda_{\text{max}} = 529\), rod outer segment: 16.2 \(\mu\)m \(\times 3\) \(\mu\)m, cone outer segment: 8 \(\mu\)m \(\times 3\) \(\mu\)m) (Yin et al. 2006). Given these photoisomerization rates, the non-color opponent ganglion cells we recorded from the dorsal retina have responses that are approximately equally divided between rods and M cone signals (Yin et al. 2006). Contrast stimulus was measured as Michelson contrast: \(C = \left(I_{\text{max}} - I_{\text{min}}\right) / I_{\text{max}} + I_{\text{min}}\), where \(I_{\text{max}}\) is maximum current, and \(I_{\text{min}}\) is minimum current.

**Bayesian analysis.** The probability of stimulus \(j\) conditional on response \(i\) is denoted \(p(s_j|r_i)\) and can be calculated by Bayes’ Rule:

\[
p(s_j|r_i) = \frac{p(r_i|s_j)p(s_j)}{p(r_i)} \quad (2)
\]

where \(p(r_i|s_j)\) is the probability of the response \(i\) conditional on stimulus \(j\), \(p(r_i)\) is the marginal probability of the response \(i\), and \(p(s_j)\) is the marginal probability of stimulus \(j\). The task was to guess which of two stimuli were presented by observing a single response \(i\). The best guess is to choose the stimulus \(j\) for which \(p(s_j|r_i)\) is greatest. Yet to fully map \(p(s_j|r_i)\) for all \(j\) is impractical because it would require too many different stimuli. Instead, consider that the marginal probability of the response \(p(r_i)\) is the same for both stimuli because it is the same response. Also, the marginal probability of the stimulus \(p(s_j)\) can be made the same for both stimuli by presenting them with equal frequency. Thus for the purposes of comparing two stimuli, Eq. 2 can be simplified into a proportionality:

\[
p(s_j|r_i) \propto p(r_i|s_j) \quad (3)
\]

Therefore, the best strategy for this particular task is to map the distribution \(p(r_i|s_j)\) for two stimuli. Then upon observing a particular response \(i\), the best guess becomes the stimulus \(j\) for which the distribution \(p(r_i|s_j)\) has the greatest value.

**Fisher’s linear discriminant.** We used Fisher’s linear discriminant to construct the two probability distributions required for the Bayesian analysis. We started by dividing recordings into \(n\) time bins, resulting in values \(x_1, x_2, x_3, \ldots x_n\). Each value \(x_i\) was multiplied by a factor \(\lambda\), and the products summed to calculate the Fisher measure \(F = \lambda x_1 + \lambda x_2 + \lambda x_3 + \ldots \lambda x_n\). The Fisher measure can be represented as the dot product of vectors \(F = \lambda \hat{x}\). When comparing two such probability distributions, the value of \(\lambda\) that produces the greatest separation (least overlap) of probability distributions is

\[
\hat{\lambda} = \left(\sum_{j=1}^{s_j} + \sum_{j=2}^{s_2}\right)^{-1}(\hat{\mu}_j - \hat{\mu}_j) \quad (4)
\]

where \(\sum_{j=1}^{s_j}\) and \(\sum_{j=2}^{s_2}\) are the covariance matrices of \(x\) for the two stimuli \(j = 1, 2\); the vectors \(\hat{\mu}_j\) and \(\hat{\mu}_j\) are the average of \(\hat{x}\) for the two stimuli (Fisher 1936).

**Ideal observer analysis.** The ideal observer analysis began by taking recordings of currents, voltages, or spikes from trials of the same contrast, and taking an equal number of trials where no contrast was presented (sham trials). Each recording was divided into \(n = 12\) time bins, with the first bin starting when the stimulus began. We choose a bin width of 40 ms because it gave the greatest sensitivity (Dinghra and Smith 2004). Currents or voltages were averaged for each bin, or the number of spikes in each bin was counted, and the resulting number \(x_i\) multiplied by the Fisher discriminant \(\hat{\lambda}\) to give the Fisher measure (e.g., Fig. 1B). The probability distributions of the Fisher measure were constructed for the contrast and sham trials (e.g., Fig. 1C). These distributions served as the likelihood functions for a Bayesian analysis: each trial was compared with probability distributions for sham and contrast stimuli, and the stimulus with the higher probability (likelihood) for that Fisher measure was chosen as the predicted stimulus. The predicted stimulus was compared with the actual stimulus and the prediction scored as correct or incorrect.

**Determination of cell type.** After extracellular recording, the patch pipette was removed, and another pipette was used in the whole cell mode to fill the cell with Lucifer Yellow. During whole cell recording, Lucifer Yellow diffused from the electrode into the cell. After filling the cell with Lucifer Yellow, the entire depth of the inner plexiform layer (IPL) was photographed using a combination of infrared illumination, epifluorescent illumination, and a cooled-CCD camera (Hamamatsu, Hamamatsu Photonics). The stratification of the fluorescent dendrites in the IPL was measured by counting the number of sections between its “top” and “bottom” edges, as marked by amacrine and ganglion cell somas respectively.

We targeted large somas for recording (15- to 25-\(\mu\)m diameter). This improved our chances of recording from OFF \(\alpha\)-cells, but also resulted in recordings from ON \(\alpha\), ON \(\delta\), OFF \(\delta\) cells, and less often from other cell types. Thus \(\alpha\)- and \(\delta\)-cells were identified as those with dendritic trees greater than 250 \(\mu\)m in diameter (Freed and Liang 2010). OFF \(\alpha\)- and OFF \(\delta\)-cells were those that ramified in the top half of the IPL, but the OFF \(\delta\)-cell ramified closer to the top edge of the
IPL than the OFF α-cell (Freed and Liang 2010; Manookin et al. 2008; Rockhill et al. 2002).

To check this method of identification, we examined seven cells identified as OFF α-cells and five cells identified as OFF δ-cells by fixing the retina in 4% paraformaldehyde, staining for choline acetyltransferase (CHAT), and photographing a series of horizontal optical sections in a confocal microscope (Olympus Fluoview 1000). CHAT forms two bands: one in the top OFF half of the IPL, and the other in the bottom ON half. All cells that we had classified as OFF δ-cells by the combined infrared/epifluorescent method were above the OFF CHAT band; all cells that we had classified as OFF α-cells and five cells identified as OFF δ-cells by fixing were below this CHAT band, thus confirming our classification (Manookin et al. 2008).

RESULTS

The ON pathway increases the accuracy with which excitatory currents in the OFF α-cell detect a dark flash. The stimulus was a 100-ms decrease in intensity throughout an OFF α-cell’s receptive field: a “dark flash.” Such an extended stimulus evokes robust responses from a ganglion cell due to timing differences between the ganglion cell’s receptive field center and surround (Frischman et al. 1987; Tokutake and Freed 2008). We varied the temporal contrast of the stimulus in random order, interleaving it with sham trials with zero contrast. Each contrast was repeated 100 times, requiring a typical recording time of 16 min.

We recorded excitatory currents from OFF α-cells by voltage clamping them at the reversal potential of inhibitory currents (E(Cl)), thus removing the driving force across inhibitory conductances. The excitatory currents responded to the dark flash with an inward transient (Fig. 1A). The currents were submitted to an ideal observer analysis that predicted whether a sham trial or a trial with contrast had been presented (MATERIALS AND METHODS). This prediction was compared with the actual trial and the accuracy of the prediction scored. The result of the ideal observer analysis was that, at the lowest contrast tested (0.25%), excitatory currents detected the flash with 61 ± 1% accuracy, just above the 50% that random guessing would produce (Fig. 1D). Accuracy increased sigmoidally with log contrast, until at the highest contrast (30%), prediction was perfect (100% accuracy).

The group III glutamate metabotropic receptor agonist, L-AP4, blocks photoreceptor input to the ON bipolar cell, and thus blocks cross inhibition from the ON to the OFF pathways (Manookin et al. 2008; Slaughter and Miller 1981). Application of L-AP4 reduced accuracy at most contrasts, but did not reduce accuracy at the contrasts that produced near chance or perfect accuracy (i.e., not at the lowest or highest contrasts tested). We concluded that normally the ON pathway increases the accuracy with which the OFF α-cell’s excitatory currents detect a dark flash.

The ON pathway increases the OFF α spike train’s accuracy at moderate contrast but reduces it at low contrast. We recorded membrane voltages by current-clamping in the whole cell mode and included Cs and QX-314 in the pipette solution to block potassium and sodium channels, respectively, thus preventing spikes. We submitted these potentials to ideal observer analysis and found that blocking crossover inhibition with L-AP4 reduced the accuracy of membrane voltages at almost all contrasts as it had for excitatory currents (Figs. 2, A and B). Thus normally the ON pathway improves the accuracy with which the OFF α-cell’s membrane voltages detect a dark flash.

Because the ON pathway improves the accuracy with which the OFF α-cell’s membrane voltages detect a dark flash, we predicted an improvement would occur for the spike train also. To test this, we recorded spikes extracellularly in the “loose patch” mode without disturbing the ionic gradients of the OFF α-cell (MATERIALS AND METHODS) (Fig. 2C). When we applied

Fig. 1. L-2-amino-4-phosphonobutyric acid (L-AP4) decreases the accuracy with which excitatory currents in the OFF α-cell detect a dark flash. A: the top traces show a 100-ms dark flash at three example contrasts. Below are excitatory currents from an OFF α-cell in control and L-AP4 conditions. B: currents from each trial were divided into n = 12 time bins, and the current averaged for each bin to give x_n. The top graph shows x_n for a sham trial (0% contrast) and a trial with 4% contrast. The bottom graph shows the Fisher discriminant λ_n (MATERIALS AND METHODS). C: the probability distributions of the Fisher measure F = ∑ λ_n for 0% and 4% contrasts. The two probability distributions were well separated in control but overlapped in L-AP4 (shaded region). D: the neurometric curves for excitatory currents in control and L-AP4 conditions. To construct neurometric curves, the data points were fit with a cumulative Weibull function: accuracy = 1 − 0.5 exp [(contrast/α)^b] (Quick 1974), where α is a scaling factor and b determines the slope. Error bars in this and subsequent figures indicate standard error of the mean. a.u., Arbitrary units.
the ideal observer analysis to the spike train, we found that blocking the ON pathway with L-AP4 decreased the accuracy of spikes at moderate contrasts, as expected (Fig. 2D). Yet contrary to prediction, L-AP4 increased accuracy at lower contrasts. Apparently the ON pathway normally increases the accuracy of the OFF α-cell’s spike train at moderate contrasts, but decreases accuracy at low contrasts, a differential effect distinct from an overall improvement for the excitatory currents and membrane voltages.

The ON pathway prevents small signals in the OFF α-cell’s membrane voltages from reaching the spike train. To determine how the ON pathway reduces the accuracy of the spike train at low contrast, we noted that L-AP4 always depolarized the OFF α-cell (by $7.3 \pm 1.6$ mV) and increased spontaneous spiking (Fig. 2C). We hypothesized that, normally, cross inhibition hyperpolarizes the cell far enough below spike threshold that small signals at low contrast do not reach the spike train. To test this, we recorded in the whole cell current-clamp mode as before, but omitted Cs and QX-314 from the pipette solution to allow spiking. When we presented the dark flash, it produced a transient depolarization, and, if the depolarization was high enough, it triggered a burst of spikes (Fig. 3A). To estimate the threshold potential for spiking, we measured the peak membrane potential $V_p$ and average spike rate $S$ in a 40-ms window that captured the acceleration of spike rate during the burst. We averaged $V_p$ and $S$ across all contrasts of dark flash and fit their relationship with a rectified power function (Priebe and Ferster 2008):

$$S(V_p) = k |V_p - V_{th}|^p$$

where $k$ is a gain factor, $p$ is an exponent greater than one, and $|V_p - V_{th}|$ is equal to the enclosed quantity, if this quantity is positive, but is otherwise equal to zero (Fig. 3B). The spike threshold was taken as the value of the parameter $V_{th}$ that produced the best fit. The result was that in control conditions, the peak of the depolarization $V_p$ was below spike threshold $V_{th}$ at low contrast and rose above spike threshold as contrast increased (Fig. 3, C and D). When the ON pathway was blocked with L-AP4, $V_p$ was above spike threshold $V_{th}$ for all contrasts. Thus we confirmed that, under control condition, cross inhibition from the ON pathway hyperpolarizes the OFF α-cell below spike threshold.

If normally cross inhibition hyperpolarizes the OFF α-cell so far below spike threshold that small signals in its membrane voltages would fail to reach the spike train, this should reduce accuracy at low contrasts that evoke small signals. If so, then depolarizing the OFF α-cell should improve accuracy at low contrast. To test this, we recorded spikes in current-clamp, applied L-AP4, and noted the degree of depolarization that this caused. We removed L-AP4, allowed the cell to repolarize, and then injected positive current sufficient to depolarize it to the level we measured in L-AP4. After the ideal observer analysis, we found that, at low contrast, depolarization improved accuracy, so that it equaled the accuracy obtained in L-AP4. At moderate contrasts, this depolarization had little effect on accuracy, presumably because, in the control condition, larger flash-evoked depolarizations were already above spike threshold (Fig. 3E). Thus we confirmed that normally cross inhibition hyperpolarizes the OFF α-cell sufficiently to reduce detection accuracy at low contrast.

The ON pathway increases the efficiency with which the OFF α-cell’s spikes detect a dark flash. Cross inhibition improves the accuracy of the OFF α-cell’s spike train at moderate contrasts: this was demonstrated by both loose-patch extracellular and current-clamp recordings of the OFF α-cell (Figs. 2D and 3F); this improvement is apparently inherited from excitatory currents and membrane voltages (Figs. 1D and 2B). Yet, at low contrast, cross inhibition also prevents small signals in the membrane potential from reaching the spike train (Fig. 3C), and we sought a rationale for this. A possible rationale was that cross inhibition, by hyperpolarizing the membrane potential, reduces the number of spikes used to detect contrast. Saving spikes is energetically important because ATP is required to reestablish the gradients that spikes expend (Alle et al. 2009; Howarth et al. 2009). Furthermore, naturalistic stimuli cause a ganglion cell to fire in bursts which
provide a sparse energy-efficient strategy for encoding information, a strategy that would be disrupted by excessive spontaneous firing (Balasubramanian and Berry 2002).

To test the idea that cross inhibition reduces the number of spikes required to detect contrast, we derived a measure of how efficiently spikes are used to detect contrast. We determined the contrast at a criterion accuracy, inverting this contrast to provide a sparse energy-efficient strategy for encoding information, a strategy that would be disrupted by excessive spontaneous firing (Balasubramanian and Berry 2002).

The ON pathway reduces tonic noise more than dynamic noise in the OFF α-cell’s excitatory currents. To improve detection accuracy of excitatory currents, cross inhibition increased the distance between the Bayesian probability distributions and reduced their widths, which is equivalent to increasing signal and reducing noise (Fig. 1C). To confirm these effects on signal and noise, we reanalyzed the excitatory currents, but, instead of the Fisher transformation, we measured signal as the average amplitude of the flash responses R and noise as the standard deviation of their amplitude σR (Fig. 4A). L-AP4 had little effect on signal at low contrast, but divisively reduced signal at moderate contrasts, i.e., as if by dividing it by a constant factor of about 2 (Fig. 4C). Thus the ON pathway normally increases signal in excitatory currents in the OFF α-cell.

Noise increased with contrast in a stepwise manner. In control (black curve, Fig. 4C), noise remained at the levels at zero contrast up to and including 2% contrast, suggesting that tonic noise at zero contrast explained the variability of responses. As contrast rose above 2%, noise rose above the tonic level, suggesting additional dynamically generated noise. Similarly, in L-AP4, noise remained at tonic levels up to and including 8% contrast; above this contrast, the dynamically generated noise appeared (Fig. 4C, red curve). It was in the range of contrasts that maintained tonic levels of noise (≤8%), that L-AP4 increased noise by a factor of 1.9. At higher contrast, L-AP4 had no significant effect on noise, indicating
that blocking cross inhibition did not greatly affect the dynamically generated noise. Thus the flash experiments indicated that cross inhibition’s main effect on noise in excitatory currents is to reduce a tonic component.

The ON pathway’s ability to reduce noise in excitatory currents depends on gap junctions. We next asked by which circuits cross inhibition reduced noise in excitatory currents. To test this, we used a stimulus that made best use of finite recording time to measure noise over multiple drug conditions. The stimulus consisted of 3 s of white noise followed by 2 s of steady intensity. We repeated the stimulus and recorded excitatory currents by clamping at the reversal potential for inhibitory currents ($I_{\text{Cl}}$) as before. The white noise evoked a series of inward events whose amplitude averaged over stimulus repeats we calculated as signal ($I_{\text{event}}$), and whose standard deviation we calculated as noise ($\sigma_{\text{event}}$) (Fig. 5A). The steady intensity evoked a tonic current ($I_{\text{tonic}}$).

L-AP4 had no consistent effect on signal, but reduced the signal associated with rare large events, which presumably corresponds to the reduction of signal at moderate contrast we found for the dark flash stimulus (Fig. 5C). L-AP4 also dramatically increased noise as it had for the flash stimulus ($\sigma_{\text{event}} = 13.8 \pm 0.4$ pA in control, $\sigma_{\text{event}} = 23.4 \pm 0.7$ pA in L-AP4) (Fig. 5C). L-AP4 shifted the baseline of the inward events inward and increased the tonic inward current ($\Delta I_{\text{tonic}} = -106 \pm 15$ pA) (Fig. 5, A and B).

The AII is a glycnergic amacrine cell that couples electrically by gap junctions with the ON bipolar cell and synapses on the OFF bipolar cell (see Fig. 7B) (Famiglietti and Kolb, 1975; Liang and Freed 2010; Petrides and Trexler 2008; Veruki and Hartveit 2009). We hypothesized this circuit allows the ON bipolar cell to reduce noise in excitatory currents. If this is true, then blocking gap junction between the AII amacrine cell and ON bipolar cells should prevent L-AP4 from increasing noise. To test this, we applied MFA, which blocks this gap junction, and then applied L-AP4 (Veruki and Hartveit 2009). In the presence of MFA, L-AP4 had no consistent effect on signal ($I_{\text{event}}$) (Fig. 5D). MFA suppressed the effects of L-AP4 on noise ($\sigma_{\text{event}} = 17.1 \pm 1.5$ pA in control; $\sigma_{\text{event}} = 18.2 \pm 1.4$ pA in L-AP4) and on tonic current ($\Delta I_{\text{tonic}} = -6.4 \pm 6.0$ pA) (Fig. 5, B and 5D).

The glycine receptor antagonist strychnine had effects that were entirely similar to those of L-AP4. Strychnine had no consistent effect on signal (Fig. 5E1) but consistently increased noise ($\sigma_{\text{event}} = 23.1 \pm 1.1$ pA in control; $\sigma_{\text{event}} = 32.3 \pm 1.6$ pA in strychnine) and tonic current ($\Delta I_{\text{tonic}} = 117.3 \pm 27.8$ pA) (Fig. 5, B and E2). In conclusion, the effects of L-AP4 on noise were blocked by MFA and similar to those of strychnine, suggesting that the AII amacrine cell is a component of the cross circuit that suppresses noise in the OFF α-cell’s excitatory currents (see DISCUSSION).

Cross inhibition provides an inhibitory current directly to the OFF α-cell that encodes information about contrast. Next we recorded postsynaptic inhibitory currents by voltage clamping the OFF α-cell at the reversal potential of excitatory currents, presented the dark flash stimulus as before, and then subjected the inhibitory currents to ideal observer analysis (Fig. 6A). The accuracy of the inhibitory currents was substantially less than those of the excitatory currents at all contrasts, yet they clearly encoded information about the dark flash (Fig. 6B).

To measure how much of the postsynaptic inhibitory signal was supplied by cross inhibition, we measured how much L-AP4 blocked the light-evoked modulation of the inhibitory current. Thus we presented the stimulus that combined white noise followed by a steady intensity and recorded inhibitory currents (Fig. 6C). To quantify light-evoked modulation, we measured the standard deviation of currents during white noise and steady intensity ($\sigma_{\text{wn}}$ and $\sigma_{\text{tonic}}$, respectively) then calculated $\sigma_{\text{wn}}/\sigma_{\text{tonic}} - 1$, which would be equal to zero if light-evoked currents were just at the level of tonic noise. Modulation was $3.6 \pm 1.1$ in control and declined to $0.4 \pm 0.2$ in L-AP4, so about 11% of modulation resisted L-AP4 (Fig. 6D). Apparently the ON pathway provides the remainder of the modulation, about 89%.

Both pre- and postsynaptic cross inhibition tonically hyperpolarize the OFF α-cell. To determine how cross inhibition hyperpolarizes the OFF α-cell, we examined the tonic currents from the steady intensity following the white noise stimulus. We had already found that L-AP4 caused the tonic excitatory current to shift inward by $\Delta I_{\text{tonic}} = -106 \pm 15$ pA, a postsynaptic effect of cross inhibition (Fig. 5B). To measure the postsynaptic effects of cross inhibition, we recorded tonic inhibitory currents while presenting the white noise/steady intensity stimulus and found that L-AP4 caused the tonic inhibitory currents to shift inward by $\Delta I_{\text{tonic}} = -522 \pm 51$ pA (Fig. 6D).

To record either excitatory or inhibitory currents, we had clamped the OFF α-cell at $E_{\text{Cl}} = -67$ mV or $E_{\text{glut}} = -0$ mV, so that the driving forces for excitatory and inhibitory conductances were opposite with absolute values equal to $E_{\text{glut}} - E_{\text{Cl}}$. Yet in the intact cell, these driving forces are set by the resting potential and the intact ionic gradients ($V_{\text{rest}} = -62$ to $-65$ mV; $E_{\text{glut}} \sim 0$ mV; $E_{\text{Cl}} = -80$ mV) (Dhingra and Smith 2004; Murphy and Rieke 2006; Zaghoul et al. 2003) (MATERIALS AND METHODS). Thus the driving force for excitation is about 3 to 4 times that of inhibition (Manookin et al. 2008). Assuming the tonic conductances under voltage clamp and intact conditions are equal, it is possible to extrapolate from the voltage-clamp recordings to the intact OFF α-cell and thus estimate the tonic effects of cross inhibition on excitatory and inhibitory currents in the intact OFF α-cell.
where $\Delta I_{\text{tonic}}$ is the current blocked by L-AP4 measured under voltage clamp, and $E_{\text{rev}}$ equals $E_{\text{Cl}}$ or $E_{\text{glut}}$ for determining inhibitory and excitatory currents, respectively (MATERIALS AND METHODS). By this equation, cross inhibition reduces the excitatory, inward currents by 82 to 86 pA, a presynaptic effect of hyperpolarizing the OFF bipolar cell. By this equation, cross inhibition increases the inhibitory outward currents by 98 to 117 pA, a postsynaptic effect on the OFF cell. Because presynaptic cross inhibition reduces an inward current in the
OFF α-cell only slightly less than postsynaptic cross increases an outward current, this implies that presynaptic cross inhibition hyperpolarizes the OFF α-cell only slightly less than postsynaptic cross inhibition does.

DISCUSSION

Our results support four conclusions about how cross inhibition from the ON pathway contributes to the detection of a dark flash by the OFF α-cell. 1) Cross inhibition decreases noise and increases signal in the OFF α-cell’s excitatory currents and thereby increases their detection accuracy: a presynaptic contribution. 2) Cross inhibition provides an inhibitory current directly to the OFF α-cell that encodes information about contrast: a postsynaptic contribution. 3) By pre- and postsynaptic contributions to excitatory and inhibitory currents, cross inhibition improves the accuracy of the OFF α-cell’s membrane voltages. 4) Cross inhibition improves the accuracy of the OFF α-cell’s spikes at moderate contrasts, but decreases their accuracy at low contrast by hyperpolarizing the OFF α-cell below spike threshold, preventing small depolarizations evoked by low contrast from triggering spikes.

Presynaptic cross circuit reduces noise in the OFF α-cell’s excitatory currents. There are many glycinergic amacrine cells, with dendrites in ON and OFF divisions of the IPL, which could mediate the effect of the ON pathway on excitatory currents in the OFF α-cell (MacNeil and Masland 1998; Menger et al. 1998). Among them, the AII amacrine cell is the most likely to mediate noise reduction for several reasons. First, the AII is the most common amacrine cell in mammalian retina (13% of total population, 20–30% of glycinergic amacrine cells) (MacNeil and Masland 1998; Menger et al. 1998). Second, the AII is coupled electrically to 80% of ON bipolar cells (Petrides and Trexler 2008). Finally, our white-noise experiments show that blocking the ON bipolar cell or glycinergic synapses increases noise in excitatory currents and that the noise-increasing effect of blocking the ON bipolar cell is suppressed by the gap junction blocker MFA, implying that the AII amacrine cell decreases noise in the OFF α-cell’s excitatory currents (Fig. 7B).

Metabotropic glutamate receptors on the axon terminals of some OFF bipolar cells bind L-AP4 and reduce transient inward currents recorded in OFF ganglion cells (Awatramani and Slaughter 2001; Higgs et al. 2002). We considered whether such “autoreceptors” could explain the effects of L-AP4 we observed, but found enough differences between these observed effects and the reported autoreceptor effects to discount this idea. First, in our experiments L-AP4 shifted tonic excitatory currents inward, but the binding of L-AP4 to autoreceptors does not (Fig. 5A). Second, in our experiments L-AP4 reduced signals at moderate contrast more than at low contrast, but autoreceptors reduce signals more at low contrast (Fig. 4B) (Awatramani and Slaughter 2001). Third, unlike the effects we observe, the autoreceptor effects of L-AP4 are immune to blocking glycinergic synapses and do not require functioning gap junctions.

How does the cross circuit reduce noise in the OFF α-cell’s excitatory currents? In previous studies, we had found that cross inhibition hyperpolarizes OFF bipolar cells so close to the threshold for transmitter release, that depolarizing the bipolar cell increases transmitter release onto the postsynaptic OFF α-cell, but hyperpolarizing the bipolar cell has a much smaller effect on transmitter release; as a result, the excitatory currents evoked by light are rectified, and the tonic rate of transmitter release is minimized (Liang and Freed 2010). Noise in excitatory currents increases as the square root of the mean current, apparently because glutamate quanta follow Poisson statistics, and so reducing tonic current should reduce noise (Freed 2005; Freed and Liang 2010). The present experiments confirm this prediction and expand upon it by showing that noise reduction increases contrast sensitivity. Furthermore, we find evidence that L-AP4 increases tonic noise at zero contrast but not noise dynamically generated at higher contrasts, suggesting that cross inhibition reducing tonic current is a sufficient explanation for its effect on noise at all contrasts (Fig. 4C).
Postsynaptic cross circuit encodes contrast information. We found that both inhibitory and excitatory postsynaptic currents encoded information about the dark flash, but the inhibitory currents detected the flash less accurately (Fig. 7A). This imbalance between inhibition and excitation may not be generally true of all stimuli or detection tasks. For example, to a bright flash, the inhibitory input produces a large outward current that encodes as much information as an (inward) excitatory current does about a dark flash (unpublished observations). For our dark flash experiments, most of the information encoded by the inhibitory currents originates in the ON pathway because L-AP4 blocked 89% of the light-induced modulation (Fig. 6D).

There are three known cross circuits that could transmit information from the ON pathway to postsynaptic inhibitory currents in OFF ganglion cells. 1) The AII amacrine cell receives electrical synapses from the ON bipolar cell and makes glycinegic synapses on the OFF α-cell (Cohen 1998; Manookin et al. 2008; Molnar et al. 2009; Nobles et al. 2012). 2) A second ON amacrine cell receives excitatory chemical synapses from the ON bipolar cell and makes glycinegic synapses on the PV-5, an OFF α-like cell in mouse (Munch et al. 2009). 3) ON amacrine cells receive excitatory input from the ON pathway and synapse electrically on OFF α-like ganglion cells in rabbit and mouse (Farajian et al. 2011; Murphy and Rieke 2011). The input from this electrical synapse can be demonstrated when light is flashed in near-absolute darkness: a transient inward current is observed at the termination of this flash (Murphy and Rieke 2011). If GABA_A and GABA_C receptors are blocked, a transient inward current is observed at the onset of this flash (Farajian et al. 2011). Circuit 3 is unlikely to have contributed to the currents that we recorded because we presented a dark flash on a much more intense background (10^4 R s^-1 for a rod), and we did not block GABA receptors. Thus of these three circuits, it is most likely that circuits 1 or 2 transmit information to the OFF ganglion cell.

In our experiments, basal illumination provided about equal isomerization rates for rods and cones, and accordingly about half the signal in the OFF α-cell was from cones and the remainder from rods (Yin et al. 2006) (MATERIALS AND METHODS). This raises the question of whether the rod signal could have come through the rod bipolar cell, which synapses on the AII amacrine cell, before it entered the α-cell’s inhibitory currents. Yet under lighting conditions similar to those of our experiments and in the same species of animal, the OFF α-cell’s inhibitory currents respond to light after ionotropic glutamate receptors are blocked (Manookin et al. 2008) (unpublished experiments). Because the rod bipolar cell → AII amacrine cell synapse is susceptible to this block (Pang et al. 2007; Trexler et al. 2005; Xin and Bloomfield 1999), the inhibitory currents could not have been driven by the rod bipolar cell. Instead, in our experiments, the rod signal most likely came through gap junctions that the rod makes with cones, mixed with cone signals, and flowed into the cone bipolar pathways.

Both pre- and postsynaptic cross circuits improve the efficiency of spike encoding. The ON pathway supplies pre- and postsynaptic cross inhibition to the OFF α-cell (Fig. 7B). Presynaptic cross inhibition tonically reduces the excitatory inward current, postsynaptic cross inhibition supplies a tonic outward current, and thus both pre- and postsynaptic cross inhibition tonically hyperpolarize the OFF α-cell. Countering pre- and postsynaptic hyperpolarizations by injecting positive current raised the OFF α-cell’s membrane potential above spike threshold, allowed small depolarizations to trigger spikes, and increased the transfer of information at low contrasts from membrane voltages to spike train. However, even when the OFF α-cell was depolarized, its spike train encoded less information for the detection task than either its V_m or its excitatory currents (Fig. 7A). Thus we confirm what has been observed before: a substantial and perhaps irreducible bottleneck in the flow of information from V_m to spike trains (de Ruyter van Steveninck and Laughlin 1996; Dhingra and Smith 2004; Haag and Borst 1998). The points we make here is that cross inhibition makes this bottleneck worse at low contrast by hyperpolarizing the membrane potential, but that this hyperpolarization has a compensating effect, which is to set the membrane potential to just below spike threshold, thus reducing spike rate (Fig. 3D), and maximizing the efficiency with which spikes encode information about moderate contrasts (Fig. 3G).

REFERENCES


