

Presynaptic Mechanism for Slow Contrast Adaptation in Mammalian Retinal Ganglion Cells

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Summary

Visual neurons, from retina to cortex, adapt slowly to stimulus contrast. Following a switch from high to low contrast, a neuron rapidly decreases its responsiveness and recovers over 5–20 s. Cortical adaptation arises from an intrinsic cellular mechanism: a sodium-dependent potassium conductance that causes prolonged hyperpolarization. Spiking can drive this mechanism, raising the possibility that the same mechanism exists in retinal ganglion cells. We found that adaptation in ganglion cells corresponds to a slowly recovering afterhyperpolarization (AHP), but, unlike in cortical cells, this AHP is not primarily driven by an intrinsic cellular property: spiking was not sufficient to generate adaptation. Adaptation was strongest following spatial stimuli tuned to presynaptic bipolar cells rather than the ganglion cell; it was driven by a reduced excitatory conductance, and it persisted while blocking GABA and glycine receptors, $K_{(Ca)}$ channels, or mGluRs. Thus, slow adaptation arises from reduced glutamate release from presynaptic (nonspiking) bipolar cells.

Introduction

The natural environment contains a wide range of possible lighting conditions. To operate across these conditions, the visual system must adapt its sensitivity to the statistics of the immediate environment. At the first stage of vision, the retina adapts to the mean intensity, a process also called “light adaptation,” through intrinsic photoreceptor properties as well as postreceptoral mechanisms (Pugh et al., 1999; Troy and Enroth-Cugell, 1993; Walraven et al., 1990). The retina further adapts to the range of intensities relative to the mean, also called “contrast adaptation.” A common hypothesis suggests that, at low contrast, the retina increases sensitivity to improve the signal-to-noise ratio, whereas, at high contrast, the retina decreases sensitivity to avoid saturating the response (Baccus and Meister, 2004; Demb, 2002). Contrast adaptation is largely absent in photoreceptors and therefore must arise through either network mechanisms or intrinsic properties of ganglion cells (Baccus and Meister, 2002; Rieke, 2001; Sakai et al., 1995).

At multiple levels of the visual system, including the retina, cells adapt to contrast over at least two time scales: a fast adaptation that acts in tens to hundreds

of milliseconds, and a much slower adaptation that acts over several seconds (Baccus and Meister, 2002; Carandini and Ferster, 1997; Maffei et al., 1973; Movshon and Lennie, 1979; Smirnakis et al., 1997; Solomon et al., 2004). The slow form of contrast adaptation was recently demonstrated in the primate magnocellular pathway in vivo (Solomon et al., 2004). Magnocellular retinal or thalamic cells were stimulated with high contrast followed by a switch to low contrast or mean luminance. Following the switch, these cells showed a suppressed response that required a recovery period of over 10 s. To put this time period into perspective, consider that under steady contrast conditions, ganglion cell responses can be predicted based on the previous ~300 ms of the stimulus (Baccus and Meister, 2002; Chichilnisky, 2001; Kim and Rieke, 2001; Zaghloul et al., 2003). Thus, a 10 s period of adaptation is relatively long.

Slow contrast adaptation in magnocellular neurons required a high-contrast stimulus effective at driving the cell (Solomon et al., 2004). This apparent activity dependence suggested that the mechanism for adaptation is intrinsic to the ganglion cell. Indeed, there is good precedent for this hypothesis: some cortical neurons express an intrinsic property for slow contrast adaptation (Sanchez-Vives et al., 2000a). When a cortical cell is stimulated strongly, either by a visual stimulus or direct current injection, the cell depolarizes and fires spikes. At the offset of stimulation, the cell hyperpolarizes and this afterhyperpolarization (AHP) recovers slowly over seconds. The AHP suppresses spiking responses to low-contrast stimuli (Carandini and Ferster, 1997; Sanchez-Vives et al., 2000a, 2000b). Ion substitution experiments demonstrated that the cortical AHP was caused largely by a potassium conductance sensitive to intracellular sodium (Sanchez-Vives et al., 2000a). This potassium conductance can apparently be activated by sodium influx caused by either synaptic input in the absence of spiking, or by spiking in the absence of synaptic input (Carandini and Ferster, 1997; Sanchez-Vives et al., 2000a, 2000b; Vidyasagar, 1990). Thus, for a cortical cell, spiking is sufficient but not necessary to drive adaptation. A retinal ganglion cell might express a similar intrinsic mechanism for slow adaptation. However, ganglion cells also show adaptive effects to small stimulus patches, implicating a possible mechanism in presynaptic bipolar cells (Brown and Masland, 2001). Cortical cells in area MT also express adaptation that is primarily caused by a presynaptic mechanism (Kohn and Movshon, 2003, 2004).

Previous studies of slow contrast adaptation in mammalian retina used extracellular recording (Brown and Masland, 2001; Chander and Chichilnisky, 2001; Smirnakis et al., 1997; Solomon et al., 2004). Here we studied slow adaptation in mammalian ganglion cells using intracellular recording. We show that slow adaptation results from a slowly recovering AHP, as shown in cortical cells and salamander ganglion cells (Baccus and Meister, 2002; Carandini and Ferster, 1997; Sanchez-Vives et al., 2000b). However, slow adaptation in ganglion cells does not arise primarily from an intrinsic property of the

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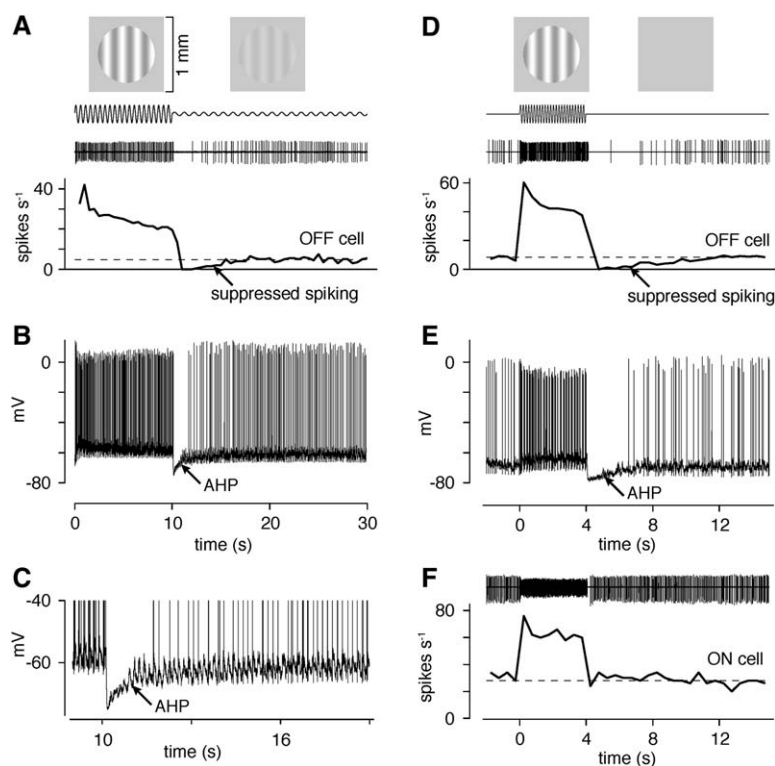


Figure 1. OFF Ganglion Cells Show Reduced Spiking following High Contrast Due to a Slow Afterhyperpolarization

(A) An OFF cell was stimulated with a drifting sine wave grating with contrast alternating between high (100%, 10 s) and low (20%, 20 s). Following high contrast, spiking was suppressed and required 7.0 s to recover to the baseline level (dashed line). Trace at top shows one cycle of the loose-patch, extracellular recording; poststimulus time histogram (psth) at bottom shows the average firing rate across four repeats (bin size, 500 ms). Grating spatial frequency was 6.7 cycles mm^{-1} and drifted at 6 Hz (stimulus trace does not show 6 Hz).

(B) Intracellular recording of the same cell and stimulus shown in (A). Following high contrast, the membrane potential showed an afterhyperpolarization (AHP) of -11.4 mV that required 5.5 s to recover to baseline. The period of suppressed spiking corresponded to the period of the AHP.

(C) Enlarged area of (B) showing the AHP.

(D) An OFF cell showed a maintained discharge (7.8 Hz) that was suppressed following a 4 s, high-contrast stimulus (drifting grating, 6.7 cycles mm^{-1} , 6 Hz). Trace at top shows loose-patch record; psth at bottom shows the average firing rate across four repeats. The spike rate recovered over 6.5 s.

(E) Intracellular recording of the cell in (D) shows an AHP following the stimulus that required 4.0 s to return to the resting potential.

(F) Extracellular recording of an ON cell does not show a prolonged reduction in spike rate following high contrast (drifting grating, 6.7 cycles mm^{-1} , 6 Hz). Following high contrast, spiking was suppressed for only ~ 100 ms, evident in the raw trace.

cell. Rather, ganglion cell adaptation arises from a network mechanism: reduced glutamate release from presynaptic (nonspiking) bipolar cells. This presynaptic mechanism complements intrinsic mechanisms for slow adaptation found at later stages of the visual pathway.

Results

Demonstration of Slow Contrast Adaptation in Intracellular Recordings of Mammalian Ganglion Cells In Vitro

We tested for slow adaptation in brisk-transient (Y-type) ganglion cells in an intact (retinal pigment epithelium-attached) in vitro preparation of the guinea pig retina (Demb et al., 1999, 2001a). We targeted Y-type cells by recording from the largest cell bodies in the ganglion cell layer (see Experimental Procedures). We focused on Y-type cells because we could target them routinely and because they are probably homologous to magnocellular pathway cells (or a subset of these cells), which show strong adaptation in vivo (Kaplan and Shapley, 1982; Levitt et al., 2001; Solomon et al., 2004). A cell was stimulated with a drifting grating that alternated between high contrast (100%, 10 s) and low contrast (5%–40%, 20 s; see Experimental Procedures). At the offset of high contrast, the spiking response in a cell, recorded extracellularly, was completely suppressed and

recovered over a 7.0 s period (Figure 1A). A whole-cell, voltage recording of the same cell showed that the period of suppressed spiking corresponded to a period of membrane hyperpolarization that slowly recovered—an afterhyperpolarization (AHP; Figure 1B), as reported in salamander cells (Baccus and Meister, 2002). Across cells, spiking was suppressed at the offset of high contrast and took 6.9 ± 0.9 s to recover ($n = 10$; see Experimental Procedures). Each cell recorded intracellularly showed an AHP during the period of suppressed spiking (AHP amplitude, -8.8 ± 0.7 mV; $n = 4$).

Many ganglion cells discharge continuously at mean luminance, and this discharge is suppressed following a period of high contrast. In one representative cell, maintained firing took 6.5 s to recover to the baseline rate (Figure 1D). An intracellular recording of the same cell showed that the period of suppressed spiking corresponded to the period of an AHP (Figure 1E). Under our conditions, suppressed spiking following high contrast was found in nearly all OFF-center Y-type cells but was weak or absent in most ON-center Y-type cells (Figure 1F; time to recovery, 0.5 ± 0.4 s; $n = 6$). However, we observed adaptation in several other types of ON-center or ON-OFF ganglion cells, suggesting that the ON pathway did express slow adaptation for certain cell types. For example, in three direction-selective ganglion cells (one ON-center type, two ON-OFF types)

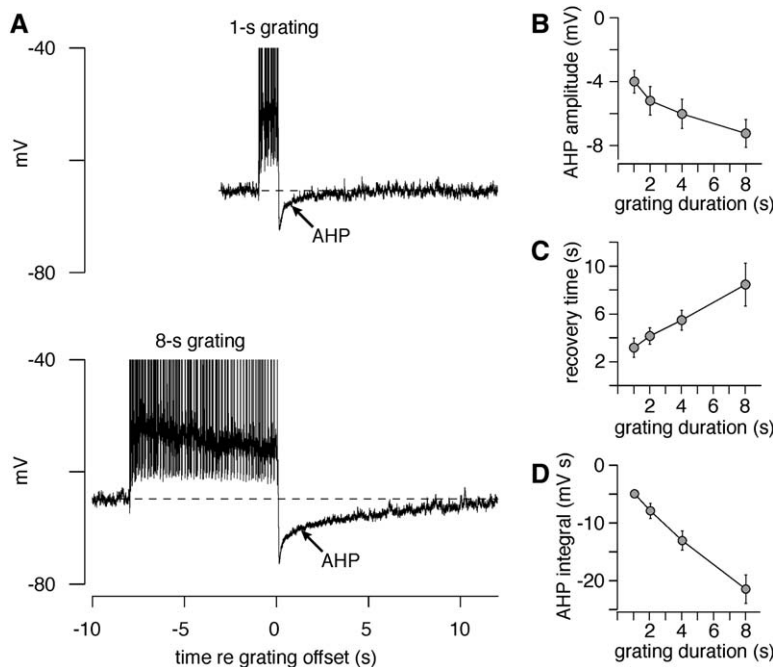


Figure 2. Longer Periods of High Contrast Evoke Larger and Longer-Lasting Afterhyperpolarizations

(A) An OFF cell was stimulated with either a 1 s or 8 s grating (100% contrast, 6.7 cycles mm^{-1} ; 6 Hz) followed by mean luminance. Compared to the 1 s grating, the 8 s grating evoked a larger AHP (-6.9 mV versus -10.8 mV) that took longer to recover (2.0 s versus 5.7 s), resulting in a larger AHP integral (-5.5 mV s versus -25.2 mV s; see [Experimental Procedures](#)). Recovery was measured as the time required to return 90% back to the resting potential (see [Experimental Procedures](#)).

(B) Longer periods of high contrast increased the amplitude of the AHP ($n = 6$ cells). Error bars indicate SEM across cells.

(C) Longer periods of high contrast increased the time needed to recover to the resting potential.

(D) Longer periods of high contrast increased the AHP integral.

recorded intracellularly, the switch from a 4 s grating (6 Hz; 6.7 cycles mm^{-1} ; 100% contrast) to mean luminance caused a large AHP (-9.5 ± 0.1 mV) that recovered over 6.2 ± 1.1 s (data not shown).

For periods of high contrast of either 1 or 8 s, the AHP amplitude increased from -4.0 ± 0.6 mV to -7.8 ± 0.9 mV and the recovery time increased from 3.6 ± 0.8 s to 7.9 ± 1.6 s ($n = 6$ OFF Y-type cells); the AHP integral increased from -5.0 ± 0.6 mV s to -20.9 ± 2.1 mV s (Figure 2). Thus, longer periods of contrast evoked larger and longer-lasting AHPs. In the following studies, we focused on OFF-center Y-type cells to investigate the mechanism for slow adaptation, because we could target these cells routinely and they showed strong adaptation as reflected by the AHP.

Spiking Is Neither Sufficient nor Necessary to Generate the Visually Evoked Afterhyperpolarization

We first tested whether the AHP results from an intrinsic mechanism (e.g., potassium channel) in the ganglion cell that is sensitive to sodium influx, as found in cortex; a cortical cell adapts following either a visual stimulus or direct current injection ([Sanchez-Vives et al., 2000a](#)). We stimulated the same ganglion cell with either the grating stimulus or a direct injection of current through the electrode (Figure 3A). Both the grating and the current step evoked a membrane depolarization and a train of spikes, and in fact the current step evoked a larger depolarization (8.3 ± 0.8 mV versus 7.0 ± 0.7 mV; a difference of 1.3 ± 0.6 mV, $p < 0.05$) and a higher spike rate (27.9 ± 4.7 spikes s^{-1} versus 15.7 ± 1.8 spikes s^{-1} ; a difference of 12.2 ± 3.3 spikes s^{-1} , $p < 0.005$). However, only the grating evoked a large and long-lasting AHP (Figure 3B). Across cells, the AHP amplitude ~ 300 ms after stimulus offset was -5.8 ± 0.6 mV following the grating versus -2.3 ± 0.2 mV following the current step (difference of 3.4 ± 0.5 mV, $p < 0.001$; $n = 9$). Four sec-

onds after stimulus offset, the AHP persisted following the grating (-1.9 ± 0.3 mV) but was nearly back to baseline following the current step (-0.30 ± 0.15 mV; difference of 1.6 ± 0.3 mV; $p < 0.001$; sampling window, 1.0 s). Furthermore, the AHP integral was about four times larger following the visual stimulus (-18.9 ± 2.3 mV s) relative to the current step (-4.4 ± 1.4 mV s; difference, 14.5 ± 0.9 mV s; $p < 0.001$). We also tried sine-wave current injection at the stimulus frequency (6 Hz, $+0.2$ nA amplitude; Figure 3C). In this case ($n = 8$ cells), current injection evoked a higher spike rate (10.3 ± 1.1 spikes s^{-1} versus 7.2 ± 1.2 spikes s^{-1}), but the grating evoked a larger AHP amplitude (-5.1 ± 0.5 mV versus -1.0 ± 0.3 mV) and a larger AHP integral (-17.3 ± 2.0 mV s versus -2.8 ± 2.0 mV s). Thus, even though current injection evoked a larger spiking response, the grating evoked a larger AHP, indicating that spiking alone is not sufficient to generate the full visually evoked AHP. Apparently the mechanism for slow contrast adaptation differs between retina and primary visual cortex ([Sanchez-Vives et al., 2000b](#)).

We next asked whether spiking was necessary to generate the AHP. We included QX-314 (5 mM) in the pipette solution to block voltage-gated sodium channels in the recorded cell ([Connors and Prince, 1982](#)). Under these conditions, a grating stimulus evoked a membrane depolarization without spiking ($n = 10$; Figure 3D). Following stimulus offset, each cell showed an AHP that was, on average, -4.8 ± 0.4 mV in amplitude and required 6.2 ± 0.6 s to return to the resting potential (AHP integral, -14.4 ± 2.2 mV s). Thus, spiking was not necessary to generate the AHP.

Spatial Sensitivity of the Afterhyperpolarization Suggests a Presynaptic, Bipolar Cell Mechanism

We hypothesized that the retinal basis for the AHP might arise from a presynaptic mechanism, possibly in the

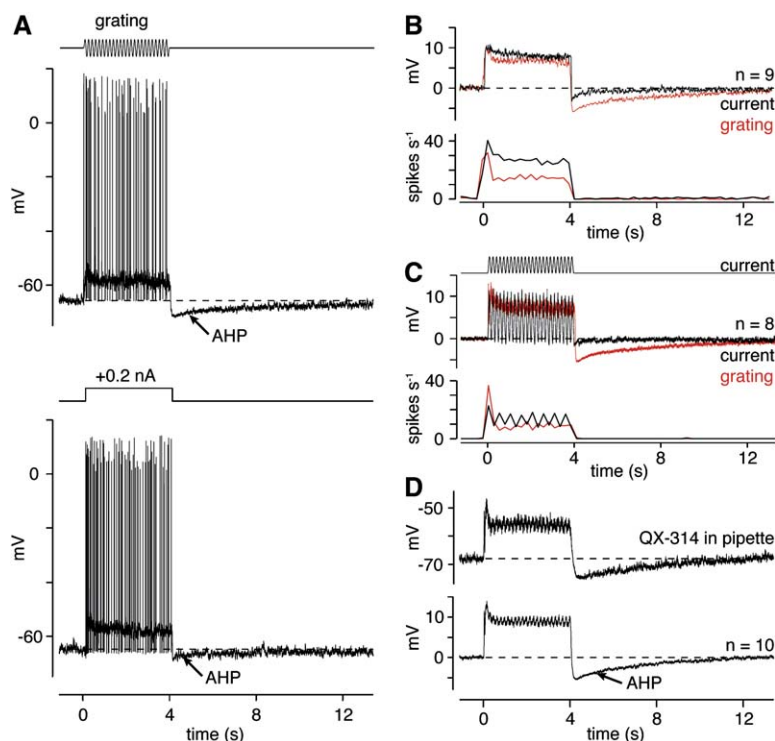


Figure 3. Spiking Is Neither Sufficient nor Necessary to Generate the Afterhyperpolarization

(A) An OFF cell was stimulated with either a 4 s grating (top; 100% contrast, 6 Hz, 6.7 cycles mm^{-1}) or a 4 s step of positive current (bottom; +0.2 nA). Both stimuli generated membrane depolarization and spiking. At the offset of the grating, there was a relatively large AHP (-5.5 mV), whereas at the offset of the current pulse, there was a smaller AHP (-3.2 mV).

(B) Average subthreshold membrane potential and spike rate for the grating stimulus and the current step ($n = 9$ cells; 100 Hz sampling). Membrane depolarization and spiking responses were larger following the current step, whereas the AHP was larger and longer lasting following the grating. Thus, spiking alone, as evoked by the current step, was not sufficient to generate the full visually evoked AHP. Stimulus responses were advanced 46 ms in time to align with the current responses. Spike rate was binned at 250 ms here and in (C).

(C) Same format as (B), except the current stimulus was a 4 s period of 6 Hz sine-wave stimulation with a peak of +0.2 nA (current injection protocol shown above response traces). Current injection evoked a higher spike rate, whereas the grating evoked a larger and longer-lasting AHP ($n = 8$ cells).

(D) An OFF cell was stimulated with a 4 s grating (100% contrast, 6 Hz, 6.7 cycles mm^{-1}) while applying QX-314 (5 mM) through the pipette to block spiking (top). On average (bottom, $n = 10$ cells), the AHP was similar to control conditions; thus, spiking was not necessary to generate the AHP.

bipolar cells that release glutamate and excite the ganglion cell. In the above experiments, we routinely used a high spatial frequency grating, with bar width of ~ 80 – 100 μm . This bar width is much narrower than the ~ 600 μm receptive field center of the ganglion cell, but similar to the ~ 100 μm receptive field center of bipolar cells (Dacey et al., 2000; Demb et al., 2001b). We directly examined the effect of spatial frequency on the AHP amplitude. A low spatial frequency (0.3 cycles mm^{-1}) evoked a strong response at the 6 Hz drift rate of the grating (F1 amplitude; Demb et al., 2001b; Hochstein and Shapley, 1976), as expected, but produced only a small AHP (Figure 4A). A high spatial frequency (6.7 cycles mm^{-1}) evoked a steady depolarization with a small 6 Hz modulation riding on top, followed by a large AHP (Figure 4A). Both spatial frequencies evoked a similar average spike rate over time (F0 component; Figure 4B), and so, as shown above, the magnitude of the AHP did not directly correspond to the preceding spike rate.

For primate diffuse bipolar cells (which synapse onto magnocellular ganglion cells), the receptive field surround strength is 1 to 1.4 times the strength of the receptive field center (Dacey et al., 2000). Assuming a similar receptive field profile in guinea pig bipolar cells (presynaptic to the Y-type cell), the surround should reduce each bipolar cell's response to the low spatial frequency, relative to the optimal spatial frequency, to

less than 30% of the maximal response (Dacey et al., 2000). Thus, the reduced ganglion cell AHP following the low-frequency grating would be explained by the reduced response of each presynaptic bipolar cell. This explanation implies that individual bipolar cells require strong stimulation in order to adapt their release rate and drive the ganglion cell AHP.

We measured grating responses to a total of seven spatial frequencies. As expected, the F1 amplitude peaked at low frequencies and gradually declined at higher frequencies (Figure 4C) whereas the AHP amplitude peaked at high frequencies (Figure 4D). To put this spatial tuning into context, we made two measurements of the “nonlinear subunit” property of the Y-type cell receptive field, where the subunits apparently represent presynaptic bipolar cells (Demb et al., 2001a, 2001b; Enroth-Cugell and Freeman, 1987; Enroth-Cugell and Robson, 1966; Hochstein and Shapley, 1976). The first subunit measure is the mean level of depolarization (F0) during the grating, and this showed the same spatial tuning as the AHP (Figure 4C). The second measure was the F2 (second harmonic) amplitude to a contrast-reversing grating (spike recordings; Figure 4E), and this also showed similar tuning to the AHP (Figure 4E) (Demb et al., 2001b). Thus, the spatial tuning of the AHP matches the tuning of the “nonlinear subunits,” suggesting that strong stimulation of bipolar cells is required to evoke the AHP.

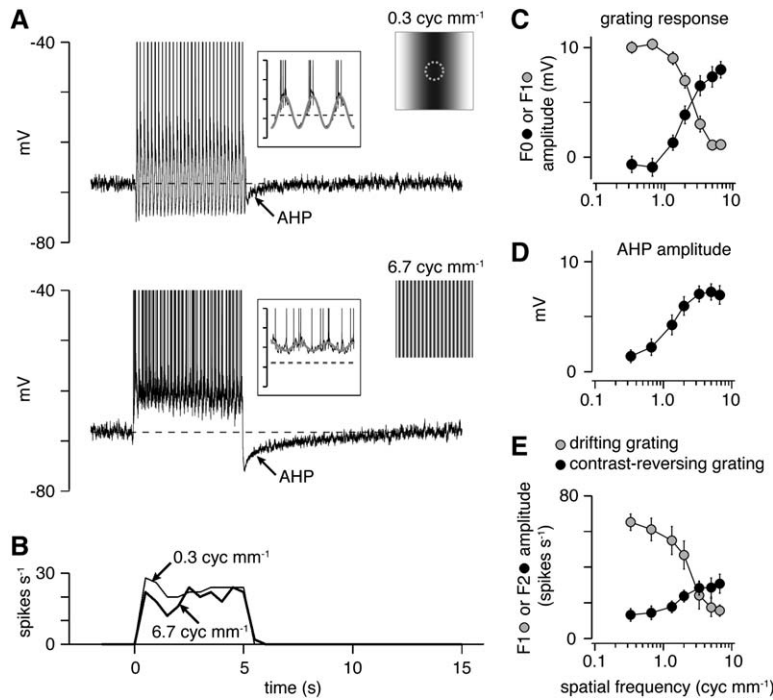


Figure 4. The Afterhyperpolarization Is Evoked Most Effectively by High Spatial Frequencies

(A) In an OFF cell, a low spatial frequency grating (0.3 cycles mm⁻¹) evoked a strong response at the 6 Hz drift rate followed by a small AHP. A high spatial frequency grating (6.7 cycles mm⁻¹) evoked a tonic depolarization plus a small 6 Hz modulation followed by a large AHP. Traces in the insets show 500 ms of response with a fitted 6 Hz sine wave (F1 response; y axis: -80 to -40 mV). In both insets, the cell fires 13 spikes (bursts of five, four, and four spikes to the low-frequency stimulus and 13 spikes dispersed over time to the high-frequency stimulus). Dashed line indicates the resting potential.

(B) A psth for the two conditions in (A) (bin size, 500 ms); mean firing rate for the two conditions was similar.

(C) The amplitude of the first Fourier harmonic (F1) of the membrane potential peaked for low spatial frequencies and declined at high frequencies, whereas the mean membrane potential (F0 component) peaked at high frequencies, reaching a peak near ~5–7 cycles mm⁻¹ ($n = 8$ cells). Error bars indicate SEM across cells.

(D) AHP amplitude increased with spatial frequency, reaching a peak near ~5 cycles mm⁻¹ ($n = 8$ cells).

(E) Spike recordings to a drifting or contrast-

reversing grating illustrate the relative size of the overall ganglion cell receptive field center and the nonlinear subunits; the subunits represent bipolar cell inputs to the ganglion cell (see Results). The F1 amplitude to a drifting grating is a measure of the overall receptive field center. This amplitude peaks at low frequencies and declines at higher frequencies. The F2 amplitude to a contrast-reversing grating reflects the nonlinear subunit response. The subunit amplitude peaks at high frequencies, similar to the pattern of sensitivity of the F0 membrane potential in (C) and the AHP amplitude in (D) (see also Demb et al., 2001b).

The Afterhyperpolarization Corresponds to a Decreased Inward Current, Consistent with a Bipolar Cell Mechanism

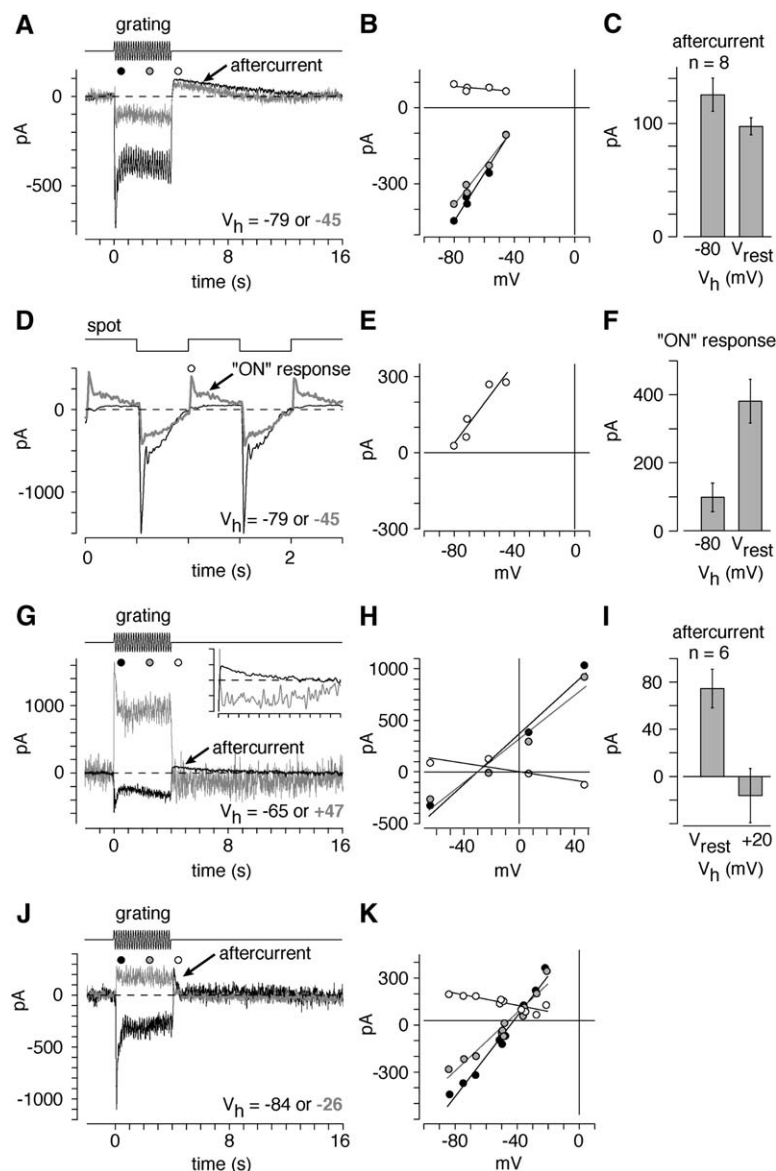
We considered two hypotheses for how bipolar cells could drive a slow AHP in a ganglion cell. First, following the offset of high contrast, bipolar cell glutamate release might be suppressed and recover slowly. Second, bipolar cells might drive inhibitory amacrine cells to release GABA and/or glycine, and this inhibition might require several seconds to subside. To test between these alternatives, we measured membrane currents under voltage clamp during and after high-contrast stimulation. The large cells under study have a low input resistance (37.9 ± 2.1 M Ω , $n = 14$) (Cohen, 2001; O'Brien et al., 2002), and so we initially used holding potentials (V_h) close to the resting potential (V_{rest}) to minimize errors in the current measurements ($n = 8$; see Experimental Procedures).

During the high-contrast grating, we measured an inward current with an estimated reversal potential (-23.9 ± 6.7 mV; $n = 8$) that suggests a mix of excitatory and inhibitory input (Figure 5B). Following grating offset, there was an outward "aftercurrent" that recovered slowly back to baseline. This aftercurrent amplitude was larger at V_h of ~ -80 mV relative to the amplitude at V_{rest} (~ -65 mV); this pattern (negative slope on an I-V plot) suggests that the aftercurrent is driven by a reduced excitatory conductance, rather than an increased inhibitory conductance (Figure 5B). Near V_{rest} , we measured similar aftercurrents using pipette solutions that

were either K⁺-based ($n = 3$) with a weak Ca²⁺ buffer (0.1 mM EGTA; 92.9 ± 11.3 pA) or Cs⁺-based ($n = 5$) with a strong Ca²⁺ buffer (10 mM BAPTA; 129.0 ± 14.0 pA; see Experimental Procedures). Thus, the aftercurrent did not depend on Cs⁺-sensitive K⁺ channels or a Ca²⁺-dependent mechanism, which is further evidence that the mechanism was not intrinsic to the ganglion cell.

We performed additional experiments with TEA in the pipette to improve the ability to clamp the dendrites at a positive holding potential (see Experimental Procedures). In the most stable recordings ($n = 6$), we measured a reversal for the aftercurrent that was $+10.3 \pm 10.2$ mV (Figures 5G–5I). This reversal is slightly positive to 0 mV, which is likely explained by an incomplete space clamp of the dendrites (i.e., in which case, an $\sim +10$ mV potential at the soma might correspond to an ~ 0 mV potential at the dendrite). The aftercurrent showed a more sustained time course at the positive holding potential, relative to the time course measured near V_{rest} (Figure 5G, inset), probably caused by an increased contribution from NMDA-receptor conductances at positive potentials (Cohen, 2000). Because of a probable NMDA-receptor contribution to the aftercurrent, the linear fits used to estimate reversal potential are approximate.

As a control, we measured an outward current that we did expect to be driven largely by direct inhibition (Demb et al., 2001a; Zaghloul et al., 2003): the OFF cell's "ON" response to a bright spot stimulus (Figures 5D–5F).



100 ms); axes represent from 4 to 16 s and from -200 to +200 pA. The time course of the aftercurrent differed at the two holding potentials, probably because of an NMDA receptor component at +47 mV.
(H) Same format as (B), showing a reversal of the aftercurrent near 0 mV.
(I) Same format as (C), showing the average aftercurrent amplitude with V_h = V_{rest} (-65.5 ± 5.6 mV) or ~+20 mV (+19.8 ± 8.3 mV, mean ± SD) across six cells.
(J) An ON cell was recorded under the same conditions as in (A). The aftercurrent was briefer than that in OFF cells but still showed a reduction at the depolarized holding potential.
(K) I-V plot of response to the grating from the cell in (G); same format as in (B).

Indeed, this outward current showed an estimated reversal negative to V_{rest}, suggesting that it was driven largely by a direct inhibitory conductance and demonstrating that our protocol was adequate to see existing direct inhibitory influences.

Following a grating stimulus, ON-center Y-type cells also showed an aftercurrent, although it was relatively brief (n = 3; Figure 5J). This brief period of the aftercurrent explains why spikes were suppressed only transiently in extracellular recordings (see above; Figure 1F). An ON cell's aftercurrent corresponded to a reduced inward current, with estimated reversal near 0 mV (Figures 5J and 5K). Thus, ON-center Y-type cells showed an out-

ward current similar to OFF-center cells, except that the recovery was much faster.

The Afterhyperpolarization Does Not Require Conductances Driven by GABA or Glycine Receptors, Calcium-Activated Potassium Channels, or Metabotropic Glutamate Receptors

The AHP does not appear to be driven by GABA or glycine release onto the ganglion cell (Figure 5); however, GABA or glycine release feeding back onto the bipolar terminal could play a role in suppressing glutamate release. This seemed unlikely based on a previous experiment with extracellular recording, in which either

Figure 5. The Afterhyperpolarization Corresponds to a Reduced Excitatory Postsynaptic Current

(A) An OFF cell was recorded under voltage clamp and stimulated with a 4 s grating (100% contrast; 6.7 cycles mm⁻¹; 6 Hz; K⁺-based solution) at a holding potential (V_h) = -45 (gray) or -79 (black). Both the inward current evoked by grating presentation and the outward aftercurrent at grating offset declined at the positive holding potential. Traces are illustrated at 100 Hz sampling. Traces here and below are leak subtracted.
(B) Current-voltage (I-V) plot of transient response (black circles) and sustained response (gray circles) to the grating and the aftercurrent (white circles) from the cell in (A). Grating responses are consistent with a mixed chloride + cation current with estimated reversal potential (transient: -23.8 ± 10.8 mV; sustained: -23.9 ± 6.7 mV; n = 8) between E_{cation} (~0 mV) and E_{Cl} (-73 mV). The aftercurrent response showed a slight decline at positive holding potentials, consistent with a reduced cation current but inconsistent with a chloride current. Fitted lines here and below show linear regressions.
(C) Outward currents following the grating (aftercurrent) at V_h near V_{rest} (±5 mV) or near -80 mV (±5 mV). Relative to V_h = V_{rest}, V_h = -80 mV increased the aftercurrent. Error bars indicate SEM (n = 8 cells).
(D) The same cell in (A) was stimulated with a spot that reversed contrast (square-wave reversal; 0.6 mm outer diameter, contrast = 100%) at V_h = -45 or -79 mV.
(E) I-V plot of traces in (D). Across cells, outward current during the positive contrast shows an estimated reversal of -86.4 ± 1.4 mV (n = 8 cells), negative to E_{Cl} (-73 mV).
(F) Outward currents during the "ON" response at V_h near V_{rest} (±5 mV) or near -80 mV (±5 mV). The response decreased at V_h of -80 mV relative to the response at V_h of V_{rest}. Error bars indicate SEM (n = 8 cells).
(G) Same format as (A) for a second cell probed at positive holding potentials (Cs⁺-based pipette solution with TEA). The aftercurrent became inward at V_h = +47 mV. Inset shows filtered traces (Gaussian filter, SD =

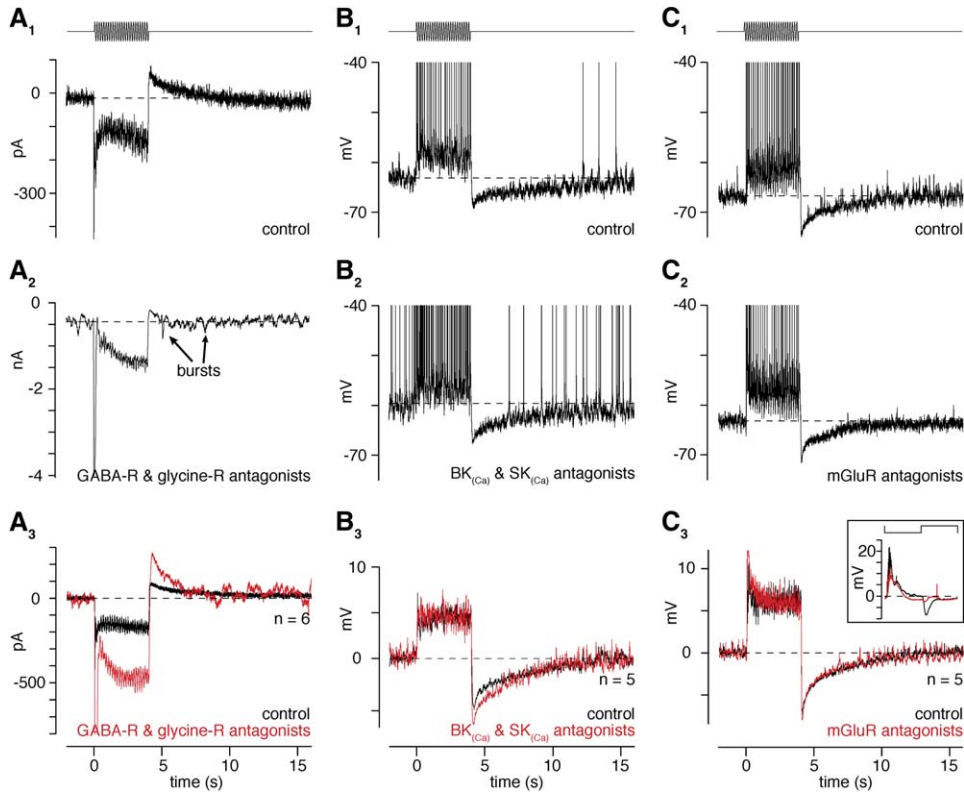


Figure 6. The Afterhyperpolarization Does Not Require Conductances Driven by GABA or Glycine Receptors, K_{Ca} Channels, or Metabotropic Glutamate Receptors (mGluRs)

(A) An OFF cell was stimulated with a 4 s grating (100% contrast; 6.7 cycles mm^{-1} ; 6 Hz) under voltage clamp ($V_{hold} = -75$ mV). The grating response was recorded under control conditions (A_1) and after adding antagonists to glycine and GABA_{A/B/C} receptors (strychnine, 2 μM ; bicuculline, 100 μM ; CGP35348, 100 μM ; TPMPA, 100 μM) (A_2). The receptor antagonists caused a large increase in responsiveness (note the different scales in A_1 and A_2). However, the aftercurrent, following the stimulus, persisted in the presence of the receptor antagonists (A_3 , average traces are leak subtracted). The arrows in (A_2) indicate bursts of inward current present during drug application, which probably represents bursts of glutamate caused by blocking all major inhibitory synapses throughout the retina. The initial inward current in the average trace in the presence of the drugs (-1.9 ± 0.53 nA) has been clipped in the figure (A_3). Without leak subtraction, the leak current was -50 ± 16 pA in the control condition and -372 ± 41 pA in the drug condition.

(B) Same format as (A), except that the cell was recorded in current clamp and we applied antagonists to two types of calcium-activated potassium channels: apamin (1 μM) to block SK_(Ca) channels, and charybdotoxin (20 nM) to block BK_(Ca) channels. Relative to control (B_1), the channel blockers caused bursting of the membrane potential and increased the maintained discharge (B_2), but did not block the AHP (B_3).

(C) Same format as (B), except that we applied antagonists to all major mGluRs: MCPG (1 mM) to block group I and group II mGluRs, and CPPG (1 mM) to block group III mGluRs. The drugs did not block the AHP following the grating stimulus (C_2 and C_3). However, during a contrast reversal of a 0.6 mm diameter spot (2 Hz, 10%–20% contrast), the drugs blocked the hyperpolarizing response to light onset in OFF cells (C_3 , inset; averaged across five cells; line above indicates time course of the contrast reversal). This was expected, because the hyperpolarizing response to light onset depends on an inhibitory synapse from an ON amacrine cell, and the ON pathway is blocked by CPPG (Awatramani and Slaughter, 2000; Zaghloul et al., 2003).

strychnine (a glycine receptor antagonist) or picrotoxin (a GABA_{A/C} receptor antagonist), when applied *individually*, did not block slow adaptation in spiking (Brown and Masland, 2001). However, this experiment left open the possibility that both GABA and glycine are involved in adaptation, in which case blocking only one class of receptors at a time would not block the total amacrine cell contribution. To follow up this result, we measured the aftercurrent, under voltage clamp, while *simultaneously* blocking glycine and GABA_{A/B/C} receptors (strychnine, 2 μM ; bicuculline, 100 μM ; CGP35348, 100 μM ; TPMPA, 100 μM , respectively).

The receptor antagonists caused, in most cases, spontaneous “bursting” and strongly increased the response to the grating (Figure 6A). However, the aftercurrent persisted and in fact the amplitude increased (control: 74.1 ± 13.1 pA; drugs: 219.5 ± 20.9 pA). Furthermore,

the antagonists altered the time course of both the grating response and the aftercurrent. The altered time courses probably arise from the extreme change in bipolar release under these conditions, caused by removing all inhibition of the bipolar terminal. Therefore, we do not draw conclusions from these conditions about the normal time course of glutamate release. However, we can conclude that the aftercurrent (and the associated AHP) does not require inhibitory synaptic transmission. This provides further evidence that the AHP arises from reduced bipolar cell glutamate release.

We checked two putative mechanisms for adaptation in bipolar cell release. Bipolar cells express Ca^{2+} -dependent K^+ channels [K_{Ca}] (Sakaba et al., 1997), which could contribute to slow adaptation (Llinas and Lopez-Barneo, 1988). We blocked K_{Ca} channels throughout the retina (bath applied charybdotoxin, 20 nM; apamin,

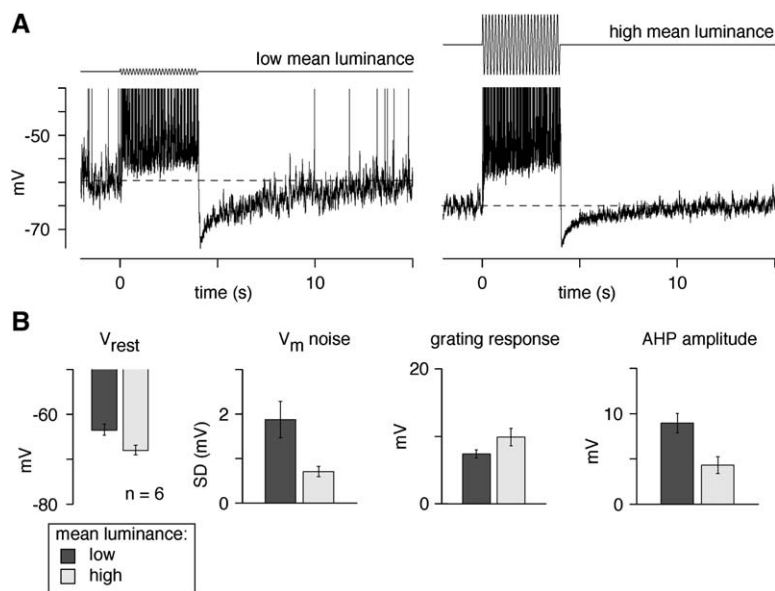


Figure 7. At Low Mean Luminance, an OFF Cell Shows a Depolarized Membrane Potential, Increased Membrane Noise, and an Enhanced Afterhyperpolarization

(A) An OFF cell was stimulated with a 4 s grating stimulus (100% contrast; 6.7 cycles mm^{-1} ; 6 Hz). The response was recorded at two different levels of mean luminance differing by a factor of ten (see [Experimental Procedures](#)). The low mean luminance caused a depolarized V_{rest} and increased membrane noise, suggesting increased presynaptic glutamate release. Low mean luminance also increased the amplitude of the AHP from -7.1 to -12.8 mV.

(B) Across cells, reducing mean luminance by a factor of ten had multiple effects: depolarization of V_{rest} , increased synaptic noise (SD of V_m measured over 2 s, before stimulus onset), and increased AHP amplitude. However, the response to the grating was similar under the two conditions. Error bars indicate SEM across cells ($n = 6$).

1 μM), and this condition caused bursting in the ganglion cell membrane potential and an increased maintained discharge ($n = 5$; [Figure 6B](#)). However, the AHP following the grating persisted (control amplitude: -4.7 ± 1.0 mV; drugs: -6.0 ± 0.6 mV). This result also rules out a role for $K_{(Ca)}$ channels in the ganglion cell, consistent with results above showing that the aftercurrent persists under voltage clamp in the presence of high BAPTA ([Figure 5](#)).

Another possible mechanism for adaptation in bipolar cells is metabotropic glutamate receptors (mGluRs) on the synaptic terminal, which could create a feedback signal and reduce release ([Awatramani and Slaughter, 2001](#)). To test this possibility, we blocked group I, II, and III mGluRs simultaneously (MCPG, 1 mM; CPPG, 1 mM) ($n = 5$; [Figure 6C](#)). This condition blocked the hyperpolarization at light onset of a spot response, as expected ([Zaghloul et al., 2003](#)) (see [Figure 6C₃](#) inset). However, the AHP following the grating persisted (control amplitude: -6.1 ± 0.9 mV; drugs: -6.8 ± 0.8 mV). This result also rules out a role for mGluRs as a post-synaptic mechanism to generate the AHP in ganglion cells.

Evidence that Basal Glutamate Release from Bipolar Cells Affects the Afterhyperpolarization

During the course of the experiments, we varied the mean luminance (see [Experimental Procedures](#)). At lower mean luminance, we observed in OFF Y-type ganglion cells two effects: a depolarized resting potential (low, -63.4 ± 1.3 mV; high, -68.0 ± 1.1 mV; $p < 0.05$; $n = 6$) and increased voltage noise (low, 1.9 ± 0.4 mV; high, 0.7 ± 0.1 mV; $p < 0.05$) ([Figure 7](#)). This pattern implies that glutamate release from OFF bipolar cells increased at the lower mean, presumably driven by increased glutamate release from cones ([Demb et al., 2004](#)). We hypothesized that the AHP in the ganglion cell would be related to the level of basal glutamate release from bipolar cells: higher basal release (at low mean luminance), when suppressed, would produce a relatively larger AHP in the ganglion cell.

We tested the effect of mean luminance on AHP size, presenting the same cell with the 4 s drifting grating (100% contrast; 6.7 cycles mm^{-1} ; 6 Hz) at two levels of mean luminance, differing by a factor of ten ([Figure 7](#)). At the two levels of mean luminance, the response to the grating was similar (low, 7.4 ± 0.6 mV; high, 9.9 ± 1.3 mV; $p > 0.10$; $n = 6$). However, the AHP was about twice as large at low mean luminance (low, -9.0 ± 1.1 mV; high, -4.3 ± 0.9 mV; $p < 0.05$). Thus, increasing the apparent basal glutamate release onto the ganglion cell, by reducing the mean luminance, increased the AHP.

Discussion

We have demonstrated a slow form of contrast adaptation in the subthreshold membrane potential of mammalian ganglion cells in vitro. Following a period of high contrast, spike rate was suppressed and required several seconds to recover; we observed suppressed spiking with both extracellular and whole-cell recordings ([Figure 1](#)). The recovery of the spike rate corresponded to a period of membrane hyperpolarization ([Figure 1](#)), and this afterhyperpolarization (AHP) depended on the period of prior visual stimulation ([Figure 2](#)). Spiking was neither necessary nor sufficient to generate the full visually evoked AHP ([Figures 3 and 4](#)). However, spiking was sufficient to generate at least a minor component of the visually evoked AHP ([Figure 3](#)). The AHP was strongest following high spatial frequency stimuli, tuned to presynaptic bipolar cells ([Figure 4](#)). Voltage-clamp analysis showed that the AHP corresponded to a reduced inward current, consistent with suppressed bipolar cell glutamate release ([Figure 5](#)). Pharmacology experiments ruled out a role for amacrine cell GABAergic or glycinergic synapses or for calcium-activated K^+ channels or metabotropic glutamate receptors in the bipolar cell (or elsewhere; [Figure 6](#)). Lowering mean luminance apparently increased basal glutamate release from OFF bipolar cells ([Figure 7](#)). At the lower mean luminance, the

AHP increased, suggesting a link between basal release and the size of the AHP (Figure 7).

Retinal Model for Slow Adaptation following a Period of High Contrast

Our results support a model where, at the offset of high contrast, bipolar cell glutamate release drops below the basal rate, and this drop in release hyperpolarizes the ganglion cell, causing the observed AHP and suppressed spiking. Glutamate release apparently requires several seconds to return to the basal level, resulting in gradual membrane depolarization of the ganglion cell back to its resting potential (Figure 8). In this model, bipolar cells require strong stimulation in order to suppress their release. So, for example, when a low spatial frequency grating is presented and stimulates a bipolar cell weakly (due to the influence of the receptive field surround; Dacey et al., 2000), there would be no suppression at stimulus offset, and the ganglion cell AHP would be weak or absent (Figure 4).

Our synaptic model for slow adaptation in ganglion cells is consistent with the conclusion reached in a previous study, based on extracellular recording (Brown and Masland, 2001). However, we also found a minor contribution to slow adaptation from an intrinsic property of the ganglion cell: current injection evoked a depolarization and spiking, followed by an AHP with an integral that was, at most, about 25% of the visually evoked AHP integral (Figure 3). However, relative to the visual stimulus, the current injection typically evoked greater depolarization and more spikes. Thus, taking into account the relatively smaller response to the visual stimulus, the intrinsic mechanism for adaptation in the ganglion cell probably contributes less than 25% of the visually evoked AHP.

Retinal Model for Slow Adaptation during a Period of High Contrast

Here, we have focused on the slowly recovering AHP following a grating stimulus, but we could also measure adaptation during the presentation of the grating itself. This side of adaptation was reflected by an initially high spike rate that declined during prolonged contrast stimulation (Figures 1 and 3). This adaptation *during* the stimulus seemed to depend on two components. First, there was an excitatory synaptic component driving the response, which is apparent in voltage-clamp recordings near E_{Cl} (Figures 5 and 8): inward currents were initially large and then declined during continued grating presentation. This decline could occur due to a decreasing glutamate release rate during the grating or postsynaptic mechanisms of receptor desensitization caused by the high release (von Gersdorff and Borst, 2002). Possible presynaptic mechanisms for a decline in release during high contrast include vesicle depletion or auto-feedback at the bipolar terminal (DeVries, 2001; Palmer et al., 2003a, 2003b; Singer and Diamond, 2006). A second component driving adaptation during the grating was a spike-frequency adaptation. This adaptation was apparent during direct current injection, which caused a high rate of spiking that then declined (Figure 3). Spike frequency adaptation seems to be a general property of many types of ganglion cells and at least partially reflects the effect of sodium channel inactivation (Kim

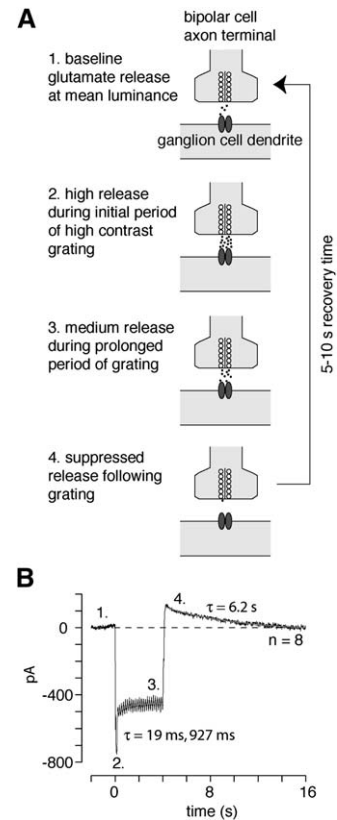


Figure 8. Working Model for the Mechanism of Slow Contrast Adaptation

(A) Glutamate release increases during periods of high contrast to a high level initially and then to a medium level for the duration of the stimulus. Following the offset of high contrast, glutamate release declines sharply. The release rate takes several seconds to recover to the initial baseline level.

(B) Time course of adaptation during and following high-contrast stimulation. Trace shows the average response, under voltage clamp, of eight cells, where V_{hold} was near E_{Cl} (trace is leak subtracted; 100 Hz sampling). The inward current is suppressed soon after grating onset causing a decrease in the inward current; gray line shows a double exponential fit with time constants of 19 and 927 ms. This decline in the inward current could reflect multiple mechanisms, including a drop in glutamate release during high contrast (see [A], stages 2 and 3) as well as possible postsynaptic receptor desensitization caused by the high glutamate release (see Discussion). At stimulus offset, there is an outward aftercurrent that slowly recovers back to baseline (dashed line); the gray line shows a single exponential fit with time constant of 6.2 s. This aftercurrent presumably reflects suppressed glutamate release that requires several seconds to recover (see [A], stages 4 and 1).

and Rieke, 2001, 2003; O'Brien et al., 2002). Furthermore, this spike frequency adaptation mechanism may explain why some cortical cells show a decline in spike rate during a stimulus with no subsequent recovery period following the stimulus (Sanchez-Vives et al., 2000b).

We examined, for excitatory synaptic currents, the relationship between the time course of adaptation *during* a high-contrast stimulus and the subsequent recovery period *following* the stimulus (Figure 8B). The onset of adaptation, during the grating, was well fit by two exponentials, with time constants of 19 ms (87%) and 927 ms (13%). The aftercurrent, following the grating, was well fit by a single exponential with a time constant of 6.2 s.

Thus, the decline of the inward current during the stimulus is apparently ~ 10 – 100 times faster than the recovery time. There are two explanations for this asymmetry in time course between these two sides of adaptation. First, as described above, the period during the grating alone could involve mechanisms of synaptic depression that relate specifically to periods of high transmitter release (e.g., postsynaptic receptor desensitization; von Gersdorff and Borst, 2002), and these mechanisms could shorten the time constant for this period of adaptation relative to the time constant for the aftercurrent. Second, even without the involvement of postsynaptic mechanisms, such as receptor desensitization, there appears to be an asymmetry between the onset and recovery from depression of presynaptic release. For example, suppressed release from the rod bipolar cell shows fast onset (<1 s) with a slow recovery time (~ 10 s; Singer and Diamond, 2006). A similar asymmetry exists at the calyx of Held, where depression can be induced in <1 s but requires several seconds to recover (von Gersdorff et al., 1997; Wang and Kaczmarek, 1998). Furthermore, a similar rapid onset with slow recovery exists, on a different time scale, for sodium channel inactivation (Colbert et al., 1997).

Comparison between Guinea Pig and Primate Retina

A recent study based on extracellular recordings in vivo suggested that the suppressed spiking following high contrast arises from a postsynaptic mechanism in the ganglion cell (Solomon et al., 2004). That conclusion was based on an experiment in which a stationary, contrast-reversing grating was positioned so as to evoke no response in the ganglion cell (because the border between grating bars was centered over the cell's receptive field, in a "null" phase). Following this stimulus, the ganglion cell responses were not suppressed, which suggests that the ganglion cell must necessarily be stimulated in order to evoke an adaptive effect. This result would be consistent with an intrinsic mechanism in the ganglion cell for adaptation, rather than a network mechanism involving bipolar cells. There are several explanations for the discrepancy between these findings in primate and ours. Two explanations relate to the different recording conditions (in vivo versus in vitro) and the different species (primate versus guinea pig). For example, the intrinsic mechanism for adaptation in the in vitro guinea pig cells was a minor component of adaptation under our conditions (Figure 3), but this component might be more prominent in the in vivo primate cells. Even within primate experiments, there are differences in slow adaptation between in vitro and in vivo conditions (Chander and Chichilnisky, 2001; Solomon et al., 2004). Another example of a difference between the guinea pig and primate studies relates to adaptation in ON and OFF cells. The primate study found adaptation in both ON and OFF magnocellular cells, whereas we found strong adaptation in OFF cells but only weak effects in ON cells; presently we cannot explain this discrepancy (Solomon et al., 2004).

We offer one further explanation for the lack of adaptation following the "null" stimulus in the primate study (Solomon et al., 2004). Magnocellular ganglion cells in the previous study were recorded at 5° – 25° eccentricity, which should have dendritic tree diameters

of ~ 40 – 140 μm (Croner and Kaplan, 1995; Perry et al., 1984). These ganglion cells probably collect from up to ~ 30 bipolar cells (Calkins, 1999; Jacoby et al., 2000), which would correspond to ~ 6 cells across the width of the dendritic tree. We also assume that each bipolar cell receptive field width is ~ 90 – 100 μm (Dacey et al., 2000). Furthermore, the central-most bipolar cells contribute the largest number of synapses onto the ganglion cell (Kier et al., 1995). Thus, based on this pattern of convergence, it is likely that the "null" stimulus for the ganglion cell was also largely ineffective at strongly driving the central-most bipolar cells. Given this weak stimulation, these central-most bipolar cells would not show an adaptive effect after the stimulus was removed, and this might explain the lack of adaptation in the ganglion cell. However, intracellular studies of magnocellular ganglion cells are clearly required to fully resolve this issue.

Conclusion

Nonspiking cells exist in many sensory systems, and these cells may also express mechanisms of slow adaptation. At present, the most likely mechanism in bipolar cells is an activity-dependent suppression of glutamate release. This mechanism apparently does not involve inhibitory synaptic feedback, K_{Ca} channels in the bipolar cell, or mGluRs at the bipolar terminal (Figure 6; Awatramani and Slaughter, 2001; Sakaba et al., 1997). Furthermore, voltage recordings from salamander retina suggest that the AHP in ganglion cells exists in the absence of an AHP in presynaptic bipolar cells (Baccus and Meister, 2002; Rieke, 2001). Thus, the most likely mechanism for the ganglion cell AHP is a depressed bipolar cell glutamate release that is not reflected by a hyperpolarization of the bipolar cell membrane potential. Further studies will be required to elucidate the mechanism for depressed bipolar cell glutamate release, as it relates to contrast adaptation. This would apparently require a novel preparation in mammalian retina: the ability to record from pairs of cone bipolar cells and postsynaptic neurons (amacrine or ganglion cell) where the bipolar voltage can be controlled while its release is read out directly by the postsynaptic neuron. Presently, such paired recordings, which require routine identification of synaptically connected cells, have only been accomplished in the rod pathway (Singer and Diamond, 2006; Singer et al., 2004).

Experimental Procedures

Recordings

In each experimental session, a guinea pig was anesthetized with ketamine (100 mg kg^{-1}) and xylazine (10 mg kg^{-1}) and decapitated, and both eyes were removed. All procedures conformed to NIH and University of Michigan guidelines. The back of the eye (retina, pigment epithelium, choroids, and sclera) was mounted flat in a chamber on a microscope stage. The retina was superfused (~ 6 ml min^{-1}) with oxygenated (95% O_2 and 5% CO_2) Ames medium (Sigma, St. Louis, MO) at 33°C – 35°C . The retina and electrode were visualized using a cooled CCD camera (Retiga 1300C, Qcapture software; Qimaging corporation, Burnaby, British Columbia). The largest cell bodies in the ganglion cell layer (20 – 25 μm diameter) were targeted for recording. A glass electrode (tip resistance, 2 – 6 M Ω) was filled with Ames' solution for extracellular recording, or intracellular recording solution. Intracellular solutions included K^+ -based solution (solution 1; in mM: K-methylsulfate, 140; NaCl, 8; HEPES, 10; EGTA, 0.1; ATP- Mg^{2+} , 2; GTP- Na^+ , 0.3; titrated to pH = 7.3); K^+ -based solution with QX-314 (solution 2), where NaCl was reduced

to 3 mM and QX-314-Br was added (5 mM); or Cs⁺-based solution (solution 3) where Cs-methane sulfonate (120 mM) replaced K-methylsulfate and BAPTA (10 mM) replaced EGTA; Cs⁺-based solution with TEA (solution 4), where TEA-Cl (5 mM) and Lucifer Yellow (0.1%) were added and QX-314-Br was reduced to 2 mM. The chloride reversal potential (E_{Cl}) indicates the reversal of the synaptic response to GABA or glycine and includes a contribution from bromide; the calculated reversal was ~ -73 mV for solutions 1 through 3 and ~ -67 mV for solution 4. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) except for BAPTA (Invitrogen; Eugene, OR), Strychnine (Fisher Scientific; Pittsburgh, PA), and (RS)-MCPG and CPPG (Tocris, Bristol, UK).

Membrane potential was amplified, continuously sampled at 10 kHz, and stored on computer using a MultiClamp 700A amplifier and pClamp 9 software (Axon Instruments, Foster City, CA; Zaghoul et al., 2005). Junction potential was corrected in all cases. We wrote programs in Matlab (The Mathworks, Natick, MA) to analyze responses in the spike rate, subthreshold membrane potential, or membrane currents. For current-clamp recordings, we balanced the bridge every few minutes in cases where we injected current. For voltage-clamp recordings, we corrected for an error in the holding potential introduced by the series resistance. The corrected holding potential ($V_{h,corr}$) was determined by the formula $V_{h,corr} = V_h - (I_{leak} \times R_s \times (1 - R_{s,correct}))$, where V_h is the apparent holding potential before the stimulus (in mV), I_{leak} is the leak current (in nA), R_s is the series resistance (18.2 ± 0.3 M Ω ; $n = 14$) and $R_{s,correct}$ is the series resistance compensation (typically 0.4–0.5). For positive holding potentials (Figure 5B), we allowed an outward current, unblocked by Cs⁺ or 5 mM TEA, to inactivate (~ 30 – 60 s) before recording the visual response. Results are from 80 cells: 16 ON cells and 64 OFF cells. The resting potential (V_{rest}) of OFF cells was similar between experimental conditions (K⁺-based solution, -66.0 ± 1.5 mV, $n = 20$; K⁺-based solution with QX-314 solution, -65.6 ± 1.5 mV, $n = 10$; Cs⁺-based solution, -64.8 ± 0.7 mV, $n = 5$; Cs⁺-based solution with TEA, -64.2 ± 2.2 mV, $n = 6$).

Visual Stimuli

The stimulus was displayed on a miniature monochrome computer monitor (Lucivid MR1-103; Microbrightfield, Colchester, VT) projected through the top port of the microscope through a 4 $\times$ objective and focused on the photoreceptors (mean luminance, $\sim 10^3$ – 10^4 isomerizations cone⁻¹ s⁻¹; resolution, 640 $\times$ 480 pixels; 60 Hz vertical refresh). The relationship between gun voltage and monitor intensity was linearized in software with a lookup table. Stimuli were programmed in Matlab as described previously (Brainard, 1997; Demb et al., 1999; Pelli, 1997). Cell type was determined using methods described previously (Zaghoul et al., 2005), and cell health was ascertained by repeated measurements of the responses to spots, annuli, and gratings.

The grating was either windowed in a circular patch (0.75 mm diameter; in most experiments) or presented over a 3 $\times$ 3 mm field (Figure 4). The gratings drifted at 6 Hz and in most cases had a spatial frequency of 5–7 cycles mm⁻¹ and contrast of 100%. All stimuli were centered on the cell body. In some cases, a neutral density filter was inserted in the light path to change the mean luminance by a factor of ten.

Analysis

Except where noted, we measured AHP or aftercurrent amplitude by averaging over 100 ms centered at times noted in the text. Recovery time of the AHP was determined by fitting a polynomial function to the AHP and determining the time required for the fit to return 90% back to the baseline response level. We used standard fitting routines in Matlab. To determine the AHP integral, we normalized the trace by subtracting V_{rest} and measured the integral of the trace, over an 8 s period, starting at grating offset. Spike poststimulus time histograms are binned at 500 ms, except where noted. Average membrane potential traces are shown with the resting potential subtracted. Data are reported as mean $\pm$ SEM.

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